FOREWORD

It gives me great pleasure and satisfaction to acknowledge the tremendous contribution of Dairy Technology Division in human resource development for the Indian Dairy and Food Industry, new knowledge being created by way of newer products and processes and its efforts in dissemination of knowledge through a number of tailor-made advanced courses under CAS Programme. In this context, the 20th short course on ‘Recent Advances in Food Additives and Ingredients’ being organized by CAS in Dairy Technology assumes great significance.

These days, tailor-made foods have caught the imagination of manufacturers and consumers in the food industry. They are largely the outcome of innovations in the R&D laboratories. Food Science & Technology have come up with new additives and ingredients that can enhance the functional properties of foods as well as provide a combination of flavour, texture, colour, shapes and potential health benefits. When these factors are varied, the possibilities for product innovation become almost infinite. Keeping in mind with the global trend of foods having natural ingredients as additives, novel dairy foods must also be designed. A host of natural flavour, colour, texture, taste and aroma imparting additives are available. Some of these are saffron, cardamom, cloves, cinnamon, cocoa, nuts (cashew, almonds, walnut, pistachio), raisins, black currant, dates etc. Many products in food and beverage industry are using these natural flavours and colours to enhance appeal, add value and offer consumers an edge over existing products. Innovation is the need of the day to provide attractive foods to the consumers. I understand that issues such as variety of ingredients available and being developed as R&D efforts, their safety concerns, standards, and new product development based on use of ingredients will be thoroughly covered in this programme.

At National Dairy Research Institute, scientists and students, strive to continually work in the direction of new product development with the incorporation of functional food additives such as cereals (rice, wheat, oat, barley etc.), milk-based nutraceuticals, host of food colours and flavourings. I sincerely hope that R&D efforts in these areas will help the Indian dairy and food industry to diversify their product range to also provide the consumer a wider choice of foods. I compliment the faculty in conceptualizing and organizing this very relevant course for the benefit of our brethren in SAUs and other ICAR institutes.

I wish the training programme a great success.

Dated: March 7, 2006

(Sushil Kumar)
ACKNOWLEDGEMENT

In recognition of the scientific contributions made by the Dairy Technology Division of National Dairy Research Institute over a period of last three decades, ICAR granted the status of Centre of Advanced Studies in the year 1995-96. Since then, Centre of Advanced Studies in Dairy Technology has strived hard to organize as many as 20 Short Courses for the faculty of SAUs and other ICAR Institutes. I express my gratitude to Indian Council of Agricultural Research (ICAR) for bestowing CAS status upon our Division and take this opportunity to thank Dr. H. S. Nainawati, ADG (HRD-II) for his keen interest in this programme and timely release of funds.

I express my sincere thanks to Dr. Sushil Kumar, Director, NDRI Karnal for his sagacious advice and guidance and also for providing all necessary facilities for organizing this course.

I sincerely acknowledge the contribution of Dr. Alok Jha, Senior Scientist, Dairy Technology Division & the Course Director for meticulously organizing this course and also for timely publication of the Course Compendium.

I express my thankfulness to the Guest Speakers like Mr. Neil Prasad, Managing Director & Mr. Ravindra Kumar, Director (Technical) from Danisco Ingredients South Asia Gurgaon, Mr. D. S. Chaddha, Advisor CII New Delhi, Dr. Ashwani Rathor, General Manager Modern Dairies Ltd. Karnal, Dr. T. K. Srinivasa Gopal, Principal Scientist, CIFT Kochi, Dr. K. D. Aparnathi, Professor (Dairy Chemistry), Dairy Science College Anand, Dr. Sudhir Singh, Senior Scientist, IIVR Varanasi, Dr. S. Singh, Former Joint Director, NDRI, and Dr. G. S. Rajorhia, Chairman IDA North Zone for their valuable contribution in this course. I also thank all the faculty of the course drawn from Dairy Technology, Dairy Chemistry, Dairy Microbiology and Dairy Engineering Divisions of the Institute.

I thankfully acknowledge the contribution of my colleagues Dr. A. A. Patel, Dr. R. S. Mann, Dr. Abhay Kumar, Dr. Dharam Pal, Dr. S. K. Kanawjia and Mr. F. C. Garg for their valuable suggestions and logistics support. Dr. Latha Sabikhi provided valuable inputs for designing the cover page of the compendium and also in content formulation. Contribution of Dr. A. K. Singh in designing the course content is sincerely acknowledged.

I also thank all the Staff of Dairy Technology Division including Ms. Prem Mehta, Mr. Hakim Singh and Ms. Kusum for their contribution in day to day affairs of CAS programme. I thank Mr. D. D. Patange, Mr. P. K. Singh and Ms. Harpreet Kaur for their valuable assistance.

Dated: March 7, 2006

(G. R. Patil)
Committees for Organization of the XXth Short Course on

Recent Advances in Food Additives and Ingredients

(March 11-31, 2006)

Organizing Committee

Dr. G. R. Patil (Director, CAS)
Dr. A. A. Patel
Dr. R. S. Mann
Dr. S. K. Kanawjia
Dr. Dharam Pal
Dr. Alok Jha (Course Director)

Reception Committee

Dr. S. K. Kanawjia (Chairman)
Dr. G. K. Goel
Dr. B. B. Verma
Dr. R. R. B. Singh

Technical Committee

Dr. Dharam Pal (Chairman)
Dr. Alok Jha
Dr. Latha Sabikhi
Dr. A. K. Singh

Hospitality Committee

Dr. R. S. Mann (Chairman)
Dr. Abhay Kumar
Dr. V. K. Gupta

Purchase Committee

Dr. A. A. Patel (Chairman)
Dr. D. K. Thompkinson
Mr. F. C. Garg
1. **Dr. Rajeev Kumar**  
   Senior Lecturer  
   Department of Animal Husbandry & Dairying, R. B. S. College  
   Bichpuri  
   Agra – 283 105 (U. P.)

2. **Sh. S. B. Jadhav**  
   Assistant Professor (Agrl. Eng.)  
   College of Agriculture  
   Ambajogai,  
   Dist. - Beed – 431 517  
   (Maharashtra)

3. **Sh. S. R. Barkule**  
   Assistant Professor (Horticulture)  
   College of Agriculture  
   Ambajogai  
   Dist. - Beed – 431 517  
   (Maharashtra)

4. **Dr. Jai Singh Yadav**  
   Reader  
   Deptt. of Dairy Science & Technology  
   Janta Vedic College  
   Barant (Baghpat)  
   (U. P.)

5. **Sh. Rakesh Kumar**  
   Assistant Professor (Dairy Microbiology)  
   S. G. Institute of Dairy Technology  
   Jagdev Path  
   Patna – 800 014  
   (Bihar)

6. **Dr. (Mrs.) Trishna Borpuzari**  
   Associate Professor  
   Department of Livestock Products Technology  
   College of Veterinary Science  
   Assam Agricultural University  
   Khanapara, Guwahati – 781 002  
   (Assam)

7. **Smt. Maya Kumari**  
   Training Associate (Home Science)  
   K.V.K., Maheshpur  
   PAKUR – 816 106 (Jharkhand)

8. **Dr. P. K. Bhardwaj**  
   Associate Professor (Dairy Technology)  
   Department of Animal Products Technology (APT)  
   CCS Haryana Agricultural University  
   Hisar – 125 004 (Haryana)

9. **Dr. Vithal Deorao Pawar**  
   Associate Professor  
   Department of Animal Products Technology  
   College of Food Technology  
   Marathwada Agricultural University  
   Parbhani – 431 402  
   (Maharashtra)

10. **Mr. Bhushan Devidas Meshram**  
    Assistant Professor  
    College of Dairy Technology  
    Warud, Post Moha  
    Pusad, Yavatmal – 445 204  
    (Maharashtra)

11. **Mr. Jadhav Vijaykumar Sharwan**  
    Superintendent  
    Satwaji Patil Agricultural School  
    Bindu College Campus  
    Tamse Road, Bhokar  
    Tq-Bhokar  
    Nanded (Maharashtra)

12. **Prof. Rahul J. Desale**  
    Assistant Professor  
    RCDP on Cattle, MPKV, huri  
    Ahmednagar – 413 722  
    (Maharashtra)
<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Position</th>
<th>Institution/University</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Sh. Sukhcharn Singh</td>
<td>Senior Lecturer</td>
<td>Sant Longowal Institute of Engineering &amp; Technology</td>
<td>SLIET, Longowal Sangrur – 148 106 (Punjab)</td>
</tr>
<tr>
<td>14</td>
<td>Dr. Devesh Gupta</td>
<td>Lecturer Senior Scale</td>
<td>Shantipuram Gali No. 2, Nehru Road Baraut</td>
<td>Baghpat – 250 611 (U.P.)</td>
</tr>
<tr>
<td>15</td>
<td>Dr. B. M. Thombre</td>
<td>Assistant Professor</td>
<td>Department of Animal Science &amp; Dairy Science</td>
<td>College of Agriculture Latur – 413 512 (Maharashtra)</td>
</tr>
<tr>
<td>16</td>
<td>Dr. Dhiraj Haraman Kankhare</td>
<td>Assistant Professor</td>
<td>Department of Animal Science and Dairy Science</td>
<td>College of Agriculture Dhule – 424 004 (Maharashtra)</td>
</tr>
<tr>
<td>17</td>
<td>Prof. M. G. Awatirak</td>
<td>Assistant Professor</td>
<td>College of Agriculture Institute of Animal Husbandry &amp; Dairy Science</td>
<td>Ambajogai Dist. Beed – 431 517 (Maharashtra)</td>
</tr>
<tr>
<td>18</td>
<td>Dr. K. D. Chavan</td>
<td>Assistant Professor</td>
<td>Animal Science &amp; Dairy Science (A.S.D.S.)</td>
<td>College of Agriculture (Dairy Farm) Mariai gate Pune – 441 003 (Maharashtra)</td>
</tr>
<tr>
<td>19</td>
<td>Dr. Chhatar Pal Mehla</td>
<td>Associate Professor</td>
<td>CCS, Haryana Agriculture University College of Agriculture Kaul</td>
<td>Dist. Kaithal – 136 021 (Haryana)</td>
</tr>
<tr>
<td>20</td>
<td>Dr. V. Chandrasekar</td>
<td>Scientist (Senior Scale)</td>
<td>Dairy Engineering Division National Dairy Research Institute</td>
<td>Karnal – 132 001 (Haryana)</td>
</tr>
</tbody>
</table>
1.0 Introduction

Despite modern-day associations food additives have been used for centuries. Food preservation began when man first learned to safeguard food from one harvest to the next and by the salting and smoking of meat and fish. The Egyptians used colours and flavourings, and the Romans used saltpetre (potassium nitrate), spices and colours for preservation and to improve the appearance of foods. Cooks regularly used baking powder as a raising agent, thickeners for sauces and gravies, and colours, such as cochineal, to transform good-quality raw materials into foods that were safe, wholesome and enjoyable to eat. The overall aims of traditional home cooking remain the same as those prepared and preserved by today's food manufacturing methods.

Our way of life has thoroughly changed in the last few decades. Activities away from the home have reduced the amount of time consumers spend in the kitchen. The use of food additives, combined with new technology, has made possible the large scale preparation of good wholesome food at economical prices. Thanks to additives we also have access to many convenience foods such as dry sauce mixes, instant mashed potato and instant desserts as well as newer products such as prepared dishes and snacks. Considerable progress has been made in the area of health and nutrition. The preparation of foods such as margarine containing poly-unsaturated fats and many low calorie products would be impossible without using food additives. In fact, many of today's foods would not exist without additives.

Over the last 50 years, developments in food science and technology have led to the discovery of many new substances that can fulfill numerous functions in foods. These food additives are now readily available and include; emulsifiers in margarine, sweeteners in low-calorie products and a wider range of preservatives and antioxidants which slow product spoilage and rancidity whilst maintaining taste.

2.0 What is a Food Additive?

A food additive is defined as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods". Many food additives are naturally
occurring and some are even essential nutrients; it is the technical purpose that leads to these being classified as food additives.

3.0 Why are Food Additives Necessary?

Additives perform a variety of useful functions in food that we often take for granted. Since most of us no longer grow our own foods, additives help keep food safe, wholesome, and appealing while en route to markets. Food is subjected to many environmental conditions such as temperature changes, oxidation, and exposure to microbes, which can change the food’s original composition. Food additives play a key role as food ingredients in maintaining the qualities and characteristics consumers’ desire.

There are six main uses for food additives:

→ **To maintain product consistency.** What makes salt flow freely? How can salad dressings and peanut butter stay smooth and not separate? Certain ingredients such as emulsifiers, stabilizers, thickeners, and anti-caking agents help ensure consistent food texture and characteristics. Examples include: alginates, lecithin, mono- & di-glycerides, methyl cellulose, carrageenan, glyceride, pectin, guar gum, sodium, or aluminosilicate.

→ **To improve or maintain nutritional value.** Nutrients can either be lacking in a food or lost during food processing. Grains, flours, milk, margarine, and other foods are enriched or fortified by adding vitamins A and D, iron, ascorbic acid, calcium, niacin, riboflavin, folic acid, zinc, and thiamin.

→ **To maintain palatability and wholesomeness.** Food naturally loses flavor and freshness, resulting from aging or exposure to natural elements like mold, air, bacteria, fungi, or yeast. Preservatives, such as ascorbic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and sodium nitrite help to slow product spoilage and rancidity while maintaining taste.

→ **To provide leavening or control acidity/alkalinity.** Leavening agents (baking soda or powder) help cakes, biscuits, and other goods to rise during baking. Other additives help modify the acidity and alkalinity of food for proper flavor, taste, and color.

→ **To enhance flavor or impart desired color.** Many spices and natural and synthetic flavors enhance the taste of food. Likewise, colors enhance the appearance of food to meet consumer expectations.

→ To provide ingredients for consumers with specific nutritional requirements, e.g. sweeteners replacing sugar for diabetics, fat replacers etc.

4.0 What are the Sources?

Food additives are manufactured in several different ways; the four groups below present the sources of some additives and the techniques by which they are obtained.
<table>
<thead>
<tr>
<th>Source</th>
<th>Additives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Products of vegetable origin</td>
<td>thickening agents, extracted from seeds, fruit and seaweeds</td>
</tr>
<tr>
<td></td>
<td>colours, isolated from seeds, fruit and vegetables</td>
</tr>
<tr>
<td></td>
<td>acidulants, such as tartaric acid from fruit</td>
</tr>
<tr>
<td>Nature-identical products (reproduced by synthesis or biosynthesis)</td>
<td>antioxidants, such as ascorbic acid in fruit and tocopherol in vegetable oils</td>
</tr>
<tr>
<td></td>
<td>colours, such as carotenoids, the colouring matter of many fruits and vegetables</td>
</tr>
<tr>
<td></td>
<td>acidulants such as citric acid present in citrus fruit</td>
</tr>
<tr>
<td>Products obtained by modification of natural substances</td>
<td>emulsifiers, derived from edible oils and organic acids</td>
</tr>
<tr>
<td></td>
<td>thickening agents, such as modified starches and modified cellulose</td>
</tr>
<tr>
<td></td>
<td>bulk sweeteners, such as sorbitol and maltitol</td>
</tr>
<tr>
<td>Man-made products</td>
<td>antioxidants, such as butylated hydroxyanisole (BHA)</td>
</tr>
<tr>
<td></td>
<td>colours, such as indigotin and quinoline yellow</td>
</tr>
<tr>
<td></td>
<td>intense sweeteners, such as saccharin</td>
</tr>
</tbody>
</table>

### 5.0 How is the Safety of Food Additives Evaluated?

All food additives must have a demonstrated useful purpose and undergo a rigorous scientific safety evaluation before they can be approved for use. The safety evaluation of additives is done by the Food Additives sub committee of Central Committee on Food Standards, Directorate General of Health Services (DGHS). Assessments are based on reviews of all available toxicological data in both humans and animal models. From the available data, the maximum level of additive that has no demonstrable toxic effect is determined. This is called the "no-observed-adverse-effect level" (NOAEL) and is used to
determine the "Acceptable Daily Intake" (ADI) for each food additive. The ADI provides a large safety margin and is the amount of a food additive that can be consumed daily over a lifetime without any adverse effect on health.

At an international level there is a Joint Expert Committee, from the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), on Food Additives (JECFA). The Codex Alimentarius Commission, a joint FAO/WHO activity which develops guidelines for food safety globally, is also drawing up new "General Standards for Food Additives" (GSFA), with the aim of establishing a harmonised, workable and indisputable international standard for world trade. Only those additives that have been evaluated by the JECFA are included.

6.0 How are Food Additives Regulated?

A true single market for food products could not exist without harmonised rules for authorisation and conditions for the use of additives. The Prevention of Food Adulteration Act (PFA) of 1955 provides the legal authority over food, food ingredients, and additives. The legislation on food additives is based on the principle that only those additives that are explicitly authorised may be used. Most food additives may only be used in limited quantities in certain foodstuffs. If no quantitative limits are foreseen for the use of a food additive, it must be used according to good manufacturing practice (GMP), i.e. only as much as necessary to achieve the desired technological effect.

Food additives may only be authorized if:

→ There is a technological need for their use,
→ They do not mislead the consumer, and
→ They present no hazard to the health of the consumer.

Prior to their authorisation, food additives are evaluated for their safety by the Food Additive sub-committee, which advises the DGHS in questions relating to food additives. Petitions for new food additive are submitted to the DGHS. The manufacturers of food or color additive must provide convincing evidence that the proposed additive performs as it is intended and is safe for human consumption. Upon approval, the DGHS issues regulations including the types of foods in which an additive can be used, the maximum amounts to be used, and how they must be identified on food labels. The use of food additives must always be labeled on the packaging of food products by their category (anti-oxidant, preservative, colour, etc).

7.0 Current Trends

The global market for the traditional food additives sectors is estimated to be worth USD22bn a year and it is expected to continue to grow at a rate of around 4 to 5 percent per year. The flavors additive category remains the most important, at about a quarter of the market with sales. Functional food ingredients, such as fat replacers and sweeteners, are also a highly significant market and key growth sector. With the current attention on obesity and its effects, plus the mounting costs to the healthcare system, fat replacers are anticipated to attain double-digit growth.
7.1 Fat Fashioning

Low-fat trends are helping create a healthier market for ingredients. Thanks to the continuing low-fat boom and the calorie-conscious consumer, things look bright for food additives. A report predicts that the demand for food and beverage additives will be positively influenced by consumer preferences for foods and beverages with reduced sugar, fat and other ingredients that require the use of more additives. The report also projects that a shift to more expensive, naturally derived food and beverage additives will spur value gains in this market.

Fat continues to be a hot button with consumers, so food companies and ingredient manufacturers are continuing to seek new ways to approach the issue. There are two approaches to the fat issue. "One is the actual replacement of fat with water in a product, and the other is to modify the structure of the fat molecule."

Taking the fat out of a product and replacing it with water leaves the product developer with two problems: keeping the water in and replacing the functionalities of the fat. This has opened up a market for fat replacement systems that can reduce fat without affecting the taste profile. Fat replacers, texturizers, bulking agents, modified starches and opacifiers have all been developed to mimic the functionalities of the lost fat.

Modifying fats can reduce calories while still retaining the functional properties of fats. Long-chain triglycerides are modified into short- and long-chain structures. Changing the structure of the molecule alters its digestibility, reducing the number of calories. Salatrim, which stands for Short and Long-Chain Triglyceride Molecules, is one such fat. It has about 5 calories per gm, compared with traditional fat, which has 9 calories per gm. Salatrim is versatile because its molecules can be arranged in ways that produce consistency from liquid to solid. Salatrim is suited for many applications that contain traditional fat.

While modified fats and fat replacers are intended for a mainstream market, structured lipids - medium-chain triglycerides - are being developed for the nutraceutical market. These special fats are metabolized differently than other fats, providing a quick supply of calories that don't increase cholesterol levels. Medium-chain triglycerides have been in use for about 40 years, but limited to the medical market. MCTs are uniquely handled by the body in comparison to typical dietary fat. It gets into the bloodstream a lot quicker, so you get more energy a lot faster. Medium chain fatty acids appear in the blood in 20 minutes. These properties can be very useful to people who need an energy-rich diet. Dietary fats are being redesigned to improve their physiological and functional characteristics, thus the name - structured lipids.

7.2 Sweetener Success

There is a lot of anticipation in the sweetener market, where different high-intensity sweeteners such as acesulfame K, alitame, sucralose, cyclamate etc are available which can find potential application for tabletop sweeteners, confections, hard and soft candies, chewing gum, dry beverage bases, dry dessert bases, baked goods, liquid beverages, yogurts, refrigerated and frozen desserts, spoon-for-spoon sugar replacement products, table syrups, sweet sauces and toppings and dry bases for dairy products.
7.3 Edible Coatings

Edible coatings are being used to provide a barrier between a food product and the elements in its environment - such as moisture or oxygen - that can cause deterioration. Attempts are being made to extend the shelf life of baked goods using edible coating. Coatings also can be used to provide a moisture barrier between different layers in a product, improving the texture of the crust on a frozen pizza, for example.

7.4 Flavor and Color Carriers

The flavor industry today can combine virtually any flavor profile desired. Some aspects of flavor remain beyond reach--baked bread aroma, for example--but the list shortens with each passing year. One area where demand remains high, however, is for new and more effective ways to modulate the contact among flavors, aromas and the human senses. Timelines, too, play a role here, although on a smaller scale.

Consider a hot capsicum (e.g., cayenne) flavor, for example. The oil will adhere to the moist environment of the mouth, and the instinctive reaction of many consumers is to seek relief by drowning their taste buds in a beverage. The problem is that water does little to dilute oil-based flavor components. Eating bread, crackers or an oily snack would help more. Low-fat foods have the opposite problem: not enough oil to help flavors adhere to the mouth. Hence, the flavor sensations associated with low-fat foods tend to be weak and insipid--an unfortunate quality that leads to the demise of all too many products. Here the task is to devise ways to help oils cling to the mouth.

Flavor chemists have a number of options at their disposal: Oil-based compounds are regularly emulsified and dispersed in water based food systems using gums and starches to aid adhesion to the tongue and mouth. Weighting agents, such as recently introduced sucrose acetate isobutyrate (SAIB), are used to adjust the density of oil-based flavor dispersions in beverages. To date, however, results have been less than fully satisfactory: Flavor delivery in low-fat systems remains a challenge.

7.4.1 Liposomes that linger

Liposomes offer one way to control flavor delivery. Liposomes consist of lecithin-based, bi-layered vesicles that are hydrophobic (water-repelling) on the inside and hydrophilic (water-attracting) on the outside. The lecithin structure can be manipulated in order to determine how a flavor or nutraceutical trapped inside the liposome will adhere to the mouth, throat or GI tract. Liposomes' direct application to the flavor industry is limited by cost at this time, although costs will drop as production increases. The relatively high cost of liposomes is somewhat offset by increased efficiency. When a flavor is allowed to adhere on the surface of the tongue for longer periods, less flavor is needed. For some applications, such as marinated meats, liposome systems could be used to help flavors integrate themselves into animal cells, for example.

7.5 Natural Colorants

If superior flavor and aroma translate into repeat sales, good color is needed to cinch the sale. A small but increasingly sophisticated industry is building around the art and science of applying natural colorants to food systems. Natural colors are notoriously unstable. Most--such as carotenones (red), luteins (yellow) or anthocyanins (blue/red)--function as natural antioxidants in fruit and vegetables. Absorbing oxygen changes their
structure and color. The natural colors are plated onto a variety of carriers, including soy proteins, salt and calcium carbonate to make them stable.

7.6 Combination Flavors Set to Rock the Sweet Flavors World

Combination flavors are more likely to tantalize taste buds as manufacturers look to increase product variety. The yogurt, sweet confectionery, and cakes and biscuits markets have all begun to incorporate combination flavors into their product offerings. Exotic flavors including mango, papaya and passion fruit it have proved particularly popular as consumers look for sweet flavors that are less predictable and more innovative. While Spain leads the market for combination flavor popularity in yogurts, the United States and the United Kingdom follow close behind with an altogether more innovative and exotic offering. Acceptance of exotic fruits is not widespread, with the United Kingdom and the United States leading in terms of the most exotic fresh fruits available. This is mirrored in the variety of exotic combinations used by manufacturers.

7.7 Regional Tastes to Become the Next Big Flavor Craze

The popularity of ethnic cuisine in Europe and the United States is greater than ever before. This increased popularity, however, is seeing consumers demand more as they crave dishes that are truly authentic rather than-meals with a manufactured spin. Ready meals and cooking sauces are now emphasizing perceived authenticity in order to satisfy consumer demands. Furthermore, regional dishes are becoming popular as consumers look for reinforced authenticity of flavor. As consumers return from exotic holiday locations, their desire to recreate specific flavors is satisfied by regional offerings.

7.8 Star-gazers

What else might make its mark on the market? In near future we'll see cell culture technology used in the flavor industry to produce some of the more expensive flavors like vanilla and saffron. Cells from plants will be grown and harvested from big vats, producing plant-derived products without growing the plants.

Bioengineered crops also will be influential in the near future. As more better-for-you products are created at the plant level, some negative characteristics like trans fatty acids can be eliminated before crops are even harvested.

8.0 Suggested Readings


1.0 Introduction

In the Prevention of Food Adulteration Rules, 1955 (proposed modifications vide Gazette Notification No. GSR 694(E) dated 25 November, 2005), the “Food Additive” has been defined as any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintenance or improving nutritional qualities.

The term ‘ingredient’ includes food additive besides other substances used in the manufacture or preparation of a food and present in the final product although possibly in the modified form.

A mention should also be made about the ‘Processing Aid’ which means a substance or material (not including apparatus or utensils) and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or its ingredients to fulfill a certain technological purpose during treatment or processing and which may result in the non-intentional but unavoidable presence of residues or derivatives in the final product.

2.0 Safety of Food Additives

From time immemorial, salt is used to preserve native type cheeses, meats and fish, herbs and spices to improve the flavour of foods, sugar to preserve fruits and vegetables like cucumber, carrots, onions, chilies, etc. The know how for food additives thus is built up over centuries. Now a day, however, improvements have been made to increase the efficiency of food additives and to ensure safety. The food laws at present strictly regulate the use of food additives and ingredients based on risk assessment. All substances that USDA or FDA generally recognized as safe (GRAS) based on their extensive history of use in foods are given GRAS status, provided that there is no scientific evidence against their application. Salt, sugar, spices, vitamins and monosodium glutamate along with several hundred other substances are classified in GRAS category.
2.1 Food Colours

Use of colours is permitted in foods, drugs, cosmetics after their testing to confirm safety. For example, of the 200 originally listed colour additives, 90 have been approved as safe and the remaining have either been removed from use by U.S. Administration or withdrawn by industry. Certified colours are man made, with each batch being tested by the manufacturer and food laws should ensure that they meet the strict specifications for purity.

Colour additives which do not require certification include pigments derived from natural sources such as vegetables, minerals or animals. Caramel colour produced commercially by heating sugars under controlled conditions for use in sauces, gravies, soft drinks, baked foods, etc. is exempt from certification, but it must meet the legal criteria for purity and other specifications.

2.2 Safety Concern

Although food additives have been used for centuries, they are most misunderstood entity and great cause of consumers worry about safety in present time food additives are reported to cause allergies and food intolerance reactions. All food additives must have not only a demonstrated useful purpose, but also must undergo a thorough and rigorous scientific evaluation before they can be approved for use. The main committee that evaluates the safety in Europe is the EU Committee on Foods and in the USA the FDA and USDA. In India, the PFA Act regulates use of food level, the Joint Expert Committee of Food and Agriculture Organization (FAO) and the World Health Organization (WHO) on Food Additives (JECFA) handles the evaluation. Codex standards are now used worldwide. A small number of people may exhibit symptoms of allergy or intolerance to some food additives. Sulfiting agents used as preservatives in permitted beverages and food products may trigger asthmatic manifestations and coughing. Reactions to colours tartrazine (a yellow food colour) and carmine (red cochinille) have been reported in sensitive to certain food additive to avoid them.

2.3. Acceptable Daily Intake

Assessments of safe limits are based on reviews of all available toxicological studies, including observations in human and in a given model. Data are analyzed to arrive at a maximum level of an additive that has no demonstrable toxic effect. The “no-observed-adverse-effect level” is used to determine the Acceptable Daily Intake (ADI) for each food additives. The ADI provides a large margin of safety which denotes the amount of a food additive that can be consumed daily in the diet, over a life time, without any adverse effect on health. The expert committees working on food additives safety also study the range of intakes across a population by consuming too much of or too many products containing a particular food additive. The JECFA have developed guidelines for food safety titled ‘General Standards for Food Additives’ with the purpose of establishing a harmonized level for acceptance by member countries signing world trade agreements for export purposes.

The EU adopted a framework directive for assessing the use of food additives by employing the three specific technical directives viz. (i) on sweeteners (ii) on colours and (iii) directive on additives other than sweeteners and colours. The purity required for these additives is laid down in directives defining specific criteria.
3.0 Approval Procedure for Food Additive

The manufacturer of food additive files an application on the prescribed proforma to the food regulatory authority by providing convincing evidence that the proposed additive performs the intended function and that it will not cause harmful effects at expected levels of human consumption. Animal experiments using large doses of the additive for long periods are often necessary to substantiate the claim. Studies of the additive in humans are also desirable. The Food Regulatory Authority may advise the government food laboratories to conduct independent risk assessment trials before deciding whether an additive should be approved. The agency considers the composition and properties of the substance, the amount likely to be consumed, its probable long term effects and various safety factors. It may be stated that absolute safety of any substance can never be proven. However, best scientific knowledge available should be used to determine whether the addition is safe.

The Ministry of Health, Govt. of India after the recommendations of the Food Additive Sub Committee of the CCFS through the Gazette Notifications invites objections from the public, trade associations, NGO's, industry users, etc. The comments received within stipulated notified time are scrutinized by the expert group. The approval is given for its use as a food additive indicating maximum amount which can be incorporated. Methods of tests are also prescribed.

3.1 Determination of Safety Margins

The toxicological tests required by the regulatory authorities include lifetime feeding studies and multigenerations that determines how the body reacts to the additive. The starting point for establishing ADI is the determination of no observed adverse effect level (NOAEL) in the most sensitive animal species. NOAEL is expressed in mg of additive per kg of body weight per day. This is then divided by a safety factor of 100. This gives a large margin of safety since the test is conducted on animals. Man is more sensitive than the most sensitive test animals. Moreover, the reliability of tests is limited by the number of animals tested. The uncertainty factor of 100 is based on a 10-fold factor for differences between animals and an average human, and a 10-fold factor to allow the differences between the sensitive sub groups of humans (pregnant women, children, elderly). The guidelines for approval of an additive are given in Annexure II of the Codex.

4.0 Modern Technology in Producing New Additives

Requests are being received to use ingredients that would replace fat or sugar in foods. Biotechnology allows the use of simple organisms to produce additives. FDA approved the first bioengineered enzyme (rennin) for use in making cheese. Rennin has traditionally been extracted from fourth stomach of the calves or the microorganisms.

JECFA reviews hundreds of fresh requests from various member countries each year. Proposals are identified as high priority or on priority for evaluation. The Codex Committee on Food Additives has developed a diagram that illustrates the procedures for consideration of the entry and review of food additives for the benefit of member countries. The codex has also developed the International Numbering System (INS) for food additive in Schedule 1.
5.0 Certification System for Food Additives

The manufacturers of food additives have to demonstrate compliance to risk assessment and near elimination of hazards. Food safety management systems like ISO, HACCP, BRC, SQF, QMS, EUREP GAP, etc. can be adopted for certification services operated by many national and international companies in India. The procedure for certification systems will be discussed during the presentation.

6.0 Suggested Readings


Prevention of Food Adulteration Act, Govt. of India, Ministry of Health.

1.0 Introduction

Dairy ingredients are preferred ingredients for their functional supremacy and good flavour, colour and nutritional profile. Production of new dairy ingredients through membrane techniques that can be used in different foods based on their functional properties is now largely spread out, particularly after the commercialization of high mechanical and physico-chemical resistant mineral membranes. The membrane filtration processes currently available include reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), microfiltration (MF) and electrodialysis. The distinction between the various membrane processes is somewhat arbitrary and has evolved with usage and time.

Ideally, RO membranes should retain all components other than the water itself. RO has widely been used to concentrate dairy fluids and also make further products like khoa (Kumar and Pal, 1994) and condensed, concentrated and dried products. RO processed milk has also been successfully used in the manufacture of yoghurt, dahi, ice cream and several other dairy products. The principal application of NF is for separation of mineral ions in the $10^{-9}$ m size. The main emerging application of NF in the dairy industry is in partial demineralization of whey. Electrodialysis is a demineralization process based on the removal of charged mineral ions from the non-charged material. This process has wide application in demineralization of whey for use in many special dietetic foods including infant formulae.

UF membranes retain only macromolecules or particles larger than 1-20 nanometers. During UF process, a portion of lactose and minerals is removed. One of the major benefits of UF technology is its ability to retain whey proteins, that are normally lost in whey in traditional manufacturing processes of cheese, chhana, paneer. UF membranes reject fat and all true proteins in milk. The proteins can be further concentrated by progressive removal of lactose and ash by a subsystem called diafiltration. During the last over three decades, UF technology has increasingly being used in the dairy industry because of many inherent advantages viz. saving on energy, improved yield of protein, enhanced nutritive value of the product and availability of a lactose stream in the form of permeate.

MF processes are designed to separate particles in the micrometer range (0.1-10 micrometers). This process retains fat globules, microorganisms and somatic cells, but allows passage of proteins in addition to lactose and minerals. The application of MF to skim milk in combination with other membrane processes and/or chromatography processes opens up possibilities in isolating and purifying caseins and the peptides derived from them that can find application in the pharmaceutical industries.
The newer application of membrane processing in the dairy industry is in the separation and fractionation of individual milk proteins having unique functional and nutritional characteristics. Fractionated milk protein isolates are finding increasing applications in infant foods formulations and dietetic products.

2.0 Insoluble and Soluble Components of Milk

Milk can be separated into its insoluble (caseins and fat) and soluble (whey proteins, lactose, peptides and NPN) components. This separation is advantageous in the sense that the casein and fat fraction can be processed separately, avoiding the inevitable interaction between the casein and whey protein fractions under the influence of heat. This approach allows reconstitution of milk with “bio-protective factors” intact. Alternatively, casein and fat rich retentate can be used for cheese production and the whey protein permeate can be ultrafiltered to obtain undenatured whey protein isolates of extremely high purity (pharmacological grade) displaying prophylactic quality.

3.0 UF Milk Retentate

UF milk retentate has widely been used for the manufacture of cheese and other fermented short shelf-life products where protein increase is desirable but lactose and ash increase is not desirable (Darghn and Savello, 1990; Green, 1990, Singh et al., 1994). In the Indian context UF retentate seems to be a highly promising base for long life paneer (Rao, 1991; Singh et al., 1994). UF milk retentate has also been used to produce low lactose powder, non-dairy whitener, rasogolla mix powder, cheese base and milk protein concentrates.

Cheese base is a paste of the same composition and pH as Cheddar cheese but without the Cheddar flavour and structure. It is used to replace the young cheese component for the manufacture of processed cheese. For the production of cheese base, Milk is pasteurized, standardized to 3.8% fat, Cooled to 50°C, ultrafiltered to 30% TS, diafiltered to reduce lactose to desired level, further ultrafiltered to 40% TS, repasteurized, Cooled and 1% Cheddar starter culture added and evaporated to 60% TS. Processed cheese is made with blending cheese base (30%) with 70% normal aged Cheddar cheese.

Milk protein concentrates (MPC) is a relatively new dairy ingredient based on ultrafiltration and drying of skimmed milk. Typically with a protein content from 50-85% of total solids, MPC can be considered as a functional ingredient to be used in the manufacture of other foodstuffs. The main application of MPC today is in spreads and dressings. It is also used as a protein base in processed or even recombined cheeses. This high protein and high calcium ingredient can be used for the preparation of many dietetic foods including foods for elderly people and sport persons.

4.0 Condensed and Dried Whey Products

By far the single largest use of whey solids on global basis is in the form of whole dry whey and it continues to grow. This is whole whey that has been condensed and spray dried as such or after blending with certain other liquid ingredients. These powdered whey products are marketed as commodity ingredients to be used in the
manufacture of confectionery, chocolates, biscuits, breads and in the manufacture of animal feed.

A major problem with many whey-based products is their salty flavour owing to their high mineral content. Demineralized sweet whey (25-65% demineralization) can be used in foods such as coffee whitener, soft serve ice cream, milk shakes, whey drinks and caramel, citrus drinks, salad dressing, animal feeds, bakery goods, confectionery coatings and dry mixes. A range of demineralized spray-dried, whey-based products for use in infant feeds and dietetic applications have been developed based on products manufactured by demineralization and ultrafiltration of whey.

5.0 Native Casein

A promising application of MF (0.2 µm pore size) of skim milk has been the selective separation of native casein micelles from the whey proteins (Sachdeva and Buchheim, 1997). If diafiltration with water is used during MF concentration, purified phosphocaseinate is obtained (Up to 90% protein in dry matter). After spray drying, such a product will easily compete with traditionally made calcium caseinates for many applications. Native casein has excellent rennet coagulating abilities and forms stronger gels at acidic pH (Famelart et al., 1996).

6.0 Casein Fractions

Membrane MF (0.2 µm pore size) allows separation of β-casein by solubilization from calcium caseinate at 5°C and from skim milk at 4°C and pH 4.2-4.6. Products obtained on both sides of the MF membrane are suitable for modifying the β-casein/αs1 casein ratio of cheese milks and consequently, texture and flavour of resulting cheeses. New cheese varieties could be created in this way. The main interest in β-casein is related to the presence of peptides with biological activities in its sequence. Indeed, β-casein peptides are thought to be involved in bio-availability of trace elements, in morphinomimetic, cardiovascular and immunostimulating activities, not only for the young but also for adults and pregnant women.

7.0 Whey Protein Concentrates

UF process is now a major means of WPC production throughout most of the dairy countries of the world. WPC with 35% protein is perceived to be a universal substitute for NFDM, because of the similarity in gross composition and its dairy character. WPC can also be seen competing with casein, egg albumin and soya proteins within the existing markets. Dietary whey proteins significantly retard the development of colon cancer and thus has potential as a functional food ingredient. Microfiltration of whey to obtain delipidised whey protein concentrates with improved properties has recently been tried out using different approaches (Gesan et al., 1995; Karleskind et al., 1995).

Due to various reasons, buffalo and cow milks are being humanised and used partly or exclusively for feeding human infants throughout the world. For humanisation, apart from making other modifications, whey proteins proportion needs to be increased in these milks. For this, a great potential lies in the application of WPC.
Whey protein based fat mimetics have recently been used in low fat variants of frozen desserts, yoghurt, fat spreads and cheese. These fat mimetics are made from concentrated cheese whey by special thermal and mechanical treatments which result in a controlled globular aggregation of denatured whey proteins termed as microparticulation (Buchheim and Hoffmann, 1994). Suspensions of such microparticles, with diameters in the range of about 0.1 to 3 μm can produce a creamy texture similar to that of globular fat particles, like the milk fat globules.

A special groups of ingredient called whey protein texturizer has been developed (Thompersen, 1994), that have unique properties of improved emulsifying property than whey protein concentrates, form gel without heat giving highly viscous solutions and give a firm gel upon heating. These can find application in comminuted meat products, mayonnaise, salad dressing and spreads, bakery products, French fries and potato products. These can also be used for preparations most suitable for heat induced structuring processes - thermoplastic expansion, microwave expansion and thermogelation. Such textured products act as extenders in various meat products.

8.0  Whey Protein Fractions

The health and medical benefits of individual whey proteins are gaining acceptance at an increasing rate. Isolation of whey protein fractions can help in preparation of infant formulae with protein compositions more reflective of human milk. From the defatted WPC, it is possible to prepare purified ß-lactoglobulin and ß-lactalbumin. ß-Lactoglobulin is the protein which is essentially responsible for the gelling property of whey protein concentrates. ß-Lactoglobulin could also have essential physiological activity as in the transport of vitamin A. This activity exists in most milk-producing animals but not in humans.

The ß-lactalbumin has a great potential market. This protein is remarkably rich in tryptophan (4 residues per mole, about 6%); such a protein could allow preparation of tryptophan containing peptides which could have physiological properties. ß-Lactalbumin is one of the main protein of human milk, so production of a formula suitable for infants could be based on native phosphocaseinate, purified ß-lactalbumin and milk ultrafiltrate. Such an infant formula will have very low or may be no allergenicity. Although it has not been clearly explained, the high ß-lactalbumin content of human milk might be indicative of the physiological role of this protein.

Defatted WPC is a very good raw material for the extraction of lactoferrin (Lf) and lactoperoxidase (Lp) through ion-exchange chromatography. Both Lf and Lp are already industrially produced, and are used in veterinary medicine to prevent diarrhea in young calves, in human eye drops, in mouthwashes, in chewing gum, and in infant foods.

Defatted whey could also be a source for preparing purified immunoglobulins through the use of UF membranes with large cut-off. However, for this family of milk components, colostrum will probably be a more suitable source than whey.

Glycomacropeptide (GMP) is separated by ultrafiltration with a membrane having a cut-off between 50,000 and 20,000 daltons. GMP suppresses appetite and consequently prevents fatty deposits (results observed with dogs), avoids adhesion of Escherichia coli cells to intestine walls, protects against influenza and finally, prevents adhesion of tartar to teeth.
9.0 Biological Peptides

Enzymatic modification of milk proteins permits development of peptides having unique physico-functional properties of pharmacological significance. These bio peptides have been implicated in physiological roles such as biotransfer of trace elements, immunomodulation, antihypertension, antithrombosis, regulation of the gastrointestinal tract and the general behaviour (Morphine like activity). UF technology is being used as the most appropriate tool for separating low molecular weight peptides and free amino-acids from proteins substrates utilizing enzymes (Gauthier and Pouliot, 1996; Leppala, A.P., 1996; Maubois et al., 1996).

10.0 Lactose

Another very important dairy ingredient is milk sugar i.e. lactose that has various applications in food and pharmaceutical industries due to its multiple functional properties. Lactose is added to salad dressing, mayonnaise, soups and sauces to enhance flavour and confer added stability to various proteins in the formulations against flocculation at acid pH and pasteurization. The hydrolysis of lactose yields a sweet syrup containing glucose and galactose that have nutritional advantages in some dietary applications. Lactose hydrolysed syrups from permeates and whey's are becoming commercially available and are being used in confectionery and ice cream. A major use of lactose is in humanised infant formulae. Lactose has been used for years in the pharmaceutical industry as a coating agent for pills and tablets. The most recent application for lactose and its derivatives is in the formulation of 'nutraceutical' health foods.

11.0 Phospholipids Enriched Fraction

Phospholipids enriched fraction can be prepared from whey and buttermilk by using microfiltration. This fraction has valuable emulsifying properties, which could be used by the cheese industry (low fat cheeses), meat industry and cosmetic industry (liposome-based products). Moreover, the nutritional value of the phospholipids will probably lead to further increased interest in this fraction in the next few years.

12.0 Dicalcium Phosphate

Whey minerals are the other whey constituents which can be recovered from the ultrafiltration permeates of acid whey. These melting salts of whey in the form of calcium-magnesium phosphate can be used as food ingredient in meat and fish products (Sienkiewicz and Riedel, 1990).

13.0 Conclusion

Production of dairy ingredients is a major activity in the developed countries like Europe and United States and provide the Indian Dairy Industry a very exciting opportunity to undertake their manufacture not only to meet the domestic demand but to get into the export markets in United States, Europe and South East Asia. Rapid developments in the range and capabilities of membranes have the potential to profoundly affect the dairy industry as a whole. Being an excellent tool for the fractionation of milk pro-
Proteins, a new range of products having unique nutritional and functional characteristics (gelling, foaming, emulsification, water holding capacity) have been developed by employing membrane processing. Membrane processing in combination with new efficient demineralization techniques and better knowledge and processes of enzymatic hydrolysis have helped in production of innumerable valuable fractionated dairy ingredients having significantly greater nutritional and biological values that have application in value added products, health foods and pharmaceutical products. More recently, membrane processes have been utilized for the preparation of enzymatic derivatives of milk proteins having pharmacological significance.

14.0 References


Green, M.L. 1990. The cheese making potential of milk concentrated up to four fold by ultrafiltration and heated in the range 90-97°C. *J. Dairy Res.*, 57: 549.


1.0 Introduction

Iron is an essential trace element in animal and human nutrition. Its deficiency is usually the result of insufficient dietary intake, poor utilization of iron from ingested food or a combination of both. The other possible reasons for iron deficiency may be hookworm infection, malaria and vitamin A deficiency. Iron participates to the structures of cytochromes and several enzymes. It is also a component of heme in hemoglobin and myoglobin in which it plays an important role in the transport, storage and utilization of oxygen. Iron deficiency induces anemia which affects more than 30% of world’s population, especially infants, women and teenagers. Around 60% women of reproductive age and 40-50% teenagers are affected by iron deficiency. Iron deficiency also results in alteration in mental development and decrease in immunity. Iron fortification of flour, bread and cereals is practiced to correct dietary iron deficiency.

The problem is more complex in developing countries than in industrialized countries where the consumption of insufficient absorbable iron is usually the only cause or may be major factor causing iron deficiency. The major causative factor in developing countries is not low iron intake, but, rather, low iron absorption. Iron intake is often relatively high, almost 20mg/day, and would easily meet the recommended dietary allowances of USA. Unfortunately, much of the ingested iron is poorly bioavailable iron from plant sources or is contamination iron from soil and includes little bioavailable iron from animal tissues. Major cereals, legumes and staple food contain high levels of phytic acid, which is a potential inhibitor of iron absorption and some, such as sorghum, also contain phenolic compounds which greatly impede iron absorption by binding iron in the gut in unabsorbable complexes. The intake of foods that enhance iron absorption such as fruits and vegetables containing vitamin C or muscle tissue is often limited.

The fortification of foods is often regarded as the most cost effective long term approach for the prevention of iron deficiency. Milk is generally considered to be a rather complete food and provides a significant part of the dietary calcium and other minerals but is a poor source of iron (0.2 mg/kg of milk) which can not be significantly increased by oral administration of iron to lactating mothers (human/animals). Fourteen percent of this iron occurs in milk fat where it is associated with the fat globule membrane. About 24 % of the iron is bound to casein, while 29% is bound to whey proteins and 32% is associated with low molecular weight fraction. In skim milk 50-65% of the iron is bound to caseins, 18-33% is in the whey protein fraction and 15-33% is in the non-protein fraction. This fraction and low molecular weight fraction could correspond to small molecules such as citric acid, orotic acid and inorganic phosphate (Pi).
Hence, iron fortification of milk and dairy products is also considered as a potential approach to prevent these disorders. Iron supplementation of infant formulae has also been recommended by most national and international agencies. UHT milk, liquid diets, infant formulas and powdered skim milk lend themselves very well suitable technologically and nutritionally as a vehicle for fortification.

However, results concerning the biophysico-chemical modifications, the organoleptic changes, and the iron bioavailability and tissue distribution are conflicting. Indeed all these modifications depend on different factors, such as the physico-chemistry of added iron (iron valence, solubility, degree of chelating, complex formation), the physico-chemistry of added iron after processing or storage, the presence of the other 81 components in the meal that can increase or decrease the absorption of iron and the physico-chemical state of the tested subject.

2.0 Iron Requirements

Net iron losses from the body through the skin, urine and blood are about 2mg/day for women, 1mg/day for men and 0.8mg/day for children; reflecting that net iron requirements are indeed very low. The Recommended Dietary Allowances (RDA) are 15 mg/day for women and 12mg/day for men which is 7.5 and 12 times higher than net iron requirements, therefore, the RDA take into account the low bioavailability of dietary iron (Herbert, 1987)

The bioavailability or more specifically the relative bioavailability (RBV) of iron in foods is measured as the incremental absorption of that iron in a standardized diet or test meal. Ferrous sulfate has been used as a reference and has an RBV of 100 %, against which other iron compounds are compared. For example ferrous fumerate, which is used in infant foods, has an RBV that is equal to Ferrous sulfate, while ferric pyrophosphate, ferric orthophosphate and reduced iron have an RBV of one half to one third of ferrous sulfate (Hurrel, 1989; Hurrel et al., 1989; Hurrel and Cook, 1990)

3.0 Criteria for Selecting Food Vehicles

While selecting an appropriate food vehicle for iron fortification the following criteria must be taken into consideration:

→ It is essential that frequency and average consumption of the food by the target group is both known and predictable, especially for young children, adolescents and pregnant women.

→ There should be no risk of over consumption.

→ The addition of iron should not affect the organoleptic qualities of the food (iron in its ionized form is an aggressive compound that interacts with vitamins, minerals and other nutrients).

→ The cost of the fortified products should not be beyond the reach of those for whom it is intended. Cost will be influenced by the type of iron compound added to the food vehicle, which depends on the nature of the food and its shelf life. Some iron compounds cost as much as a complete vitamin/ mineral premix that need to be considered in designing micronutrient concepts and priorities.
4.0 Nature of The Added Iron/Iron Fortificants

In iron fortification of foods/milk and milk products, three categories of iron compounds have been used.

→ The first category that is the most used is the iron salts. These salts have two oxidation states (Fe$^{2+}$ and Fe$^{3+}$), are completely dissolved in water and in milk. However, they have the disadvantage of freely interacting with the constitutive elements of milk, which may alter its sensory properties.

→ The second category (elemental iron i.e. Fe O) is obtained by reduction under H$_2$ or CO$_2$ by electrolysis or by the carbonyl process. These compounds are powders with various particle size, poorly soluble or insoluble in water and chemically inert. They have the disadvantage of being used only in solid dehydrated foods because they do not dissolve in neutral liquids.

→ The third category is iron complexed to proteins or phospholipids. The binding iron sites are mainly amino acids such as Pser (phosphoserine), Asp and Glu. The iron bound to these compounds is generally not exchanged and does not react with components of milk.

The presentation of iron can also vary. Thus FeSO$_4$ microencapsulated with lecithin has the same bioavailability as FeSO$_4$ but has the advantage of being coated with phospholipid membrane, in the same way; lipid microencapsules of FeSO$_4$, alone or with ascorbic acid to fortify cheese and other high moisture foods with iron is developed. This keeps the iron away from coming in contact with the food vehicle preventing the undesirable interactions that take place when conventional FeSO$_4$ is used.

A number of iron compounds are listed as “Generally Recognized As Safe”(GRAS) by the US Food and Drug Administration (Code of Federal Regulation, 1994). Iron fortificants listed as GRAS are reduced iron, ferric phosphate, ferric pyrophosphate, ferric sodium pyrophosphate, ferrous gluconate, ferrous lactate and ferrous sulfate. However, some non-GRAS iron fortificants are also being recommended and used. These compounds may be safe, but in some cases more data are needed to corroborate their safety. Example of these includes carbonyl iron, iron amino acid chelates and sodium-iron-EDTA. As of today sodium-iron–EDTA is not manufactured on commercial scale. Furthermore, the compound is very expensive to produce and its superior bioavailability has only been shown in diets high in phytate.

5.0 Optimization of the Iron Compound

This necessitates the careful selection of both the food product to be fortified and the iron fortification compound to be added. Clearly the iron compound must be first optimized with respect to relative bioavailability. However, if the food vehicle contains potent inhibitors of iron absorption, the added iron will have little or no impact on the iron status of the consumer. The success of food fortification programme thus depends heavily on the absorbability of the added iron and its protection from major dietary absorption inhibitors. The commonly used iron compounds can be divided into four groups:

→ Those that are freely water soluble.

→ Those that are poorly water soluble but soluble in dilute acids such as gastric juice.

→ Those that are water insoluble but poorly soluble in dilute acids.
Protected iron compounds.

In general, the freely water soluble compounds are highly bioavailable in rodents and humans, as are compounds that are poorly soluble in dilute acids, however, have only a low to moderate bioavailability. This is because of variable dissolution in gastric juice owing to both the characteristics of the compound itself and the meal composition. Although, it would be logical to always use iron compounds of highest bioavailability, they often cause unacceptable color and flavor changes in many foods. Optimization, therefore, means selecting the iron compound with the highest potential for absorption without causing subsequent organoleptic problems in food vehicle.

6.0 Bioavailability

The absorption of fortification iron depends primarily on its solubility in gastric juice. Water soluble compounds such as ferrous sulfate dissolve instantaneously in gastric juice, whereas, more insoluble compounds, such as elemental iron, rarely dissolve completely. Once dissolved the fortification iron enters the common pool, where its absorption (like that of all pool iron) depends on the content of enhancing or inhibitory substances in the meal and on the iron status of the subject. For example, phytate and polyphenols or a satisfactory iron status in an individual will diminish absorption, whereas vitamin C or low iron status will enhance the iron absorption.

7.0 Organoleptic Problems

In addition to causing unacceptable changes in color and flavor when added to foods, iron compounds may also provoke precipitation, such as when added to fish sauce or when iron fortified sugar is added to tea. Many iron compounds are colored and can not be used to fortify light colored foods. In addition, the more soluble iron compounds often react with substances in foods, causing discoloration. Infant cereals have been found to turn gray or green on addition of ferrous sulfate and dark blue if bananas are present. Phenolic compound have often been implicated and Douglas et al. (1981) reported that ferrous sulfate, ferrous lactate, ferrous gluconate and ferric ammonium citrate, as well as the less soluble ferrous fumerate and ferric citrate produce off – colors when added to a chocolate milk drink. Similarly, salt fortified with ferrous sulfate or other soluble iron compound become yellow or brown.

Off-flavor can also result from the metallic taste of the soluble iron itself, particularly in beverages. However, the catalytic effect of iron on fat oxidation in cereals during storage is the major problem. As in the case of product discoloration, the water soluble compounds, such as ferrous sulfate promote fat oxidation and reduce product shelf life.

8.0 Protecting and Enhancing the Absorption of Fortification Iron

Many food vehicles for iron fortification contain substances that inhibit iron absorption. Cereals contain phytic acid and occasionally polyphenols, milk contains calcium and casein, and chocolate drinks, coffee and tea contain polyphenols. In addition, many diets in developing countries to which iron fortified salt, sugar, or other condiments are added are often high in phytate and polyphenols from cereals and legume foods. To ensure a level of absorption that is high enough to improve or maintain iron status, it is necessary to prevent the fortification iron from reacting with the absorption inhibitors. This can be
accomplished by adding absorption enhancers. The most common enhancer is vitamin C. Alternatives would be bovine hemoglobin and NaFeEDTA where iron is in protected form.

8.1 Vitamin C

Vitamin C can increase the absorption of both native and fortification iron several fold when added to foods. Its effect appears to be related to both its reducing power and its chelating action. It can reduce ferric to ferrous iron and/or maintain ferrous iron in the ferrous state and so prevent or decrease the formation of insoluble complexes with absorption inhibitors or with hydroxide ion in the gut. In addition, it can form soluble complexes with iron at low pH that remains soluble and absorbable at the more alkaline duodenal pH.

8.2 Hemoglobin

Hemoglobin is a form of food iron that is naturally protected from major inhibitors of iron absorption, such as phytic acid and polyphenols. The iron is contained within the porphyrin ring of the heme molecule, which is split from the globin moiety during digestion, and is taken up intact into mucosal cells. The iron is released within the mucosal cell by the action of heme oxygenase and is prevented from reacting with the inhibitory and enhancing ligands within the intestinal lumen.

8.3 Sodium Iron EDTA

The use of NaFeEDTA as a food additive has recently been reviewed by the International Nutritional Anemia Consultative Group (INACG) and was strongly recommended as most suitable iron fortificant for use in developing countries. The provisional acceptance of the compound by the joint FAO/WHO Expert Committee on Food Additives for use in supervised fortification programme in iron deficient populations has cleared the way for large scale fortification trials. However, before general use of NaFeEDTA can be recommended, more systematic studies are necessary to ascertain potential organoleptic problems in a variety of foods.

9.0 Food Vehicles for Iron Fortification

9.1 Cereal Products

Cereal flours are currently the most frequently used vehicle for iron fortification that reach the entire population. Wheat flour enrichment is mandatory in many countries (USA). In the United States, corn meals, corn grits and pasta products also have standards for voluntary iron enrichment, and these commodities are mostly enriched by manufacturers. Other commonly fortified foods are breakfast cereals and infant cereals.

9.2 Salt

Iodine fortified salt has successfully eradicated iodine deficiency in many countries, so salt would also seem a highly suitable vehicle for iron fortification. Almost all of the development work for the fortification of salt with iron has been conducted in India. Color
changes during storage have been the major problem, because salt in India is relatively crude and contains up to 4% moisture.

9.3 Sugar

Sugar is an alternative vehicle for iron fortification in reasons of the world where it is produced, such as Caribbean and Central America. But in other developing countries refined sugar consumption is more common in the middle and upper socio-economic segments of the population. Iron fortified sugar would be expected to be well absorbed if consumed with citrus drinks but poorly absorbed from coffee and tea owing to Phenolic compounds or if added to cereal products owing to phytate.

9.4 Milk

Infant formulas are usually milk based with added vegetable oils, minerals and vitamins. Iron is almost always added as ferrous sulfate from 5-12 mg/lit and its absorption can be improved considerably by the addition of 100-200mg of vitamin C per liter. The relatively low iron bioavailability from milk products can be assumed to be due to the presence of two inhibitory factors, calcium and the milk protein casein. In a series of fortification trials in Chile in which iron fortified formulas were fed to infants, the improvement of iron status was only modest in the absence of vitamin C but improved considerably when it was added to formula. The wide spread consumption of iron-fortified (and vitamin C-fortified) formulas by infants in the United States is regarded as the reason for the dramatic fall in the prevalence of anemia over the last 30 years.

Whole milk could also be considered as a vehicle for iron fortification, but because of the presence of calcium and casein, an absorption enhancer should be added to improve absorption. Unfortunately it is difficult to add vitamin C to fluid milk as it has been reported to degrade rapidly to diketogluconic acid leading to changes in flavour. Many soluble iron compounds rapidly produce off-flavors when added to milk, owing to the promotion of lypolytic rancidity, oxidative rancidity by the oxidation of free fatty acids and the partial or complete loss of vitamins A, C and B-carotene.

After evaluation of a series of compounds, the addition of ferric ammonium citrate and ferric pyrophosphate has been proposed for liquid milk and for skim milk, skim milk concentrate and dry milk powder. The addition of NaFeEDTA would appear to be an interesting alternative, but it has not been evaluated extensively for organoleptic properties in milk. The usefulness of milk as a vehicle for iron fortification has been demonstrated in a Mexican school feeding programme. The hemoglobin level of children fed 200 ml milk containing 20 mg iron as ferrous chloride improved by 1g/dL. In three months this study demonstrated that with high level of added iron, the addition of vitamin C was not essential. As with iron fortified sugar; when iron fortified milk is added to tea, coffee or cocoa, the beverage undergo unacceptable color changes.

10.0 Conclusions

One strategy to overcome the high prevalence of iron deficiency anemia in developing countries is to fortify various food products with iron. There are several options with respect to the iron compound used and the food products to be fortified. Various factors, including cost effectiveness of the fortification in raising absorbable iron intake in the targeted population, the palatability of the fortified food, and the etiology of iron
deficiency must be considered before initiating a fortification programme. As most iron fortified foods contain potential absorption inhibitors, it is essential to protect the fortification iron so as to ensure adequate absorption. This can be achieved easily in the food industry by adding vitamin C, although NaFeEDTA and possibly, hemoglobin would seem better options for developing countries.

11.0 Suggested Readings


1.0 Introduction

Sweeteners can be divided into two main groups: bulk and intense sweeteners. Simple sugars such as glucose, fructose, sucrose and maltose; sugar alcohols such as sorbitol, maltitol and lactitol; starch and starch hydrolysates such as glucose syrups; and high-fructose corn syrups, all belong to the class of bulk sweeteners. They confer body and texture to foods. Intense sweeteners form a very heterogenous chemical group. Some are naturally occurring molecules extracted from plant material (glycyrrhizin, steviosides, thaumatin), whereas others are artificial compounds obtained by organic synthesis (aspartame, acesulfame-K, sucralose, saccharin, cyclamate, etc.). Unlike bulk sweeteners, they are generally not metabolized by the body and are excreted unchanged. For this reason, and due to the fact that they are used at very low levels in foods, they are also referred to as nonnutritive or noncaloric sweeteners (Prodolliet, 1996). These low calorie artificial sweeteners are used to aid in the management of diabetes and dental caries, weight control and create a variety of good tasting foods and beverages (www.caloriecontrol.org, 2004).

According to a notification amending the PFA rules, 1955 and issued by the Ministry of Health and Family Welfare on June 25, 2004, the use of artificial sweeteners has been allowed in food items as per the limits prescribed. Present notification permits the use of four artificial sweeteners like saccharin, aspartame, acesulfame-K and sucralose in combination, within prescribed limits. Use of artificial sweeteners has been allowed for the first time in sweets like halwa, khoya, burfi, rasogolla, gulabjamun and other milk products. Quantitative information on the sweeteners degradation/decomposition in dairy systems would help to establish any excess required to compensate for processing and storage losses. Degradation and hydrolysis of these sweeteners during prolonged heat treatment has been of concern due to the formation of some potentially toxic metabolites.

2.0 Stability of Sweeteners

Long-term storage and heat stability are important factors for the use of intense sweeteners in many food products and beverages. The sweeteners have been found to have different response behaviour towards heat and pH. Sweeteners are highly sensitive to extreme conditions of pH and temperature and consequently break down to their respective moieties.
2.1 Acesulfame Potassium (Acesulfame K)

It is approved for use in a wide range of products, including tabletop sweeteners, desserts, puddings, baked goods, candies and soft drinks. Acesulfame potassium occurs as a colorless to white-colored, odorless crystalline powder with an intensely sweet taste. It dissolves readily in water, even at room temperature. The approximate sweetening power is 200 times that of sucrose. Acceptable daily intake (ADI) is 15 mg/kg body weight/day. It blends well with other sweeteners and is synergistic with aspartame and sodium cyclamate but less so with saccharin.

Stability In aqueous media, acesulfame-K is distinguished with very good stability. After several months of storage at room temperature, virtually no change in acesulfame-K concentration was found in the pH range common for beverages. Prolonged continuous exposure to 30°C did not cause losses exceeding 10%, the threshold for recognition of sweetener’s difference (Hoppe and Gassmann, 1985). Even at temperatures of 40°C, the threshold for detection of sweetness differences was exceeded after several months, only for products having pH 3.0 or less (Von Rymon Lipinski, 1988 and 2004). Acesulfame-K containing beverages can be pasteurized under normal pasteurization conditions, without loss of sweetness. Sterilization is possible without losses under the normal conditions (i.e., temperatures around 100°C for products having lower pH levels and 121°C for products around and greater than 4.0 pH). In baked foods, no indication of decomposition of acesulfame-K was found, even when biscuits with low water content were baked at high oven temperatures for short periods. This corresponds to the observation that acesulfame-K decomposes at temperatures well above 200°C.

Decomposition: Acesulfame-K is a derivative of oxathizin. Decomposition of acesulfame-K occurs only under extreme conditions of pH. In acidic conditions (pH 2.5) acesulfame-K breaks down to acetone, carbondioxide, ammonium hydrogen sulphate, amidosulphate, acetoamide and acetoamide N-sulfonic acid, while it decomposes to acetoacetic acid and acetoacetamide N-sulfonic acid in alkaline conditions (pH 3-10.5) (Arpe, 1978).

2.2 Aspartame

It occurs as off-white, almost odorless crystalline powder with an intensely sweet taste nearly 200 times that of sucrose (www.nutrasweet.com, 2005). It is slightly soluble in water and sparingly soluble in alcohol but insoluble in fats or oils. ADI of 50 mg/kg body weight/day. Aspartame has a sweet taste with minimal bitterness. Its onset of sweetness may be slightly slower than sucrose and the sweetness may linger. Studies have demonstrated that the taste profile of aspartame closely resembles that of sucrose. It enhances various food and beverage flavors, especially fruit flavors. Aspartame is non cariogenic and helps in production of sugar free frozen desserts (Jana et al, 1994). Although aspartame may hydrolyze with excessive heat, it can withstand the heat processing used for dairy products and juices, aseptic processing, and other processes in which high-temperature, short time and ultra-high-temperatures are used.

Stability Under dry conditions, the stability of aspartame was excellent. However, it was affected by extremely high temperatures that were not typical for the production of dry food products. At 25°C, the maximum stability was observed at pH 4.3. Aspartame functioned very well over a broad range of pH conditions, but was most stable in the weak acidic range in which most foods existed, i.e. between pH 3.0 and 5.0. Since, aspartame had lower free moisture; the shelf-life stability of aspartame exceeded the predictable shelf-life stability of these products (Beck, 1978). Fellows et al. (1991) observed that 60% of the
original aspartame remained after 6 months at 21.1°C. Based on the tests, shelf-life estimates for the fruit preparations in yoghurt were 1.5 months at 32.2°C, 4-6 months at 21.1°C and >6 months at 4.4°C.

Keller et al. (1991) evaluated three commercial yoghurt cultures and strains of L. bulgaricus and S. thermophilus, for their effect on aspartame degradation during and after fermentation. The rate of aspartame loss was related to growth of organisms. Aspartame degradation rates were correlated to metabolic rate of the culture. As the rate decreased, loss of aspartame decreased. Results indicated that aspartame remained stable in yoghurt, provided it was added after fermentation. Microbial content has been shown to affect the degradation rate of aspartame in some but not in all food products. Higher levels of initial microbial population and subsequent increase in population over time resulted in higher rates of aspartame degradation.

The major problem with aspartame is that, at the inherent pH of milk (6.6), the rate of aspartame degradation is extremely rapid, even under refrigeration. This degradation is accompanied by sweetness loss, which reduces the sensory shelf life of the product (Tsoubeli and Labuza, 1991). Aspartame degradation is minimal in the pH range of 4-5 (Bell and Labuza, 1991), which is below the pH of protein stabilization. Processing of milk at UHT is necessary as inherent dairy microorganisms can degrade aspartame.

Prodolliet and Bruelhart (1993) detected aspartame and its degradation products: diketopiperazine, aspartylphenylalanine and phenylalanine in commercial products viz. tabletop sweeteners, candy, cream, yoghurt, powdered beverages and cola samples. Leonard and Theodore (1994) observed the kinetics of aspartame degradation in low to intermediate moisture systems, by incorporating aspartame into agar/micro crystalline cellulose model systems, prepared at pH 3.0, 5.0 and 7.0, equilibrated after freeze-drying to three water activities (0.34, 0.56 and 0.66) and stored at 25°C to 45°C. Aspartame was most stable at pH 5.0 and became less stable as pH decreased or increased. They also evaluated the stability of aspartame in commercially sterilized skim milk beverages. The pH and storage temperature appeared to be the two most important factors. The half-lives were 1 to 4 days at 30°C and 24 to 58 days at 4°C. Decreasing the pH from 6.7 to 6.4 doubled the stability of aspartame. Aspartame losses were more in flavoured dairy drink than in lassi (Kumar, 2000)

Decomposition: Aspartame is a dipeptide composed of two amino acids, L-aspartic acid and the methyl ester of L-phenylalanine. In liquids and extreme conditions of moisture, temperature, and pH (<3.0 and >6.0), the ester bond is hydrolyzed, forming the dipeptide, aspartylphenylalanine, and methanol. Ultimately, aspartylphenylalanine is hydrolyzed to its individual amino acids, aspartate and phenylalanine. Methanol is hydrolyzed by the cyclization of aspartame to form its diketopiperazine. Other products formed include phenylalanine aspartic acid from diketopiperazine and β-aspartylphenylalanine (Mazur, 1974).

### 2.3 Saccharin

It occurs as a white crystalline odorless powder, or has a faint aromatic odor. It is slightly soluble in water, sparingly soluble in alcohol. It is about 500 times as sweet as sucrose. ADI is 15 mg/kg body weight/day. Saccharin is stable under the normal range of conditions employed in food formulations. It is commercially available in three forms: acid saccharin, sodium saccharin and calcium saccharin. Sodium saccharin is the most commonly used form because of its high solubility and stability. It is freely soluble in water and sparingly soluble in alcohol. It has a metallic aftertaste that is detectable above
concentrations of 0.1% in solution. Calcium saccharin, however, might be chosen for a “sodium-free” product. In its bulk form, saccharin and its salts have been shown to be stable for several years (Nabors, 2001). Saccharin is the foundation for many low-calorie and sugar-free products around the world. It is used in table top sweeteners, baked goods, jams, chewing gum, canned fruit, candy, dessert toppings and salad dressings.

→ Stability: In its bulk form, saccharin and its salts show no detectable decomposition over long periods. They are also highly stable in aqueous solutions over a wide pH range. DeGarmo et al. (1952) studied the stability of saccharin in aqueous solutions. They found that saccharin solutions buffered at pH’s ranging from 3.3 – 8.0, were essentially unchanged after heating for 1 hour at 150°C. Recent work using high performance liquid chromatographic techniques has confirmed their findings (PMC Specialties Group, 2004). Only under severe conditions of high temperature, high and low pH, over extended period does saccharin hydrolyze to a measurable extent.

→ Decomposition: Saccharin is a derivative of isothiazol. DeGarmo et al. (1952) showed that only under severe conditions of high temperature, high and low pH over an extended period does saccharin hydrolyze to a measurable extent. The only hydrolysis products are 2-sulfobenzoic acid and 2-sulfamoylbenzoic acid.

2.4 Sucralose

It occurs as anhydrous, white, crystalline, orthorhombic needle-like crystals with an intensely sweet taste. It is a chlorinated sucrose derivative that is 500 to 600 times sweeter than sucrose. It has no calories and is exceptionally stable (Lawson, 2000). ADI is 5 mg/kg body weight/day. Sucralose is the only non-caloric sweetener made from sugar. Its unique combination of sugar-like taste and excellent stability allow sucralose to be used as a replacement for sugar in virtually every type of food and beverage, including in home cooking and baking. Sucralose is not used for energy in the body and does not break down as it passes rapidly through the body. The sweetest of the currently approved sweeteners, it has a clean, quickly perceptible sweet taste. The solubility and aqueous stability of the sweetener allow it to be provided as a liquid concentrate for industrial use. Studies in model food systems, demonstrate that sucralose can be used in dry food applications, with no expectation of discoloration when food products are handled in normal food distribution systems. The actual usage level varies with the sweetness level desired and the other ingredients and flavor system used in the specific formulation.

Stability: Solubility was measured in a thermostatically controlled jacketed glass vessel. The temperature range studied was 20°C - 60°C. Sucralose is freely soluble in water and ethanol. As the temperature increases, so does the solubility of sucralose in each of the solvents. The data demonstrated that sucralose is easy to use in food operations (Jenner and Smithson, 1989). Dry neat sucralose was sealed in glass vials, stored at 75°F, 86°F, 104°F and 122°F, and monitored for the development of colour. The time to first discolouration was 18 months at 75°F, 3 months at 86°F, 3 weeks at 104°F and less than 1 week at 122°F. However, dry neat sucralose had sufficient shelf life if the product was properly packaged, stored, and handled at appropriate temperatures.

Simple aqueous solutions were evaluated for stability of sucralose over various pHs, temperatures and times. After 1 hour of storage at 100°C, there was 2% loss at pH 5.0, a 3% loss at pH 7.0, and a 4% loss of sucralose at pH 3.0 (Anonymous, 1987). The stability of sucralose solutions stored at 30°C for up to 1 year at pH 3.0, 4.0, 6.0 and 7.5 showed no measurable loss of sucralose after 1 year of storage. At pH 3.0, there was less than 4%
loss of sucralose after 1 year of storage (Mulligan et al., 1988). These data revealed that sucralose withstands heat processing conditions typical of most food processes, but under extreme conditions would slowly break down to its component moieties.

Decomposition: Sucralose is a chlorinated disaccharide. Under conditions of low pH and high temperature, sucralose breaks down slowly into its component moieties, 4-chlorogalactose and 1, 6-dichlorofructose. However, in extreme cases of high pH, 3, 6-anydro-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galacto pyranoside is formed (Goldsmith and Merkel, 2004).

3.0 Conclusion

Artificial sweeteners can be used to expand markets for existing low-calorie products, such as jams and jellies, chewing gum, and carbonated soft drinks. Development and approval of a variety of safe, low-calorie sweeteners and other low-calorie ingredients will help to meet this consumer demand. The ideal sweetener should be stable in both acidic and basic conditions and over a wide range of temperatures. Long-term storage and heat stability are important factors for the use of intense sweeteners in many food products and beverages.

4.0 Suggested Readings


1.0 Introduction

Cereals are very important part of human diets. The three major species, namely wheat, maize and rice, account for a large proportion of the calories and protein human diets. Apart from being used as staple food, cereal grains are also finding increasing use as value added ingredients in different type of food products, giving rise to new type of products in the market. This has also led to exploring new uses of cereal grains with product formulations, which offer better nutritional properties. Product development using grains as food ingredient can also enable newer uses for lesser-known types of cereals. Some of such cereal grains may offer increased benefits such as more healthful properties and better functional properties. Using different grain combinations to compensate for possible shortages in certain grain areas can also lead to the development of products that offer special functionality or health benefits. Some of the cereal grains being increasingly used as food additives are described in this paper.

2.0 Wheat

Around 10,000 years ago, man discovered that wheat could be planted, grown, and harvested. Still development of new wheat varieties and the ingredients that are derived from them is one of the prime R&D objectives of major wheat producing countries in the world. Soft wheat flours with stronger protein strength are being developed. Innovative uses for wheat-based ingredients continue to be found, such as their potential use as a shortening substitute. New wheat four acts as shortening substitute in bread. Wheat flour from a new waxy durum wheat cultivar, developed by researchers in USA, can reportedly replace vegetable shortenings or other fats used in breads to improve crumb softness, volume, texture, and storage qualities. The flour is said to work best as a shortening substitute when it is used at 20% of a dough formulation. The reason behind the flour’s fat-replacing capacity has been ascribed to a type of starch found in this particular wheat cultivar. Unlike most bread wheat cultivars which have about 24% amylase and 76% amylopectin, this new cultivar contains an unusual type of starch that is 100% amylopectin.

A texture wheat protein called Wheatex has been developed, which is said to successfully mimic the textural characteristics and appearance of meat, fish, and poultry products. It is available in a variety of sizes and colors and can be easily formed into patties and other shapes. The ingredient has a neutral taste and excellent water-binding capacities. Wheat protein isolates have been developed for frozen dough, frozen par-baked, and freshly baked goods. Some wheat protein isolates can reportedly help improve dough machinability. The filmforming properties of these products can help control water migration while improving product softness, freshness, and shelf life.
3.0 Oats

Oats may be used in a variety of foods that are quite familiar with such as oatmeal, oatmeal cookies, bread, granola, and snack bars, just to name a few. Because of its potential health benefits, especially its cholesterol-lowering abilities, oats may find increasing use in a broader range of applications, such as beverage formulations, fortified cookies, nutraceutical foods, and even food supplements.

In addition to its health benefits, oats offer functionality. Toasted oats can add a nutty taste and crunchy texture to salads, snacks, and other foods. Oat flour may be used as a thickener for soups, gravies, sauces, and puddings, and is suitable as a coating for fish, meat, and poultry products. Oat oil may be used as an alternative for shortenings in breads.

An oat bran concentrate, suitable for use in capsules, bars, chewables, drink mixes, and health-promoting foods, has been developed and marketed under the name OatVantage by Nurture Inc., USA. The manufacturer describes the ingredient as oat bran concentrated to 50% oat beta-glucan (soluble fiber) by weight. Derived from oats, it is available as a free-flowing powder, which has a neutral taste and is water soluble. Use of 1.5 g of the oat bran concentrate in the formulation, claimed to offer benefits for hypertension, diabetes, and obesity.

A series of new beverage formulations containing an oat-derived soluble dietary fiber that reduces the risk of coronary heart disease and lowers blood cholesterol have gained popularity in US and European markets. The beverage formulations are said to be formulated with Nutrim, the newest generation of oat bran, which contains highly concentrated amount of oat beta-glucan, that lowers cholesterol. The ingredient was originally developed by the USDA as a food additive to act as a healthy replacement for butter, oil, cream, and other fats.

Oat oil, which makes up about 65 of most dehulled oats, is rarely sold commercially but, according to current researches in European Countries, the ingredients may offer potential value in the bakery products. The oil is rich in phospholipids and glycolipids, which when combined with water, can lubricate bread dough to help it rise evenly and bake into a uniformly soft loaf. The oil may be used as an alternative to vegetable shortenings and other such additives to increase loaf size, improve texture, and lengthen the shelf life of bread. Bread made with oat oil or its components could become and alternative dietary food for people who need to vegetable shortening which contain trans fatty acids.

4.0 Barley

Barley is used commercially for animal feed, to produce malt for beer production, for seed, and as an ingredient in human food applications. World over, nearly 55% of the crop is used for animal feed, about 40% for malt production, about 3% for seed and about 2% for human food. But it is expected that consumption of barley as food ingredient may increase due to various healthful properties that is has to offer.

First, there has been an increased effort to get more whole grains into the diet. Barley is a very much source of antioxidants, vitamins, fiber, protein, and other nutrients. Improved processing methods may also help in retaining the nutritional value of barley, which previously might have been lost or reduced.

Researchers are continuing to make progress in improving different barley varieties, enhancing traits that will prove useful in the development of health-promoting foods. Barley is recognized as having a higher beta-glucan content than oats, which can be effective in lowering serum cholesterol levels. Work is being done to isolate and
concentrate components of barley, which may find use in the creation of novel nutraceuticals.

In addition to its potential health value, barley offers a variety of functional properties. Because of barley’s neutral flavor and texture properties, it may be added to a variety of dishes, including soups, sausages, crackers, casseroles, hot and cold ready-to-eat cereals, snacks, baked goods, and granola. Furthermore, it can be milled into various fractions, producing different cuts, pearls, flours, grits, and flakes. As a functional food ingredient, barley may be used as a soluble fiber or as a fat replacer. Barley flours may also be added to flours from other grains.

Barley-based ingredient aids in glucose transporter activity. A barley-based ingredient that increases the non-insulin dependent glucose uptake in peripheral muscle tissue has been developed and marketed under the name Maltrim by FutureCeuticals, USA. The ingredient is said to be a product of liberation and activation of low-molecular-weight active substances hidden in a macromolecular matrix of barley seed proteins that actively increases the body’s Glut-4 (glucose transporter) activity. The product is useful in maintaining healthy blood glucose levels and aids in weight management. In addition to blood glucose management, it may also be effective in decreasing total lipid levels in blood triglycerides and cholesterol with and improvement in the HDL/LDL ratio. The ingredient may be used in health bars or beverages, or as a stand-alone supplement in capsules and tablets.

Barley deta-glucans have been found effective in reducing LDL cholesterol and helping to modulate blood sugar levels by slowing the rate of carbohydrate digestion and glucose absorption. It can stimulate immune function and can increase short-chain fatty acid production through fermentation in the colon. Furthermore, barley beta-glucans are significant source of antioxidant activity and can provide a satiety effect that can contribute to weight loss.

Hulless barley varieties may be utilized as a source of cholesterol-lowering beta-glucans, which can be incorporated into a number of foods, including breads, ready-to-eat hot and cold cereals, muffins, cookie, biscuits, noodles, and granola bars. Hull-less barley can be ground, pearled, cracked steamed, flaked, or cut into grits.

5.0 Corn

Starches have always offered functionality. But increasingly, starches are being developed that offer potential health benefits. In particular, resistant starch derived from high amylose corn can help lower the glycemic response and provide probiotic and prebiotic benefits. Corn starch may be used to provide enhanced texture, flavor, and color in a variety of applications, including corn rolls, corn pan bread, baking powder corn biscuits, and corn pancakes. A modified corn flour may be used to formulate Mexican-style low-fat brownies, soft pretzels etc. Novation 9230, which is newly developed modified corn starch variant, has been used for aseptic puddings, dips, sauces, and salad dressings because it can withstand high temperatures, high shear, or low-pH food processing systems. Novation 9260 is suitable for foods such as retorted soups and gravies. This ingredient exhibits low to moderate tolerance to temperature and shear and shear at neutral pH. As per FDA regulations in USA, food producers, who want a 100% organic product label can use these starches as food ingredients.

High-amylose resistant starches derived from corn (Novelose) resist digestion by human digestive enzymes and ferment in the large intestine. Human clinical studies have shown that these starches act physiologically as fiber with significantly higher butyrate production than other fibers. Butyrate is the preferred energy source for healthy colon cells and has been associated with anti-cancer benefits.
Resistant starches also lower the glycemic response when used as a substitute for flour and other rapidly digested carbohydrates. Incorporating resistant starch into foods such as breads, baked goods, and other processed foods can lower the glycemic response while maintaining the high quality and taste of those foods. Researchers have indicated that moderating the glycemic response of carbohydrates may be important in reducing the risk of numerous health conditions, such as diabetes, heart disease, and obesity.

6.0 Seeds

Two seeds products, namely, sunflower and flax, are playing an increasingly important role in product formulation as potential us as food additives. Some of their roles as food additives to promote healthful properties are discussed below:

6.1 Sunflower

Studies have identified compounds present in sunflower seeds as offering a variety of potential health benefits, including protection against heart disease and cancer, as well as playing a role in memory and cognitive functions. It has been found that sunflower seeds contain high levels of tocopherols (vitamin E), choline, betaine, and phenolic acids, as compared to other nuts. In addition, the kernel is a good source of lignans and arginine.

6.2 Flax

Flax is particularly rich in lignans – a compound which may protect against certain types of cancer by interfering with the effects of estrogen. Flax seeds, especially when ground, can increase the fiber content of muffins and other baked products, while adding omega-3 polyunsaturated fatty acids. Its light, nutty flavor complements many whole grains. Also, whole seeds may be used as a topping, providing texture and flavor benefits.

7.0 Conclusion

There is a renewed focus on ingredients development, using whole grains. Grains and grain-derived ingredients offer health benefits such as to lower cholesterol levels in blood. Individual components isolated from grains may be used in the development of nutraceutical foods and beverages as well as supplements.

7.0 Suggested Readings


1.0 Introduction

Consumer’s interest in healthy eating has been gaining momentum since the 1980s and one of the most important features has been the trend towards low fat and fat free foods. Excessive intake of high fat products has been associated with increased risk of obesity, certain forms of cancer, high blood cholesterol, diabetics and coronary heart diseases. Nutrition Experts recommend that the total intake of dietary fat should be limited to not more than 30 per cent of daily energy intake. Health conscious consumers are now aware of the link between diet and health and the adverse effects of intake of high fat food. Consequently, the demand for low fat or no fat food products but with a good taste has increased. Fat contributes to flavour (combined perception of mouth-feel, taste, and aroma), appearance, creaminess, palatability and lubricity of food. Fat also carry lipophilic flavour compounds, act as a pre-cursor for flavour development and stabilize the flavour. It serves as a source of fat-soluble vitamins, essential fatty acids, pre-cursor for prostaglandin, and a carrier for lipophilic drugs. In other words, it contributes to overall acceptability of a product. Hence, the challenge before the food processor in the development of low fat or no fat products is to match the overall product characteristics in terms of physical, structure, physical stability and sensory characteristics with the full fat variant. Fat in food products may be replaced by traditional techniques such as substituting water or air for fat, using lean meat in frozen entrees, skim milk instead of whole milk in frozen desserts, and baking instead of frying for manufacture of snack foods or by reformulating foods with lipid, protein or carbohydrate based ingredients (commonly known as fat replacers), singly or in combination. Foods formulated with the fat replacers possess the potential to provide a suitable alternative to familiar high fat product variant and meet the expectation of the consumers and requirements of a healthy food. Development of food products by replacing fat, wholly or partially, therefore, provides one of the major developmental areas for the food industry.

2.0 Fat Replacers

Substances, which chemically resemble fats, proteins or carbohydrates and possess certain desirable physical or organoleptic properties of fat, are known as fat replacers. Use of fat replacers in food products lower the caloric value and at the same time possess the potential to impart desirable qualities to food. Fat replacers are categorized in two groups, i.e., Fat substitutes and Fat mimetics.
2.1 Fat Substitutes

Fat substitutes are fatty acids based products in which ester bonds are resistant to lipase-catalyzed hydrolysis. These are synthetic chemical entities prepared by either chemical synthesis or derived from conventional fats and oils by enzymatic modifications. Fat substitutes resemble fat in physical and thermal properties and often referred to as lipid or fat based fat replacers. They can replace fat on gram for gram basis. Many Fat substitutes are stable at cooking or frying temperatures. Hence they can be used as frying medium for foods. However their use in food products requires legislative approval.

2.2 Fat Mimetics

The substances, which can imitate physical or organoleptic properties of fat are called Fat mimetic or referred to as texturizing agents. These substances are often proteins or carbohydrates based fat replacers. Fat mimetic binds water and denature or caramelize at higher temperatures. Hence they are not suitable for frying applications. Many of these products, however, are suitable for baking or retorting applications. Caloric value of these compounds range between 0-4 kcal / g. Fat mimetic carry only water soluble flavour compounds and not the lipid soluble flavour compounds. Hence, they are generally less flavorful than the fat they are intended to replace. Formulations containing Fat mimetic require use of emulsifiers for successful incorporation of lipophilic flavour compounds. Fat mimetic cannot replace fat on gram for gram basis. It is suggested that the following three ingredients system is necessary for a good Fat mimetic:

→ A thickening agent for lubricity and flow control
→ A soluble bulking agent for control of adsorption/ absorption of the food on to taste perceptors of tongue
→ A micro particulate generally insoluble agent that acts as a ball bearing to create smoothness.

3.0 Ideal Fat Replacer

An ideal fat replacer should impart creamy, oily and fat like mouth feel to the food products and contribute to its appearance, texture, emulsion stabilization, bulking, flavour modifications and preservations. It should be colourless, bland, heat stable, low in caloric value, nutritionally equivalent and biodegradable. It should have a shelf life of 1 year and should not possess any binding properties for micronutrients, vitamins or flavour. Its consumption should not produce any side effects like laxative effects or osmotic diarrhea.

In developing formulations for low fat or no fat dairy products the processors face different challenges as the problems differ with the products. For example, reduction of fat in cheese changes its micro-environment influencing the flavour, body & texture, appearance and overall quality of the resulting cheese. Similarly it influences viscosity, syneresis, mouth-feel, starter activity in fermented milk products and ice crystal formation, melt-down characteristics, shelf stability etc. in frozen products. Therefore type of product, level of fat reduction, multifunctional effect of fat as an ingredient on processing and on final quality of product, availability of fat replacing ingredient, legislative requirements etc. must be kept in mind while selecting a fat replacer for a food system.
Table 1: Differences in the Characteristics of Fat Substitutes and Fat Mimetics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fat substitutes</th>
<th>Fat mimetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Fatty acid derivatives</td>
<td>Carbohydrates, protein based</td>
</tr>
<tr>
<td>Miscibility</td>
<td>Lipid phase</td>
<td>Aqueous phase</td>
</tr>
<tr>
<td>Digestibility</td>
<td>None or low</td>
<td>Full or partial</td>
</tr>
<tr>
<td>Adverse affects</td>
<td>Removal of oil soluble vitamins</td>
<td>None</td>
</tr>
<tr>
<td>Flavour impacts</td>
<td>None</td>
<td>Of flavours, gummy texture, starchy taster</td>
</tr>
<tr>
<td>Food applications</td>
<td>Frying, cold and hot</td>
<td>Cold and hot</td>
</tr>
<tr>
<td>Targeted fat replacement</td>
<td>75-100%</td>
<td>50-75% with carbohydrates based</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75-100% with protein based</td>
</tr>
<tr>
<td>Regulatory Category</td>
<td>Food additives</td>
<td>GRAS</td>
</tr>
</tbody>
</table>

4.0 Types of Fat Replacer

Consequent to the attempts made by food technologists a wide range of ingredients are commercially available to replace fat in food products and beverages. The existing fat replacers (substitutes, alternatives, analogs, mimics, mimetics, fat sparing products, artificial fats, synthetic fats) can be classified into three main groups. These include; fatty acid based products, protein based products and carbohydrates based products

4.1 Fatty Acid Based Fat Replacers (Fat Substitutes)

Sucrose fatty acid polyester - This is commonly known as Olestra. It is manufactured from saturated and un-saturated fatty acids of chain length of 12-carbon atoms and higher obtained from edible fats and oils. The process involves hydroxylation and methylation fatty acids to form fatty acids methyl esters. The esters thus formed are then added to sucrose for trans- esterification or to sucrose octaacetate for ester interchange using catalyst, such as alkaline metals or their soaps in anhydrous conditions and high vacuum. The crude Olestra is purified by washing, bleaching and de-odourising to remove free fatty acids and odors followed by distillation to remove un-reacted fatty acid methyl esters and sucrose esters with low degree of fatty acid substitution. Replacement of conventional fat with Olestra benefits people at high risk of coronary heart disease, colon
cancer and obesity. Food And Drug Administration (FDA) has approved its use in USA in savory snacks (salty and piquant) to replace fat up to 100% and for frying applications. Since its use may produce some side effects like gastrointestinal effects it is mandatory to write the statement “The product contains Olestra” on the label of the product.

4.1.1 Sucrose fatty acid esters

Mono-, di-, tri-, esters of sucrose with fatty acids made in a manner similar to sucrose polyester possess excellent emulsification and surfactant functionality. The hydrophilic and lipophilic properties of an emulsifier enable it to stabilize the interface between fat and water droplets through hydrogen bonding. Thus by acting as surface-active molecules emulsifiers can replace up to 50% of the fat in a food formulation. They are easily absorbed and hydrolyzed by digestive lipases and hence are caloric food ingredient. But they possess excellent emulsifier and surfactant functionality. They provide and stabilize aeration, promote and stabilize foam, control syneresis, carry flavours, control rheology and provide lubricity. They form complex with starch, interact with protein and modify the crystallization characteristics of other fats. Emulsifiers are most effective in replacing functionality of fat when used in combination with other ingredients. These are useful in margarines, baked food, frozen desserts, dairy products, spread and shortening, whipped toppings and confectionaries.

Other carbohydrate fatty acid esters and polyol fatty acid esters also hold promise for fat replacing systems. Such replacers are prepared by reacting one or more fatty acids esters with a polyol containing at least four hydroxy groups in the presence of a basic catalyst. Examples of such type of fat substitutes include Sorbitol, Raffinose, Trehalose etc. Sorbestrin or Sorbitol polyster is another such product, which is a mixture of tri, tetra and penta esters or Sorbitol and Sorbitol-anhydrides with fatty acids. It is intended for use in salad dressing, baked foods and frying.

4.1.2 Acaloric synthetic compounds

Although these compounds possess functional and sensory properties similar to fat, have low caloric value, none of them are currently approved by FDA for use in food products. Dialkyl dihexadecylmalonate (DM), Esterified propoxylated glycerol (EPG), Trialkoxytricarballylate (TATCA), Trialkoxyxycitrrate (TAC), and Trialkoxyglycerol ether (TGE) are some of the examples of this group. DDM is fatty acid alcohol dicarboxylic acid ester of malonic acid and alkylomalonic acid, synthesized by reacting a malonyl dihalide with a fatty alcohol. It may be used for replacement of oil in food formulations and for frying purpose. It is a non-caloric ingredient for food. EPG are synthesized by reacting glycerol with propylene oxide to form a polyether polyol followed by esterification with fatty acids. It may be used in frozen desserts, salad dressings, baked goods, spread and for cooking and frying. TATCA, TGE, and TAC are polycarboxylic acids with two to four carboxylic acid groups etherified with saturated or unsaturated alcohol having straight or branched chains of 8-30 carbon atoms.

4.1.3 Structured lipids
designer lipids

Structured lipids are fat based replacers. These are triglycerides (fat) prepared by chemical and enzymatic synthesis or random trans-esterification of short chain fatty acids and/ or medium chain fatty acids and long chain fatty acids. Structured lipids are developed for specific purpose, such as, reducing the amount of fat available for metabolism and low
caloric value. Caprenin and Salatrim are few examples of structured lipids. Caprenin is manufactured from glycerol by esterification with caprylic (C 8:0), capric (C 10:0) and behenic (C 22:0) fatty acids. It has a caloric value of 5 Kcal/g and possesses functional properties similar to cocoa butter. It is suitable for use in soft candy, and confectionery coatings. Salatarim is composed of a mixture containing at least one short chain fatty acids (C 2:0, C 3:0, C 4:0 fatty acids) and at least one long chain fatty acid (C 18:0) randomly attached to glycerol backbone. Its commercial name is Benefat and has a caloric value equal to 55% of the value of conventional fat. It is designed for use in chocolate flavoured coatings, deposited chips, caramels and toffees, fillings, baked goods, peanut spreads, savoury dressings, dips, sauces and dairy products such as sour cream, frozen desserts and cheese.

4.2 Carbohydrate Based Fat Replacers (Fat Mimetics)

Carbohydrates and carbohydrate based products form a major group of food ingredients and are used as texturizing agents and for the partial replacement of fats and oils in food products. These compounds in foods improve the textural quality of water phase and produce creamy & smooth consistency and a more fatty mouth-feel. Since these compounds have water binding capacity, viscosity of the product increases which imparts creamy & smooth consistency. In some cases gels are formed which give a fatty mouth-feel. The interaction between lipids in food products and carbohydrate based fat mimetics is enhanced in the presence of a fatty acid based emulsifier and result into product which has properties more like fat. These fat mimetics, however, do not interact with gluten and starch in baked foods like conventional fat. Carbohydrate based fat mimetics are useful ingredient for replacement of fat in baked foods, frozen desserts, mayonnaise, sauces, whipped toppings, spreads, salad dressings, ice-cream etc. It is not suitable for frying application. The caloric value of fat mimetic is very low (4 Kcal/g) on dry basis except for polydextrose which provides only 1 Kcal/g energy.

Gums, starches, pectin, cellulose and other carbohydrate ingredients perform the functions of fat in foods by binding water. They also provide texture, mouth-feel, and opacity. Corn syrups, syrup solids and high fructose corn syrups are used as fat replacers in many fat free and reduced fat cookies to control water activity (aw). Polyols such as sorbitol and maltitol as well as fructo-oligo-saccharides may also be used to control water activity. Fat free salad dressings contain xanthan gum and carrageenan as stabilizers. Carbohydrate based fat mimetics are suitable for frying but can be used as fat barriers for frying and for baking.

4.2.1 Gums

Gums are used as thickeners, stabilizers, and gelling agents @ 0.1-0.5%. Gums that are used in fat replacing systems with other gums, fat replacers or bulking agents include guar, xanthan or locust bean gum, carrageenan, gum arabic, and pectins. Gums are used in salad dressings, icings and glazes, desserts and ice cream, ground beef, baked goods, dairy foods, soups and sauces.

4.2.2 Starches

Although starches from different sources (common corn, high amylase corn, waxy maize, wheat, potato, tapioca, rice and waxy rice) can sometimes be used in their native form to replace fat, they are commonly used in modified form. Starch can be modified by
acid or enzymatic hydrolysis, oxidation, dextrinization, cross linking or mono-substitution to obtain modified starch and achieve desired functional and sensory properties. Starch is also available in pre-gelatinized or instant forms which perform well in high moisture foods such as mayonnaise, spreads, salad dressings and sauces, baked foods, frostings and fillings, sauces. However these do not perform well in low moisture foods such as cookies. Modified starch is a reduced caloric fat replacer supplying 1-4 Kcal / g energy. The ingredient is manufactured as a fine powder, which on addition of liquid yields slurry. This slurry on shearing forms smooth creamy substance that has similar properties to shortenings. Modified starch can also be used as bulking agent and texture modifier. It is used in combination with emulsifiers, gums, and other food starches to make dairy products, salad dressings etc.

4.2.3 **Cellulose**

Cellulose is available in various forms and are frequently used in combination with other hydrocolloids, such as gums and pectins to replace fat. Cellulose based replacers can be obtained in any of the following forms.

- Powdered cellulose obtained by mechanical grinding is useful in reduced fat sauces, fried batter coatings and fried cake doughnuts, increasing the volume of baked goods as it stabilizes air bubbles and minimizes after baking shrinkages.

- Microcrystalline cellulose/ cellulose gel obtained by chemical depolymerisation and wet mechanical disintegration is non-caloric replacers used in aqueous systems to contribute body, consistency and mouth feel. It stabilizes emulsions and foams, controls syneresis; aids viscosity adds gloss and opacity to foods. Their applications include salad dressing, frozen desserts, sauces and dairy products.

- Sodium carboxyl methyl cellulose/ cellulose gum, methyl cellulose/ modified vegetable gum and hydroxy propyl methyl cellulose/ carbohydrate gums are chemical derivatives of starch. These are surface acting agents and can hydrate in water, form films in solutions and gel upon heating as a result of methoxy and hydroxy propyl constituents. These compounds impart creaminess, lubricity, air entrapment and moisture retention in baked goods, frozen desserts, dry mix sauces and pour able and spoonable sauces and dressings.

4.2.4 **Maltodextrin**

These are obtained from corn or potato starch by partial hydrolysis. These are not sweet; provide 4 kcal/g on a dry basis. These are also obtained from oat, wheat, rice, tapioca and are used to build solids and improve viscosity, bind/control water and contribute smooth mouth feel in fat replacing system for spreads, margarines, imitation sour cream, sauces, frozen dressings, fillings, baked goods etc.

4.2.5 **Polydextrose**

It is a randomly bonded polymer of glucose, sorbitol and citric or phosphoric acid. It is available in liquid or powder and acidic or neutralized forms. It is partially metabolized and provides only 1 kcal/g. It is used in several food products like baked goods, baking mixes, chewing gums, confectionery, salad dressings frozen dairy desserts and mixes, puddings and fillings hard and soft candy, peanut spread, fruit spread, sweet sauces,
toppings, and syrups. Polydextrose contribute slight smoothness in high moisture formulations. It also produces some side effects and hence requires mention on the label.

4.2.6 Oatrim

It is made by partial enzymatic hydrolysis of starch containing portion of the hull or bran obtained from whole oat and/or corn flour. It is thermally stable and can withstand high temperature short time processing but not suitable for frying applications. In small concentrations it is suitable as fat replacer for improving the texture of low fat processed cheese of acceptable sensory quality. It may be used in other dairy products, confectionary, frozen desserts, cereals baked goods and meat products.

4.2.7 Z-trim

It represents a zero calorie fat replacer and has been developed for blending with Oatrim. It is an indigestible insoluble fiber made from the high cellulose portion of the hulls of oats, soybeans, peas, rice or bran from corn or wheat. The hulls are processed in to broken cellular fragments and purified followed by drying and milling into powder. The powder may then be re-hydrated into gel for use. It contributes smoothness, moistness, and fiber of variety of foods including cheese, baked goods etc.

4.3 Protein Based Fat Replacers (Fat Mimetics)

This group of fat mimetics are derived from a variety of protein sources like egg, milk, whey, soy, gelatin and wheat gluten. Some of these mimetics are micro-particulated, i.e., sheared under heat to form microscopic coagulated round deformable particles (0.1-2.0 micron) that mimic the mouth-feel and texture of fat. Some F M are processed to modify other aspects of ingredients functionality, such as water binding, and emulsifying properties. These are not suitable for frying applications but can be used as ingredient in foods that may go for cooking, UHT processing or retorting. These are generally used in dairy products, salad dressings, frozen desserts and margarines.

4.3.1 Whey protein concentrate

Products containing more than 50% concentration of whey protein exhibit functional properties of proven fat replacers. Some whey proteins aggregate and precipitate at their iso-electrical point (pH 4.5-5.2) but others are soluble over a wide range of pH (2-11). Hence, these are suitable for many liquid or moist food systems. The protein concentration, pH, and temperature each play an important role in determining the nature of the aggregate which is formed, i.e., small, soluble aggregate or a gel lattice structure. Controlled thermal denaturation results in a functional protein with fat like properties.

4.3.2 Microparticulated proteins

Simplesse is one example of micro-particulated protein. It is manufactured from whey protein concentrate or milk and egg protein by micro particulation process. It is used in frozen desserts, yoghurt, cheese spreads, cream cheese and sour cream and in products that do not require frying like baked foods, dips, frostings, salad dressings, mayonnaise, margarines, sauces, and dips. The caloric value is 4 Kcal/ g on dry basis. It imparts
creaminess in high moisture application and tends to mask the flavour. Use of Simplesse in food products retains the biological value of proteins.

4.3.3 K-Blazer, Ultra-Bake, Ultra-Freeze, Lita are some other protein based fat mimetics which are prepared from different protein sources as well as different processes. Some blends of protein and carbohydrate based FM are used in baked goods and frozen desserts.

5.0 Conclusion
Fat reduction is associated with changes in perceived product characteristics. No permitted single ingredient is commercially available, which can directly replace fat in all food applications. Hence the only viable route is to apply a holistic approach in fat reduction whereby, a fat replacing system needs to be devised together with appropriate processing changes for each of the products. Combinations of fat replacers together with procedural changes in product manufacture will help develop good quality low fat and fat free variants to cater the needs of health conscious consumers.

6.0 Suggested Readings
1.0 Introduction

Lactulose is a semi synthetic disaccharide comprised of the sugars D-galactose and D-fructose. It is not found naturally. A beta glycoside linkage making it resistant to hydrolysis by human digestive enzymes joins the sugars. There is no disaccharide in the microvillus membrane of small intestine enterocytes that can hydrolyze lactulose; nor is the disaccharide absorbed from the small intestine. Lactulose is, however, fermented by a limited number of colonic bacteria. This can lead to changes in the colonic ecosystem in favor of some bacteria, such as lactobacilli and bifid bacteria, which may confer some health benefits.

Lactulose is a solid substance that is very soluble in water and has a sweet taste. It is sweeter than lactose but not as sweet as fructose. Lactulose is also known as 4-0-beta-D-galactopyranosyl-D-fructofuranose. The structural formula is:

![Structural formula of lactulose](image)

- Molecular formula: \( C_{12}H_{22}O_{11} \)
- Molecular weight: 342.30
- Appearance: A clear, colorless to pale brownish-yellow
- Refractive index: \( 20 ^\circ C \) : 1.451
- Relative density: 1.38
2.0 Mechanism of Working

Lactulose reaches the colon unchanged, where the bacteria of the intestines metabolise it, producing a number of short chain fatty acids. This process initiates a large number of effects in the gastrointestinal tract. It has three type of working as mentioned below

- Laxative-osmotic
- Ammoniac binding
- Pre-biotic

2.1 Laxative-osmotic

Lactulose is a carbohydrate and energy source for colonic saccharolytical bacteria. Bifido bacteria and Lactobacilli metabolise Lactulose into several SCFA (short chain fatty acids). Total biomass, stool volume and osmotic pressure is increased and pH is decreased resulting in a accelerated bowel movements and shorter transit time.

2.2 Ammoniac Binding

- Inhibits bacterial ammonia production by acidifying the content of the bowels.
- Promotes growth of colonic flora. The growing biomass uses ammonia and nitrogen from amino acids to synthesize bacterial protein, which in turn inhibits protein degradation to NH₃.
- Leads to less ammonia by inhibiting acterial urea degradation.
- Reduces colonic transit time, thus reducing the time available for ammonia production and expediting ammonia elimination.

2.3 Prebiotic

In the colon, undigested lactulose carbohydrates are the ideal nutritional basis for health-promoting bacteria in the bowel flora, e.g. bifido bacteria and lactobacilli. This can result in a large number of positive effects:

- Intact intestinal barrier
- Stabilization of the immune system
- Strengthened immune system
- Reduced susceptibility to illness

3.0 Where can Lactulose be Used

- In human medicine
- As a food refining additive
- And as feed supplement.

3.1 Lactulose as a Drug

Lactulose is used in the treatment of constipation and hepatic encephalopathy. The efficacy of lactulose in these conditions is based on its fermentation in the colon by certain bacteria and the increase of the biomass of these bacteria in the colon. The
products of fermentation are mainly organic acids, such as lactic acid and small-chain fatty acids, which, by exerting a local osmotic effect in the colon, result in increased fecal bulk and stimulation of peristalsis. The higher doses used for hepatic encephalopathy lower the colonic pH, and the bacteria for amino acid and protein synthesis use ammonia, in the form of ammonium ions. This lowers the serum ammonia levels and improves mental function.

The stimulation of the growth of bacteria, such as bifidobacteria, may have other health benefits, such as protection against cancer of the colon. Lactulose is referred to as a bifidogenic factor. Substances such as lactulose that promote the growth of beneficial bacteria in the colon are called prebiotics. Prebiotics are typically nondigestible oligosaccharides. In addition to its uses in treatment of hepatic encephalopathy and constipation, lactulose is used in Japan in functional foods and as a nutritional supplement. These uses of lactulose are being explored in the United States, as well.

Fig. 1 Mechanism of Action in Constipation

3.1.1 *Salmonella infections*

Lactulose has been applied in the treatment of intestinal infections since 1975. Lactulose reduces the pH value to make the milieu less favorable for salmonella and expedites transit time. Both actions are important pre-conditions for the regeneration of intestinal flora.

3.1.2 *In the prescription market*

As life expectancy of the population grows, the number of patients with many diseases will increase. A laxative with minimal drug inter-action potential and good tolerability is needed in these cases. Lactulose is a multi-faceted substance with the potential for use in diverse clinical areas. Lactulose is already an important drug and as research goes on, the discovery of more indications will enlarge its potential.
3.1.3 Leading in self-medication

As the financial pressures of health care increase, more and more patients will be encouraged to self-medicate. The patient has in part to evolve from being a doctor's patient to being an auto-synchratic consumer. Since Lactulose is an effective product with a good safety record that causes no physical addiction, it meets the needs of this market perfectly.

Beside the above, known effects, it is also considered having further positive influences on saccharolitic bacteria:

- Prevention of diverse infection pathways
- Calcium absorption
- Prevention of diarrhea
- Prevention and therapy of auto-immune transmitted illnesses
- Hyper cholesterol levels
- Diabetes mellitus
- Various intestinal illnesses
- Creation of carcinomas

3.2 Lactulose for Healthy Livestock Breeding

3.2.1 Lactulose as livestock feed

The trend in livestock breeding not only continues to take the important factor of quantity into account, but now also focuses on quality – the health of the animals must be ensured if the target of economic breeding taking all parameters into account is to be achieved. In the future, lactulose, with its diverse pre-biotic effects will play an important role in prophylactics for and health maintenance of the gastro-intestinal tract.

3.2.2 Lactulose and livestock health

Across the world, the great increases in livestock production have been achieved by quickly digestible feeds with high levels of carbohydrates, proteins and fats. However the fundamental needs of ruminants are insufficiently met by such regimes, which lack raw fibers, resulting inevitably in metabolic diseases. In the case of pigs and poultry, the discrepancy between low-age and final-weights is particularly apparent. The condition of the digestive organs due to husbandry developments is widely off the performance targets.

The concomitant loss of the immunological function of the gastro-intestinal tract causes major livestock and economic problems. Only more frequent veterinary intervention can uphold stable health scenarios, which are being jeopardized by the rapid increases in antibiotic resistance with concomitant high costs. The public acceptance of animal feeds with "turbo effects" is therefore falling worldwide. The prohibition of the use of antibiotics to maintain performance parameters means that preventive measures which do not impact on performance, are now more necessary than ever.

3.2.3 Effects during peripartus

During the peripartus phase, it is particularly vital that livestock is given adequate feeding. The oral ingestion of lactulose can help by considerably reducing infections...
during this sensitive period. Delays in delivery caused by endotoxins released during metabolism and the formation of nitrogen monoxide are two factors, which can lead to an increasing probability of stillbirths or weakness. Lactulose has been successfully investigated as a preventive measure in these cases. The application of lactulose during food changeover in the peripartus period as well as at weaning may help positively affect the gastro-intestinal flora of the mother animal at the right time, and also helps the brood via the colostral milk.

In the swine and poultry industry only large numbers are economic. The use of lactulose as a nutritional supplement can help in salmonella prevention and be a general aid to maintaining the health of the gastro-intestinal tract as an immuno organ.

3.2.4 Effects in the prevention of salmonella

In the swine and poultry industry only large numbers are economic. The use of lactulose as a nutritional supplement can help in salmonella prevention and be a general aid to maintaining the health of the gastro-intestinal tract as an immuno organ.

3.2.5 Effects in osmotic protective function

Destruction of the microbial ecological equilibrium of the gastro-intestinal flora leads in many cases to damaged intestinal cells. The osmotic protective function of lactulose makes it the ideal additive to feed in concentrations, which are effective prebiotic ally.

Over and above applications in agriculture, lactulose is also a beneficial additive for pet foods, e.g. for dogs and cats. The enrichment of often unbalanced and rapidly digested canned food diets with indigestible carbohydrates and the improved milieu for bifido bacteria and lactobacilli can help promote the health of the gastro-intestinal tract in both dogs and cats, leading to a plus in quality of life and life expectancy.

3.3 Lactulose as a Logical Food Additive

3.3.1 Lactulose in prophylactic therapy

It is now known that many bowel irritation symptoms and illnesses are closely related to the living and eating habits common in modern society. On the other hand, fitness and health are also part of our present day lifestyles. Health has therefore more than simple medicinal aspects. Our mobility and our enjoyment of travel, sport etc. depends on personal well being, for which we must each play our part. Food, metabolism and the immune system must be seen as an inter-active process. If we can balance our food and digestion with this background in mind, we can contribute to stabilizing health. The prebiotic effect of lactulose can play its part not only in therapy but also as a sensible preventive agent in order to maintain the health of the immune system, the gastro-intestinal tract and hence to underpin and enhance general well-being. In the future, lactulose will therefore be increasingly added to foods as a nutritional supplement because of its preventive actions.

3.3.2 The food design trend

The trend in the food industry is moving towards clearly defined ingredients, put together according to nutritional/scientific food design specifications as a sensible nutrition supplement. Obviously, the safety of the product in both its effect and its interaction are of primary importance. Lactulose has a very high safety profile even over
long ingestion periods. Food design is feasible for individual target groups and market
niches:

For children and young people of various ages, for expectant and breast-feeding
mothers, for high performance sportsmen and women and more cerebral workers as
well as for senior citizens: each with their specific and special requirements. The ability
to positively influence the digestive and immune systems makes lactulose a key player
in food design, as an agent between nutrition and health.

3.3.3 Health care needs health food

In food production, health aspects are gaining in importance. Today the
gastrointestinal tract has been identified as a central organ of the immune system, and
consequently food research and production have gained more significance. Growing
health awareness and the desire and the will for illness prevention are broad-based
social movements. The prevention of illnesses in the gastrointestinal tract is the field in
which lactulose plays an outstanding role: in its liquid form it can be easily and
unproblematic ally added to many food to promote overall health and quality of life.

Lactulose promotes the development of Bifidobacteria Lactobacilli Streptococci
Lactulose and inhibits the growth of Clostrides E. coli. bacilli Salmonella Enterococci.

4.0 Lactulose in the Food Industry

Following the successful introduction of minerals, electrolytes and vitamins as
nutritional supplements in the production of health food, lactulose, with its high safety
profile and its health-promoting pre-biotic effects can also now become a very inter-
esting product for the industry. In pure health food, fast foods, industrial catering or
hospital cuisine: The use of lactulose has no limits from medical or nutritional science
view-points. Lactulose is a particularly attractive product under the aspect of illness
prevention, with glowing economic prospects in a broad and differentiated market place.

5.0 Concerns and Cautions

- Lactulose should be stored at room temperature away from light. If lactulose has
  been prescribed to you in a clear plastic bottle, be sure to store it in a cabinet or
  inside a bag. If lactulose is not stored properly, it can become cloudy or
darkened but this does not affect the efficacy of the drug.

- Lactulose is very sweet tasting and can be objectionable to some animals.

- Because it is syrup, it can create a sticky mess in the fur around the face.

- Diabetics should not take Lactulose. Even though the mammalian intestine does
  not readily absorb lactulose, there is still a small percentage, which is absorbed.
  In addition, there may be some unbound fructose or galactose in the syrup,
  enough to be a problem for a well-regulated diabetic.

6.0 Suggested Readings

WWW.pdrhealth.com
1.0 Introduction

Milk is the source of a wide range of proteins that deliver nutrition to the most promising new food products today. Isolated milk proteins are natural, trusted food ingredients with excellent functionality. Separation technologies provide the basis for adding value to milk through the production of proteins that provide the food industry with ingredients to meet specific needs, not possible with milk itself or with other ingredients. The global functional food and nutraceutical market is currently worth about US$50 billion and is growing at some 8% annually. This huge and rapidly growing market, driven by consumer demands for health-promoting foods, is creating an almost insatiable desire on the part of food manufacturers for new and novel ingredients with which to formulate these foods. Dairy constituents, notably the proteins and peptides, provide the food technologist with a rich selection of potential ingredients for functional foods. Dairy proteins and peptides are truly multi-functional components, providing desirable features such as physical functional traits, nutritional qualities and an increasing array of substantiated bioactivities. Their promise is clear. The challenge for science and technology is to isolate these ingredients in a cost-effective manner while maintaining their inherent functional and nutritional traits.

2.0 Biological Activity of Major Milk Proteins

Milk contains two major protein groups - caseins and whey proteins, which differ greatly with regard to their physico chemical and biological properties. Casein which accounts for 80% of the total protein in bovine milk, exist primarily in large complexes termed micelles. The multiple functional properties of caseinate derivatives allow them to be used in several food products. The caseins are known to exhibit biological activity, such as carrying of calcium, zinc, copper, iron and phosphate ions in body (Table 1). Also casein acts as precursor of a number of different bioactive peptides. The whey proteins, which accounts for 20% of total proteins, represents an excellent source of functional & nutritious proteins and exhibit a wide range of biological activity (Table 1).
Table 1: Functions of Bioactive Milk Proteins

<table>
<thead>
<tr>
<th>Protein Fraction</th>
<th>%</th>
<th>Biological Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>2.7</td>
<td>Ion carrier (Ca, PO₄, Fe, Zn, Cu), antioxidant, precursor of bioactive peptides</td>
</tr>
<tr>
<td>β-CN</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>αs₁-CN</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>αs₂-CN</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>κ-CN</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Whey proteins</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>β-Lg</td>
<td>0.4</td>
<td>Retinol carrier, fatty acid binding, antioxidant, precursor of bioactive peptides</td>
</tr>
<tr>
<td>α-La</td>
<td>0.15</td>
<td>Lactose synthesis in mammary gland, Ca carrier, immunomodulation, anticarcinogenic, precursor of bioactive peptides</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>0.10</td>
<td>Immune protection</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>0.05</td>
<td>Binds fatty acids and small molecules</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>≤0.01</td>
<td>Antimicrobial, antioxidative, immunomodulation, iron absorption, anticarcinogenic</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>&lt;0.01</td>
<td>Antimicrobial</td>
</tr>
</tbody>
</table>

3.0 Novel Separation Techniques of Bioactive Proteins

Separation technologies used to produce protein ingredients derived from milk include screening based on size differences: centrifugation based on density differences; membrane processes based on size differences, such as ultrafiltration, diafiltration, nanofiltration, and reverse osmosis; ion exchange based primarily on charge differences; and affinity chromatography based on specific binding to a matrix. Owing to unique functional and biological properties of many of the whey proteins, a number of pilot and industrial scale technological methods have been developed for their isolation in a purified form. Improved separation technologies and emerging markets have resulted in fractionation of milk proteins into ingredients that are enriched in specific proteins, or peptides, or both to fill those new opportunities. This is especially true for milk protein fractions that are in very low concentrations in the native state that require further concentration. These ingredients may be especially useful in the developing market of physiologically functional foods, or nutraceuticals. Separation technologies are available to prepare fractions that are enriched in the following milk components: β-CN, κ-CN, β-LG, α-LA, casein phosphopeptides, lactoferrin, lactoperoxidase, and immunoglobulins and other minor proteins with special functional properties. Many of these products are commercially available in limited quantities.

4.0 Whey Protein-derived Products

Whey proteins of particular interest and currently commercially available are α-LA, β-LG, lactoferrin and lactoperoxidase.
4.1 β-lactoglobulin

β-lactoglobulin is approximately 50% of the total whey protein content in bovine milk. It is capable of binding hydrophobic molecules and may function in vivo as a transport mechanism for retinol into small intestine. It also binds fatty acids and may stimulate the activity of pancreatic lipases. β-lactoglobulin has numerous binding sites for minerals, fat soluble vitamins and lipids and can be used to incorporate desirable lipophilic compounds such as tocopherol and vitamin A into low fat products.

4.2 α-lactalbumin

It represents about 25% of the total whey protein in bovine milk. Seventy percent of protein in human milk is like whey protein and 41% of that protein is α-lactalbumin. α-lactalbumin accounts for 28% of the total protein in human milk. It is a calcium modulating protein and may function as a metal carrier. It also plays an important role in the newborn. Recently, it has been associated with apoptosis of transformed cancer cell lines in vitro and is under consideration in design of new anti tumor agents. Addition of bovine α-lactalbumin is strongly advocated to humanize infant formulae and create other products for people with limited or restricted protein intake.

4.3 Immunoglobulins

In bovine colostrum, the protein content is 3-4 times higher (up to 15 grams per litre vs. 3-4 grams per litre) than in normal milk. This is primarily attributed to high concentration of whey proteins. Among colostral whey proteins, the Ig’s represent up to 75% of total protein nitrogen in the first milking as compared to about 10% in normal milk. The content of Ig’s varies individually, ranging from 20 to over 100 g/l. After post partum, the content of Ig’s decreases below one gram per litre over the course of one week.

4.3.1 Biological characteristics

The Ig’s are a heterogeneous group of proteins with a variable high molecular weight. Three classes of Ig’s have been identified in bovine milk: IgG, IgA and IgM. The IgG is represented in colostrum by two sub-classes, IgG1 and IgG2. IgG1 is the principal immunoglobulin in respect to the passive immunization of calves. IgA, which occurs mainly in its secretory form SIgA, is more abundant in colostrum than in normal milk, and IgM is an important agglutinating antibody in blood. In milk or whey, even 80% of the immunoglobulins consists of IgG1. IgG molecules have a relatively larger content of basic amino acids than the other whey proteins and therefore, their isoelectric point is also higher (pI = 6.3-7.0).

4.3.2 Technological properties

Ig’s have interesting technological properties such as the cold agglutination of fat globules in milk and binding of fatty solutes and bacteria in desalted acidified whey. During processing, the stability of Ig activity in colostrum or milk is influenced by thermal treatment. They are composed of four polypeptide chains joined by disulphide bridges and show a higher denaturation (unfolding) temperature than α-la and β-lg. In the presence of other whey proteins, however, the Ig’s are very thermolabile, which may be related to the
activity of thiol groups of β-Ig and BSA. As a result of modern technology, it has been possible to overcome thermolability by means of membrane sterilization.

During high temperature short time (HTST, 72°C / 15s) or batch pasteurization (63°C/ 30min) only 0.5 -10 % of Ig activity is lost, whereas ultrahigh temperature(UHT) treatment (138°C/ 4s) and evaporation processing destroy the specific immune activity of milk. Microfiltered bovine colostral Ig's fraction have been shown to be the most heat-stable among the whey proteins with irreversible thermally induced unfolding not dependent on pH and stabilized against aggregation by NaCl in phosphate buffer.

4.3.3 Applications

Recent commercial and industrial applications have involved the targeted immunization of cows, such that bovine milk can be used to recover high levels of specific antibodies raised against predetermined species of bacteria and viruses. Such hyperimmunized milk products are currently being developed for use within both the pharmaceutical and feed industries. Also, preparations containing specific colostral Ig's (antibodies) may in the future find applications in the prevention and treatment of human microbial diseases. Further, Ig-enriched preparations have been produced in many countries as calf milk replacers.

4.4 Lactoferrin

Lactoferrin is an 80 kDa iron-binding glycoprotein present in the colostrum and has been identified in milk of number of mammalian species such as human, bovine, buffalo, ewes, goat and camel. Bovine colostrum contains approximately 2 mg of lactoferrin per ml whereas normal milk contains 20 to 200µg/ml). Lactoferrin is also present at high concentrations (up to 100 mg/ml) in the mammary secretions of the non-lactating cow. Apart from being present in milk, lactoferrin is generally found in the products of the exocrine glands located in the gateways of the digestive, respiratory and reproductive systems, like saliva, tears, nasal secretions and seminal plasma. The occurrence of lactoferrin in these biological fluids suggests a role in the non-specific defense against invading pathogens.

4.4.1 Chemical composition

Lactoferrin is a single chain glycoprotein folded into two globular units, each of which can bind one ferric ion (Fe3+) together with a bicarbonate ion. In natural state, bovine lactoferrin is only partly saturated with iron (15 - 20%) and has a salmon pink colour, the intensity of which depends on the degree of iron saturation. Iron depleted lactoferrin with less than 5% iron saturation is called apo-lactoferrin. Iron saturated lactoferrin is referred to as holo-lactoferrin. Colostrum lactoferrin from buffalo, cow, ewe, goat and camel is low in iron as compared to mature milk lactoferrin. Unlike the ruminants, the lactoferrin present in human milk is essentially apo-lactoferrin. Bovine lactoferrin is composed of two homologous lobes, called N- and C - lobe, referring to N-terminal (1-333 amino acid residues) and C-terminal (345-676 amino acid residues) part of the molecule respectively, connected by a short alpha helix (consisting of 10–12 residues), which shares 40% identical sequence. This appears to be one of the distinguishing features between lactoferrins and transferrins.
4.4.2 Bio-functions of Lactoferrin

One of the first functions attributed to lactoferrin was the ability to inhibit bacterial growth. Because of its high concentration in the milk of some species, lactoferrin was also thought to be involved in the delivery of iron into milk. The low degree of iron saturation of lactoferrin in human milk and the exceptionally high affinity constant of lactoferrin for iron also led the investigators to suggest that lactoferrin is a bacteriostatic agent. Though iron binding is considered an important molecular property of lactoferrin, a number of biological functions are independent of this metal binding property. Specific & non-specific interactions of lactoferrin with cells, coexistence with a variety of biomolecules at different levels, molecular heterogeneity and structural flexibility confers a spectrum of multifunctional properties of lactoferrin molecule in-vivo viz. increase in intestinal iron absorption, antimicrobial activity, antiviral activity, antioxidant/anti-inflammatory activity, antitumor activity and immunomodulating property.

4.4.3 Technological characteristics

Pasteurization at 72°C / 15s had no influence on its antibacterial activity; however, UHT treatment (135°C/4s) abolishes the ability of iron saturated lactoferrin to bind bacteria as well as bacteriostatic activity of apo lactoferrin. It can be heated at 90°C (pH 4) and 80°C (pH 6.0) without significant loss of iron binding capacity or antibacterial activity.

4.4.4 Applications

The major use of lactoferrin is in Infant formulae with the expectation of contributing to the babies’ defense system in the gut against harmful microorganisms, i.e., inhibition of the growth of Enterobacteriaceae and promotion of the growth of bifidobacteria. Several kinds of lactoferrin tablets are now available to promote or maintain human health. Lactoferrin saturated with excess iron is expected to be useful supplement to prevent anemia. Many kinds of commercial products containing lactoferrin are now being developed and some have appeared in the market such as makeup and cosmetics for facial skin, chewing gum and Infant formula, yoghurt, sports foods, nutritional supplements and special therapeutic diets for fish and animals.

The worldwide production of bovine lactoferrin has increased tremendously in the last decade with current estimates ranging from 50 to 100 metric tonnes per year for product purities over 90%. The production from cow’s milk, or the whey from cheese factories, is mainly due to the economy of scale in the dairy industry together with industrial developments in chromatographic separation technology. Lactoferrin is currently manufactured on an industrial scale by various companies in Europe, USA, Japan and Germany.

4.5 Lactoperoxidase (LP)

4.5.1 Biological significance

Lactoperoxidase (EC 1.11.1.7) is a member of the peroxidase family, a group of natural enzymes, widely distributed in nature and found in plants and animals, including man. LP is an oxido-reductase secreted into milk, and plays an important role in protecting the lactating mammary gland and the intestinal tract of the newborn infants against pathogenic microorganisms. The enzyme LP is a normal component of bovine and human
milk and is present in all mammalian milks, which have been tested so far. The biological significance of LP is its involvement in the natural host defense system against invading microorganisms. In addition to its antimicrobial action, degradation of various carcinogens and protection of animal cells against peroxidative effects.

4.5.2 Physico-chemical properties of Lactoperoxidase

Bovine LP is a single polypeptide chain of 78 kDa containing 612 amino acid residues, with a high isoelectric point of 9.6, 10% carbohydrate and one haem group in the catalytic centre of the LP molecule, protoporphyrin IX, covalently bound to the polypeptide chain through an ester bond. The iron content of LP is 0.07%, corresponding to one iron per LP molecule, being part of the haem group. Lactoperoxidase has an absorbance maximum at 412 nm and its purity ratio is 0.95 measured as A412/A280.

4.5.3 Technological significance

Lactoperoxidase is one of the most heat stable enzymes in milk and its destruction has been used as an index of pasteurization efficiency of milk. Lactoperoxidase is only partially inactivated by short time pasteurisation at 74°C, leaving sufficient activity to catalyse the reactions between thiocyanate and hydrogen peroxide. It is reported that LP retains its activity during normal pasteurisation of cow milk (63°C for 30 min or 72°C for 15 s) but is destroyed at 80°C in 2.5 s. However, activation of the LP system in cow milk heated at 80°C for 15 s had little or no effect on these bacteria. Lactoperoxidase is less heat stable under acidic conditions (pH 5.3) and the calcium ion concentration has a large influence on its heat stability.

4.5.4 Use of Lactoperoxidase system for the preservation of raw milk

The most widely recommended industrial application of the LP system in food production is in the dairy industry for the preservation of raw milk during storage and/or transportation to processing plants. Antimicrobial agents of the LP system in milk cause inhibition of various spoilage and pathogenic organisms, thus enhancing the microbiological quality of milk. The antibacterial activity of the LP system in milk against psychrotrophic and mesophilic spoilage organisms has been widely investigated. Experiments conducted in India showed that activation of the lactoperoxidase system extended the keeping quality of raw buffalo milk at 30°C under both farm and field conditions. Apart from its importance in the preservation of raw milk, the LP system can also be used to extend shelf-life of pasteurized milk.

4.5.5 Other applications

However, other novel applications of the LP system are being explored. If the LP system is activated immediately prior to application of approved thermal processes, the shelf-life of dairy products may be extended significantly and high-temperature processes may be replaced with more economical lower temperature treatments. In addition to energy savings, LP low temperature thermal processes may provide better nutrient and/or quality retention for highly heat-sensitive foods such as salad dressings, spreads, beverages, dips and desserts. The shelf life of paneer is extended on treatment with LP-system. The LP system can provide a broad-spectrum antimicrobial activity against bacteria, yeasts and
moulds and have the potential for use in cosmetics, oral hygiene, toothpastes and mouth rinses, fish farming, meat, milk replacers for calves and functional foods.

4.6 Lysozyme

Lysozyme is an enzyme present in the milk of some species, especially human milk. There are two types of lysozyme. One type is found in the hen egg-white and is known as chicken-type or c-lysozyme. The other type is found in the goose egg-white and is known as goose type or g-lysozyme. Human and equine lysozymes are considered to be the c-lysozyme type. However, cow milk may contain both c- and g-lysozymes because both types are found in various other body fluids and in the stomach tissue of the cow. Lysozyme kills bacteria by disrupting the glycosidic bond between the two components of peptidoglycan, a constituent of the bacterial cell wall. Lysozyme activity is low in cow milk, but very high in human milk (0.12 grams/liter). The concentration of lysozyme is highest in human colostrum and pre-colostral milk. The limited lysozyme activity in cow milk increases due to mastitis and high somatic cell counts. Heating cow milk at 75°C for 15 minutes destroys 25 percent of the activity of this enzyme. However, human milk lysozyme is more heat stable than cow milk lysozyme. Lysozyme is used as a food preservative in some countries.

5.0 Casein Derived Products

In the dairy foods industry, major interest has been directed to the separation and enrichment of β-CN, casein glycomacropeptide, and casein phosphopeptide, which have potential applications in functional foods.

5.1 β-CN

Both ion exchange and membrane processes have been used to produce β-CN–enriched fractions. Potential uses include improved functionality over sodium caseinate in some applications, supplementation of infant formula, and insulin-dependent diabetes.

5.2 Glycomacropeptide (GMP)

GMP, the glycosylated portion of caseinomacropeptide (CMP) is present in sweet whey formed following k-casein cleavage and casein precipitation by rennin. This protein is absent from acid whey formed when caseins are precipitated by lowering the pH to 4.6. The GMP constitutes about 10 to 20% of the total proteins in cheese whey. It has been shown to possess powerful biological activity and health benefits such as prevention of stomach disorders caused by bacteria and viruses, particularly Cholera, E. Coli and salmonella. GMP can suppress the appetite via stimulation of the pancreatic hormone cholecystokinin (CCK) release, prevent heart burn and reduce the gastric acid output in the stomach, lower the heart stroke by inhibiting the platelet aggregation and binding of I fibrinogen to platelets thereby reducing the risk of blockage of the arteries or blood clots, prevents cavities by inhibiting adhesion of tartar to teeth, alters pigment production in melanocytes, acts as prebiotic and has immunostimulatory actions. Physiologic activity of GMP depends upon its glycosylation.
6.0 Conclusion

Dairy manufacturing technology has expanded tremendously in recent years and the emphasis on identifying, recovering, and/or supplementing bioactive proteins and peptides as functional ingredients will remain at forefront of future. Ultimately these approaches will improve the quality of food products containing such constituents.

7.0 Suggested Readings


STRATEGIES TO ENHANCE CONJUGATED LINOLEIC ACID IN MILK

Dr. Vivek Sharma
Scientist (Senior Scale)
Division of Dairy Chemistry
N.D.R.I., Karnal

1.0 Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. Since CLA is a product of ruminant animals, bovine milk and milk products are among the richest dietary sources. Biomedical studies with animal models have demonstrated a variety of beneficial health effects from CLA, including anticarcinogenic, antiatherogenic, antiobesity, immune system enhancement and antidiabetic (McGuire and McGuire, 2000; Pariza et al., 2001). The various health effects of CLA may relate to specific CLA isomers, it has been established that the cis-9, trans-11 CLA is anticarcinogenic when included in the diet as a natural component of food (Ip et al., 1999), and cis-9, trans-11 C_{18:2} is the major CLA isomer in dairy products (Parodi, 1997). The amount of CLA found in whole milk is generally about 4.5 to 5.5 mg/g fat, although variation of as much as 2.5 to 18 mg/g fat has been reported. The CLA content of meat and dairy products is altered little by processing, storage, or cooking and hence, the concentration in food depends primarily on the concentration in the raw material. CLA intake necessary to produce anti-carcinogenic effects in humans may be about 3g per day (Ip, et al., 1994). CLA enriched milk produced through manipulation of the dairy ration has an advantage over this type of product in that it can be promoted as a “natural” source of CLA. It may also be easier for CLA enriched milk to gain acceptance since milk already has a wide distribution and consumers are well accustomed to seeing a broad variety of dairy products in the shops.

2.0 Boi Synthesis of CLA

Conjugated linoleic acid is formed in the rumen as an intermediate product in the digestion of dietary fat. The forages and grains fed to dairy cows are characterized by a relatively high content of linoleic (18:2) and linolenic (18:3) acid. Kepler and Tove (1967) showed that cis-9, trans-11 18:2, the major isomer of CLA, is the first intermediate formed in the biohydrogenation of linoleic acid by the rumen bacteria, butyrivibrio fibrisolvins. This initial reaction involves the isomerization of the cis-12 double bond to trans-11 by cis-9, trans-11 isomerase. The next step is the conversion of this diene to the trans-11 monoene (trans-11 18:1). These initial steps occur rapidly. The cis-9, trans-11 is the predominant CLA isomer in bovine milk, other isomers can be formed with double bonds in positions 8/10, 9/11, 10/12, or 11/13.

Recent evidence has shown that oleic acid can also be converted to trans 18:1 isomers by rumen bacteria (Mosely, et al 2001). Because of the extensive biohydrogenation
of linoleic and linolenic acid to 18:1 trans-11, several studies have suggested that there may be little accumulation of CLA in the rumen. Although it is accepted that CLA is formed in the rumen,

There is good evidence that much of the cis-9, trans-11 CLA found in bovine milk is actually synthesized within the mammary gland from 18:1 trans-11 (Griinari and Bauman, 1999). This is possible through the action of stearoyl-CoA desaturase (Δ⁹-desaturase), an enzyme capable of adding a cis-9 double bond to 18:1 trans-11 to give cis-9, trans-11 CLA. Some researchers have also reported variation associated with breed. Variation in Δ⁹-desaturase may explain much of this difference between breeds.

Table 1: CLA Content of Selected Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Total CLA (mg/g fat)</th>
<th>Cis-9,trans-11 isomer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy Products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenized Milk</td>
<td>5.5</td>
<td>92</td>
</tr>
<tr>
<td>Butter</td>
<td>4.7</td>
<td>88</td>
</tr>
<tr>
<td>Sour Cream</td>
<td>4.6</td>
<td>90</td>
</tr>
<tr>
<td>Plain Yogurt</td>
<td>4.8</td>
<td>84</td>
</tr>
<tr>
<td>Nonfat Yogurt</td>
<td>1.7</td>
<td>83</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>3.6</td>
<td>86</td>
</tr>
<tr>
<td>Sharp Cheddar Cheese</td>
<td>3.6</td>
<td>93</td>
</tr>
<tr>
<td>Mozzarella Cheese</td>
<td>4.9</td>
<td>95</td>
</tr>
<tr>
<td>Colby Cheese</td>
<td>6.1</td>
<td>92</td>
</tr>
<tr>
<td>Cottage Cheese</td>
<td>4.5</td>
<td>83</td>
</tr>
<tr>
<td><strong>American Processed Cheese</strong></td>
<td>5.0</td>
<td>93</td>
</tr>
<tr>
<td><strong>Meat (uncooked)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh Ground Beef</td>
<td>4.3</td>
<td>85</td>
</tr>
<tr>
<td>Beef Round</td>
<td>2.9</td>
<td>79</td>
</tr>
<tr>
<td>Veal</td>
<td>2.7</td>
<td>84</td>
</tr>
<tr>
<td>Lamb</td>
<td>5.6</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-----</td>
<td>---</td>
</tr>
<tr>
<td>Pork</td>
<td>0.6</td>
<td>82</td>
</tr>
<tr>
<td>Poultry (uncooked)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>0.9</td>
<td>84</td>
</tr>
<tr>
<td>Fresh Ground Turkey</td>
<td>2.5</td>
<td>76</td>
</tr>
<tr>
<td>Seafood (uncooked)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmon</td>
<td>0.3</td>
<td>—</td>
</tr>
<tr>
<td>Lake Trout</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Shrimp</td>
<td>0.6</td>
<td>—</td>
</tr>
<tr>
<td>Vegetable Oils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safflower</td>
<td>0.7</td>
<td>44</td>
</tr>
<tr>
<td>Sunflower</td>
<td>0.4</td>
<td>38</td>
</tr>
<tr>
<td>Canola</td>
<td>0.5</td>
<td>44</td>
</tr>
<tr>
<td>Corn</td>
<td>0.2</td>
<td>39</td>
</tr>
</tbody>
</table>


3.0 Strategy to Enhance CLA

3.1 Manipulation of the Diet

The concentration of CLA in bovine milk fat can vary quite substantially depending on the feeding strategy adopted. For instance, pasture feeding has been found to result in a much higher milk fat CLA concentration than that achieved with typical total mixed rations (TMR) based on conserved forage and grain (Dhiman, et al., 1999; White, et al., 2001). Dhiman, et al. (1999) reported the CLA concentration of milk to be 22.1mg/g fat with pasture feeding compared to 3.8mg/g fat with TMR feeding. Kelly et al. (1998) supplemented the basal diet with 53g/kg dry matter (DM) of peanut oil (high oleic acid), sunflower oil (high linoleic acid), or linseed oil (high linolenic acid). CLA concentrations were 13.3, 24.4, and 16.7 mg/g milk fat, respectively. The increase in CLA levels observed with the sunflower oil treatment represented levels approximately 500% greater than those typically seen in traditional diets. It appears that the availability of the oils to the rumen microbes is an important determinant of subsequent CLA production. The amount of CLA, and type of CLA isomers, produced as a result of feeding supplemental fat varies to a large extent depending on the ruminal conditions. The amount of CLA, and type of CLA isomers, produced as a result of feeding supplemental fat varies to a large extent depending on the ruminal conditions. Dietary fish oil supplementation has also been found to increase the concentration of CLA in bovine milk from 0.2-0.6% in control diets to 1.5-2.7% in
supplemented diets (Chilliard, et al., 2001; Allrede et al, 2006). It is thought that the supplemental fish oils interfere with the biohydrogenation of 18:2 and 18:3 from the basal diet, specifically inhibiting the conversion of trans-11 18:1 to 18:0. As discussed already, trans-11 18:1 can be desaturated to cis-9, trans-11 CLA in the mammary gland.

### 3.2 Feeding Synthetic CLA

Conjugated linoleic acid can be synthesized in the laboratory from vegetable oils like sunflower. As noted earlier, CLA produced in this way tends to contain a mixture of CLA isomers. This type of product is already available commercially for feeding to swine because of its ability to improve lean gain in the growing animal. Synthetic CLA could be used to increase the CLA concentration in bovine milk if protected in some way from the rumen environment. Methods available to reduce biohydrogenation in the rumen include encapsulation of the fat in formaldehyde-treated casein or feeding the fat as a calcium salt.

### 3.3 Fermentation

The studies have demonstrated that strains of lactic acid bacteria used as starter cultures in the dairy industry (lactobacillus acidophilus, L. delbreuckii sub sp. Bulgaricus, L. delbreuckii. Subsp. Lactis; lactococcus lactis subsp. cremoris; lactococcus lactis subsp lactis and streptococcus salivarius subsp. thermophilius) has the ability to convert linoleic acid (LA) to CLA. The addition of 0.1% LA in the sterilized skim milk and fermenting that with lactic culture startes, leads to a sharp increase in the CLA content. (Lin et al, 1999, Alonso et al, 2003)

### 3.4 Crystallization

Milk fat is hydrolysed into fatty acids, followed by crystallization. The long chain unsaturated fatty acids along with CLA gets concentrated after crystallization (Kim et al, 1999)

### 3.5 Using Enzymes

A continuous flow reactor containing an immobilized lipase has been used to enrich the butter oil with CLA. A mixture of partially purified CLA and butter oil is fed at a variety of flow rates to a tubular reactor packed with an immobilized lipase from candida antarctica. Using a 1:10 ratio of CLA to butter oil mixture as the feed stock, one can obtain a product containing 9% esterified CLA at a reactor space time of 3 hrs. (Garcia et al, 1999)

### 3.6 Supercritical Fluid Processing

Another means to alter the milk fat content of CLA is to selectively fractionate milk fat during the manufacturing processes. Supercritical fluid processing is one method of fat fractionation, and several studies have described this technique. The supercritical (SC)-CO2 fractions of milk fat are unique in their physical and chemical properties, and this procedure has been used to obtain milk fat fractions suitable for applications in different food formulations and nutritional supplements. The extraction was conducted over a pressure range of 24.1 to 2.4 MPa and temperature of 40 to 60°C. The highest
concentration of CLA attained showed an increase of about 89% and occurred in the raffinate fraction (S1). (Romero et al, 2000)

3.7 Processing Treatments

Processing treatments like heating of cheddar cheese in the presence of whey protein concentrate can be used as a technique to enhance the total CLA. An increase of about 35% in the CLA has been reported by Shantha, 1992.

4.0 Suggested Readings


1.0 Introduction

Ghee is a clarified milk fat which occupies a prominent place in Indian diet. Ghee production forms the largest segment of milk components and utilization pattern in India. Chemically ghee is a complex lipid of mixed glycerides together with a small amount of free fatty acids, phospholipids, sterols and their esters, and fat soluble vitamins A, D, E and K. Due to low moisture content, ghee has better shelf life than other indigenous dairy products. The monosaturated and polyunsaturated fatty acids of ghee are susceptible to oxidative free radical reaction which is referred to as autoxidation and is a multi-step process. The rate of oxidation increases by itself as the reaction proceeds under usual processing and storage conditions. Several workers have carried out exhaustive work to improve the stability of ghee against autoxidation by altering processing parameters using proper packaging practices and storage conditions, addition of milk components and use of antioxidants. The oxidative rancidity in ghee is one of the severe problems thereby affecting the sensory characteristics and loss of nutritional quality. Such undesirable changes often create economic losses to dairy industry.

Ghee no doubt contain natural antioxidants like tocopherol, carotene, phospholipids but their concentration is too low to inhibit or delay oxidative deterioration for a sizeable period under proper storage conditions.

Various methods like vacuum or atmosphere packaging or packaging under inert gas to exclude oxygen or/and refrigeration /freezing are commonly employed to enhance the shelf life of fat rich dairy products.

2.0 Antioxidants

Antioxidants are only one means of fending off oxidation because they are generally inexpensive, easily applied and more effective when compared with other methods. Addition of antioxidants or utilization of antioxidants naturally present in food can minimize oxidation of fat and thereby maximize food quality. Antioxidants are often grouped together with the larger group of food additives that extend shelf life. Antioxidants are defined as any substance, which is capable of delaying, retarding or preventing the development of rancidity in fats/oils or other flavour deterioration due to oxidation. For all practical purpose, none of the available antioxidants entirely prevent autoxidation rather they delay it. Delaying the onset of rancidity means that the foodstuff is acceptable for a longer period.
An ideal antioxidant must meet the following requirements:

a) Safe in use
b) Should impart no odour
c) Effective at low concentration
d) Should be easy to incorporate
e) Should survive cooking processes such as frying and baking
f) Should be available at a low cost in use

During the last few decades, an intensive testing of safety of synthetic antioxidants like BHA, BHT and TBHQ has been carried out and it has been found that prolonged use of these synthetic antioxidants possess some toxic activity and causes health hazards such as teratogenic, carcinogenic and mutagenic effects in animal and primates. BHA a permitted antioxidant in ghee @0.02 % as per PFA Act 1954 has shown to cause lesion formation in rat fore stomach. Moreover several studies have shown that BHT may cause internal and external haemorrhaging at high dose that is severe enough to cause death in some strains of mice and guinea pigs. These findings together with consumer preference for natural antioxidants have reinforced the interest in natural antioxidants.

3.0 Natural Antioxidants

The best method of application of natural antioxidants is to isolate them from natural sources e.g. cereals, nuts, fruits, vegetables because they are regarded as safe and no special approval for their application is necessary. Another possibility is to use natural food ingredients such as herbs and spices. Green tea contains a high percentage of catechin which possess antioxidant activity comparable to synthetic antioxidants. Similarly phenolic antioxidants isolated from rosemary like carnosic acid and rosemarinic acid have been used in processed food for decades. Hydroxycinnamic acids such as p-coumaric acid caffeic acid, chlorogenic acid and ferulic acid are present in a larger variety of fruits, vegetables, seeds, herbs, tea and coffee etc.

4.0 Sources of Different Antioxidants and Their Use in Foods

Antioxidants in food may originate from compounds that occur naturally in the food stuff or from substances formed during its processing. Natural antioxidants are primarily plant polyphenolic compounds that may occur in all parts of the plant. Plant phenolics are multifunctional and can act as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers (Shahidi et al., 1992). Examples of common plant phenolic antioxidants include flavonoid compounds, cinnamic derivatives, coumarins, tocopherols and polyfunctional organic acids (Pratt and Hudson, 1990).

4.1 Hydroxycinnamic Acids

A major class of phenolic compounds are hydroxycinnamic acids which are found in almost every plant.
4.2 Ferulic Acid

Ferulic acid (FA) is found in the seeds and leaves of most plants, especially in the brans of grasses such as wheat, rice, and oats. The concentration of ferulic acid in various foods is shown in the table (Table 1). Its chemical structure strongly resembles that of curcumin, the substance responsible for the yellow color of the spice turmeric (Clifford, 1999).

Table 1: Ferulic Acid Content in Foods

<table>
<thead>
<tr>
<th>Type of Food</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavedo oranges</td>
<td>40-50 mg/kg</td>
</tr>
<tr>
<td>Blood oranges juice</td>
<td>30-64 mg/l</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>5-15 mg/kg</td>
</tr>
<tr>
<td></td>
<td>(glucosides of Ferulic acid)</td>
</tr>
<tr>
<td>Sugar beet fiber</td>
<td>9 g/kg</td>
</tr>
<tr>
<td>Oat meal</td>
<td>200-300 mg/kg</td>
</tr>
<tr>
<td>Barley barn</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td></td>
<td>(bound form)</td>
</tr>
<tr>
<td>Rice endosperm</td>
<td>2.5 g/kg</td>
</tr>
</tbody>
</table>

Due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential. UV absorption by ferulic acid catalyzes stable phenoxy radical formation and thereby potentiates its ability to terminate free radical chain reactions. Its addition to foods inhibits lipid peroxidation and subsequent oxidative spoilage. Ferulic acid neutralizes free radicals (superoxide, nitric oxide and hydroxyl radical) which could cause oxidative damage of cell membranes and DNA. It helps to prevent damage to cells caused by ultraviolet light. It is often added as ingredient of anti-aging supplements. The safety of ferulic acid is well-established both by animal studies and by its consumption as part of people’s daily diet for thousands of years - it being a significant component of grains, seeds, leafy vegetables and other food plants. Because of these properties and its low toxicity, ferulic acid is now widely used in the food and cosmetic industries. It is used as the raw material for the production of flavour component vanillin and preservatives, as a cross-linking agent for the preparation of food gels and edible films, and as an ingredient in sports foods and skin protection agents. (Ou and Kin-Chor Kwok, 2004).
4.3 P-Coumaric Acid

P-Coumaric acid has been suggested to exhibit antioxidant properties (Laranjinha et al., 1996; Castelluccio et al., 1996). It was reported that P-Coumaric acid in vitro can provide antioxidant protection to LDL as a result of the chain-breaking activity (Castelluccio et al., 1996). Diet supplementation with a crude extract of P-Coumaric acid isolated from pulses resulted in the reduction of ester cholesterol, providing a protective mechanism against the development of atherosclerosis (Sharma, 1979). The ability of P-Coumaric acid to prevent excessive lipid peroxidation on the basis of its chain-breaking activity of α-tocopherol oxidation has also been demonstrated (Laranjinha et al., 1996). Castelluccio et al. (1996) reported that P-Coumaric acid was effective in enhancing the resistance of LDL to oxidation. The concentration of P-Coumaric acid in various foods is shown in the table (Table 2).

Table 2: P-Coumaric Acid Content in Foods

<table>
<thead>
<tr>
<th>Type of Food</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oranges</td>
<td>8-12 mg/kg</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>20-70 mg/kg</td>
</tr>
<tr>
<td></td>
<td>(glucosides of P-coumaric acid)</td>
</tr>
<tr>
<td>Peas</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Peanut flour</td>
<td>500-1350 mg/kg</td>
</tr>
<tr>
<td>Barley barn</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>Rice endosperm</td>
<td>0.5 g/kg</td>
</tr>
</tbody>
</table>

4.4 Caffeic Acid and Chlorogenic Acid

The major representative of hydroxycinnamic acids is caffeic acid, which occur in plants mainly as an ester with quinic acid called chlorogenic acid (5-caffeoyl quinic acid). Coffee beans are one of the richest dietary sources of chlorogenic acid and for many consumers this will be their major dietary source (Clifford, 1999). It has been estimated that coffee drinkers might ingest as much as 1 g per day cinnamate esters (mostly chlorogenic acid) and 500 mg per day cinnamates (mostly caffeic acid). Other dietary sources of chlorogenic acid include apples, pears, berries, artichoke. Coffee could supply as much as 70% of the total dietary source of this group of antioxidants. Chlorogenic acid and caffeic acid are antioxidant in vivo and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds as they are inhibitors of the N-nitrosation reaction in vitro. Further, chlorogenic acid can inhibit DNA damage in vitro (Olthof, 2001). The concentration of Chlorogenic acid in various foods is shown in the table (Table 3).
Table 3: Chlorogenic Acid Content in Foods

<table>
<thead>
<tr>
<th>Type of Food</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green coffee</td>
<td>6-10 % (dry wt basis)</td>
</tr>
<tr>
<td>Arabica coffee</td>
<td>70-200 mg/200 ml cup</td>
</tr>
<tr>
<td>Robusta coffee</td>
<td>70-300 mg/200 ml cup</td>
</tr>
<tr>
<td>Apple</td>
<td>30-60 mg/kg (flesh and skin)</td>
</tr>
<tr>
<td>Pears</td>
<td>60-280 mg/kg whole fruit</td>
</tr>
<tr>
<td>Pears juice</td>
<td>240 mg/l</td>
</tr>
<tr>
<td>Blueberries</td>
<td>0.5-2 g/kg</td>
</tr>
<tr>
<td>Blackberries</td>
<td>70 mg/kg</td>
</tr>
<tr>
<td>Broccoli</td>
<td>60 mg/kg</td>
</tr>
<tr>
<td>Potato tubers</td>
<td>500-1200 mg/kg (dry wt basis)</td>
</tr>
<tr>
<td>(at harvest)</td>
<td></td>
</tr>
<tr>
<td>Potato peels</td>
<td>2-5 g/kg</td>
</tr>
<tr>
<td>Corn salad</td>
<td>1 g/kg</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>100-500 mg/kg</td>
</tr>
</tbody>
</table>

It has recently been demonstrated that humans absorb about 33% of ingested chlorogenic acid and about 95% of ingested caffeic acid (Olthof, 2003). This implies that part of chlorogenic acid from food will enter into blood circulation, but most will reach colon. Thus, the ingested chlorogenic acid that is absorbed could have biological effect in blood circulation. A study on human chlorogenic acid metabolism showed that the unabsorbed chlorogenic acid which reaches the colon is hydrolysed to caffeic acid and quinic acid by the colonic microflora (Olthof, 2003).

5.0 Antioxidant Effect In Fat/Oils

Zia-ur-rehman et al. (2003) evaluated effect of the natural antioxidant-potato peel extract in soybean oil during the storage at 25 and 45 °C for 60 days. Free fatty acids (FFA), peroxide value (PV) were used as criteria to assess the antioxidant activity of potato peel extract. After 60 days of storage at 45 °C, soybean oil containing 1600 and 2400 ppm of petroleum ether extract of potato peels, showed lower FFA (0.120, 0.109 %) and PV (10.0, 9.0 meq/kg) than the control samples (FFA 0.320 %, PV 59 meq/kg). These results illustrated that potato peel extract, at various concentrations exhibited very strong
antioxidant activity and their activity was almost equal to synthetic antioxidants (BHA and BHT) and therefore can be used as a natural antioxidant to suppress lipid oxidation. The strong antioxidant activity of petroleum ether extract of potato peels is due to the presence of chlorogenic acid, cinnamic and ferulic acids (Onyenecho and Hettiarachchy, 1993). The autoxidation of kinetically pure triacylglycerols and methyl esters of olive oil in the presence of four different concentrations (0.02, 0.05, 0.10 and 0.20 wt %) of p-coumaric acid, ferulic acid and caffeic acids at 100 °C was studied by Marinova et al. (1996). Oxidation was carried out at 100 ±0.2 °C by blowing air through the samples in the dark at a rate of 100 ml/ min. The process was followed by withdrawing samples at measured time intervals and subjecting them to iodometric determination of the peroxide concentration. The addition of phenolic antioxidants raised the induction periods in both the systems. Methanolic extract of oat was tested in soybean and cotton seed oils held at 30 and 60 °C in the dark and at 30 °C in the light. During storage, the peroxide values of the oils were generally significantly lower with the addition of extract than was the control. In addition, the extract was added to emulsions of the same oils and held at 30 ℃ in the light and at 60 ℃ in the dark. The peroxide values of the emulsions containing the extract were significantly lower than those containing TBHQ and the control. Antioxidant activity was due to ferulic acid, p-coumaric acid, caffeic acid and their derivatives (Tian and White, 1994). Phenolic acids contribute to the antioxidant activity of peanut products. The application of peanut meal and flour to food formulas can contribute to the oxidative stability. p-Coumaric acid is the predominant phenolic acid (84 %) followed by ferulic (8.7 %), caffeic (2 %) and 5 % of sinapic acid (Dabrowski and Sosulski, 1984). The methanolic extracts of peanut hulls showed antioxidant activity equal to BHA and stronger than α-tocopherol (Duh, 1992). The methanolic extracts of peanut hulls also showed good inhibitory activity in lard oxidation compared with BHA (Yen and Duh, 1993). Phenolic compounds isolated from barley were tested for its antioxidant properties. Main Phenolic acids include ferulic acid and caffeic acid. They reduced DDPH radical, inhibited oxidation of β-carotene and reduced lipoxygenase activity (Goupy et al., 1999).

Singh (2004) studied the effectiveness of caffeic acid at different concentrations (0.01, 0.015, 0.02, and 0.025 %) in preventing the oxidation of ghee. Caffeic acid at all concentrations delayed the oxidation of ghee. Ghee samples containing BHA (0.02%) showed peroxide value higher than samples containing caffeic acid. At a concentration of 0.025 % caffeic acid treated ghee samples showed slower rate of formation of conjugated dienes and minimum development of free fatty acids.

5.1 Rosemary

Rosemary antioxidants have been used in processed foods for decades. Carnosic acid and carnosol are the most important active components of rosemary extracts. Carnosic acid and carnosol have been suggested to account for over 90% of the antioxidant properties of rosemary extract and are also powerful inhibitors of lipid peroxidation in microsomal and liposomal systems, and are good scavengers of peroxyl radical and superoxide anion. Its use is recommended in fats and lipids, in spices, meat and fish foodstuffs, prepared foods, sauces and in aromatic and cosmetic composition. Rosmarinic acid, the water-soluble component of rosemary extract has shown also antioxidant properties against lipid peroxidation and superoxide anion.

Chang et al (1977) extracted rosemary leaves (Rosmarinus officinalis L) with hexane, benzene, ethyl ether, chloroform, dioxane, ethylene dichloride and methanol. The extracts were (0.02 %) were tested during the oxidation of lard at 60 °C in the dark. It was found that greatest antioxidant activity was located in the methanol extract. The methanol...
extract was further purified, and the resultant fraction showed an outstanding activity in potato chips fried in sunflower oil and held at 60 °C in the dark for 60 days. Marinova et al. (1991) and Chen et al. (1992) found that the hexane extract from rosemary were better antioxidants for lard, rapeseed and sunflower oils, than methanol or ethanol extract. Hexane extract (0.05 %) caused a 35-fold increase of the oxidative stability of lard determined at 100 °C, and the use of 0.05 % ethanol extract resulted in a 20-fold increase (Marinova et al., 1991). In bulk rapeseed oil hexane extracts from rosemary were more active than acetone extract. Rosemary antioxidant was suitable for deep frying in edible oils especially in the presence of ascorbyl palmitate (Gordon and Kourimska, 1995). Barbut et al. (1985) found that rosemary was as effective as the combination of BHA, or BHT with citric acid in suppressing oxidative rancidity. Wada and Fang (1992) observed a strong synergistic effect between rosemary extract (0.02%) and α-tocopherol in sardine oil at 30 °C. They suggested that rosemary extract functions as a hydrogen donor regenerating the α- tocopheroyl radical to α-tocopherol.

Carnosol and carnosic were as active as tocopherol in bulk oils (Hopita et al., 1996). Carnosic acid was even more potent than BHA and BHT in soybean oil (Richheimer et al., 1996). Carnosol showed lipoxygenase-inhibitory activities (Chen et al., 1992). Amr (1990) studied the effect of addition, rosemary (Rosmarinus officinalis L) on the oxidative stability of ghee. When these herbs were added at 7.5 % level to the ghee, only rosemary showed an antioxidative effect equivalent to that of BHA + BHT (1:1) added at rate of 250 ppm, while the other herbs were less effective.

5.2 Catechins

Catechins are a group of natural polyphenols found in green tea. There are four main catechin derivatives, including epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). Su et al. (2004) studied the antioxidant activity of catechin derivatives in canola oil. Oxidation was conducted at 95 °C by monitoring the oxygen consumption and decreases in the linoleic and α-linolenic acids of canola oil. All the four derivatives were found to be effective against the lipid oxidation of canola oil. α-linolenic acids in the samples without any antioxidants decreased from 5.9 to 4.4% in canola oil heated for 36 h, but decreased only to 5.5 to 5.5% in samples containing catechin derivatives. The study also revealed that catechins were more powerful than BHT as an antioxidant in heated canola oil.

The addition of 200 ppm of green tea extract (GTE) to conjugated linoleic acid (CLA) decreased the oxygen uptake as compared with the control CLA samples during incubation for 4 h at 90 °C (Ziang and Chen, 1997). Oxidative stability of CLA concentrate stored in air at 45 °C up to 44 days was assessed by peroxide value. Addition of 200 ppm of GTE lowered the peroxide value of the sample compared to the control CLA samples (Lee et al., 2003).

6.0 Future Trends

There are now increasing reports which suggests that natural antioxidants are generally preferred by consumers, and may gain legislative approval more easily than synthetic additives do. No rational or scientific or technical arguments can be given for natural antioxidants: they are more acceptable to consumers mainly on emotional grounds. While it is important for manufacturer to meet the requirement of consumer, it is imperative that safety of additives that are not generally recognized as safe (GRAS) be tested before
use. The safest approach is to avoid the use of synthetic antioxidants and replaced them by natural antioxidants.

7.0 Suggested Readings


1.0 Indian Food Sector

- India is one of the major food producers in the world
- The food sector contributes 25 - 28% to India’s GDP
- In terms of world ranking India stands
  - 1st in the production of Cereals, Milk & in Livestock population
  - 2nd in producing vegetables & 3rd in producing fruits
  - Ranks amongst the top 5 in producing Rice, Wheat, Groundnuts, Tea, Coffee,
  - Tobacco, Spices, Sugar & Oilseeds

The processing level in India is extremely low - around 2% in fruits & vegetables, 14% in milk, 4% in fishery, 1% in meat and poultry. 30 % of Fruits & Vegetables go waste due to lack of proper processing and packaging resulting in annual revenue loss of Rs. 30000 Crore annually. Some of the methods to reduce the losses could be:

- Creation of adequate infrastructure
- Cold Chain
- Processing (Additives)

2.0 Why to use Additives (extraneous chemicals)

- Can we avoid
- Perhaps not
- Why not

Because use of additives has become necessary in processing:

- To prevent losses due to spoilage
- To prolong shelf life and make available in off-season
- To improve appearance, taste, flavor, organoleptic quality etc
- To have consistency of a product throughout the season as applicable to product
3.0 Safety of Use of Food Additives

3.1 General Principle

The data provided by Joint Expert Committee on Food Additives & Contaminants (JECFA) by Acceptable Daily Intake (ADI) and maximum level of use of additives is based on dietary pattern of a region and thus taking into account the total intake of a particular additive through all sources which should be below ADI. Same principle is also adopted for Maximum Residue Level (MRL) of Pesticide & Toxins etc. We can ensure safety by adoption of Food Safety Management System GHP/GMP/HACCP so that food additives does not present a hazard to health while serving a technological function. Safety is ensured through Principle of Risk Assessment System.

3.2 International Scenario

3.2.1 Codex (definitions)

“Food” means any substance, whether processed, semi-processed or raw, which is intended for human consumption, and includes drinks, chewing gum and any substance which has been used in the manufacture, preparation or treatment of “food” but does not include cosmetics or tobacco or substances used only as drugs.

“Ingredient” means any substance, including a food additive, used in the manufacture or preparation of a food and present in the final product although possibly in a modified form.

“Food Additive” means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include “contaminants” or substances added to food for maintaining or improving nutritional qualities.

“Contaminant” means any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination.

“Processing aid” means a substance or material not including apparatus or utensils and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or its ingredients to fulfill a certain technological purpose during treatment or processing and which may result in the non-intentional but unavoidable presence of residues or derivatives in the final product.

4.0 Carry-Over of Food Additives Into Foods

4.1 Compliance with the carry-over principle (Other than by direct addition)

An additive may be present in a food as a result of carry-over from a food ingredient, subject to the following conditions:
The additive is permitted in the raw materials or other ingredients (including food additives) according to this General Standard.

The amount of the additive in the raw materials or other ingredients (including food additives) does not exceed the maximum amount so permitted.

The food into which the additive is carried over does not contain the food additive in greater quantity than would be introduced by the use of the ingredients under proper technological conditions or manufacturing practice.

4.2 Ingredients and raw materials as carriers for additives

An additive is permitted in a raw material or other ingredient if the raw material or ingredient is used exclusively in the preparation of a food, which is in conformity with the provisions of the standard.

5.0 Indian Scenario

5.1 PFA Act & Rules (Use of Various Additives)

5.1.1 Coloring matter

- Definition: Substance when added, adds or restore color in food
- Function
  - To offset colour losses during manufacture, storage
  - To have consistent quality
  - To provide colorful identity of to foods associated with food
  - To provide appealing, organoleptic properties
- Classification
  - Natural Colour – Chlorophyll, Carotenes, Caramel etc
  - Synthetic Color – Erythrosine, Sunset Yellow etc
  - Inorganic pigments Titanium Dioxide

5.1.2 Preservatives

- Definition: Preservative means a substance which when added to food, is capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food.
- Classification
  - Class – I (e.g. Common Salt, Sugar, Vinegar, Honey etc.)
  - Class – II (e.g. Benzoic Acid & salts, Nitrates, Nitrites, Sorbic Acid & its salts, Sulphurous acid & its salts etc.)
5.1.3 **Antioxidants**

→ **Definition** 'Antioxidant' means a substance which when added to food retards or prevents oxidative deterioration of food and does not include sugar, cereal oils, flours, herbs and spices. (Rule 58)

→ **Function** to prevent rancidity of oils – oxidative, hydrolytic etc

→ **Use** of antioxidants: Lecithin, Ascorbic Acid and Tocopherol may be added to any food unless otherwise provided in the rules.

→ Use of other antioxidants (e.g. Gallates, BHA, TBHQ, Citric Acid, Tartaric Acid etc.)

→ Certain foods are allowed antioxidants e.g. Rasogolla, Flavours, Ghee etc.

5.1.4 **Anticaking agent**

→ **Function**: to prevent lump formation in solid foods

→ **Types** of anticaking agents e.g Carbonates/Phosphates of Calcium, Magnesium, Silicates of Calcium & Magnesium, Palmitates or Stearates of Aluminium, Calcium, Ammmonium, Potassium or Sodium

→ **Food permitted** to contain anticaking agents – common salt, onion powder, garlic powder, fruit powder etc.

5.1.5 **Emulsifying and stabilizing agents**

→ **Definition**: Emulsifying agents and Stabilizing agents mean substances which when added to food are capable of facilitating a uniform dispersion of oils and fats in aqueous media or vice versa and / or stabilizing such emulsions

→ Pectins & Alginates -permitted in fruit products

→ Certain emulsifying & stabilizing agents prohibited in Milk & Cream

→ Use of Starch phosphates, modified starches in certain foods

→ Flavoring agents allowed to contain emulsifying & stabilizing agents

→ Xanthum Gum deleted (rule 6E) but added in foods

5.1.6 **Antifoaming agents**

→ **Definition**: means substance, which retards deteriorative change and foaming height during heating.

→ **Type** of antifoaming agent & Level of use

→ Dimethyl Polysiloxane (food grade) allowed in edible oils and fats for deep frying

→ Maximum limit = 10 ppm.

→ Mono and Diglycerides of fatty acids of edible oils in jam, jellies, and marmalade.
5.1.7 **Raising agent**

→ **Definition:** A substance or combination of substances, which liberate gas and thereby increase volume of a dough e.g. Baking powder, Sodium bicarbonate etc.

→ Release agent: Dimethyl Polysiloxane if used, as a release agent in confectionery shall not exceed 10 ppm of the finished product.

5.1.8 **Flavoring agents and related substances**

→ **Definition:** Flavoring agents and flavors substances, which are capable of imparting flavoring properties, namely taste or odor or both to food and include flavor extracts, or flavor preparation.

→ **Classification**

   → Natural Flavors and Natural Flavoring Substances: Flavour preparation & single substance respectively obtained exclusively by physical process from vegetable substance.

   → Nature Identical Flavoring Substances: Chemical isolated from aromatic raw Materials or obtained synthetically; Chemically identical to substance present in natural products either processed or not.

   → Artificial Flavoring Substances: not identified in natural products (processed or not).

→ **Use of other additives in flavouring agents:** The flavoring agents may contain permitted anti-oxidants, emulsifying & stabilizing agents, and food preservatives.

5.1.9 **Flavor enhancers**

→ Food permitted to use Monosodium Glutamate (MSG)

5.1.10 **Sequestering and buffering agents (Acids, Bases and Salts)**

→ **Definition:** Sequestering agents are substances which prevent adverse effect of metals catalyzing the oxidative breakdown of food forming chelates; thus inhibiting decoloorization, off taste and rancidity (Rule 70)

→ **Buffering agents** are materials used to counter acidic and alkaline changes during storage or processing steps thus improving the flavor and increasing the stability of food (Rule 71)

→ **Use** of sequestering and buffering agents in foods & their level of use.

→ Restriction on use of certain additives Ester gum Sucrose acetate isobutyrate.

5.1.11 **Sweeteners**

→ **Definition:** A non sugar substance which imparts a sweet taste to a food
### Classification

- **Bulk Sweetener**
- **High Intensity Sweetener**

- **Bulk sweeteners** include sugar alcohol viz Sorbitol, Mannitol, Maltitol, Xylitol etc & fruit oligosaccharide such as Inulin derivatives found in natural foods mostly indigestible but contribute to weight & volume of foods.

- **High intensity sweeteners** – Saccharine, Acesulfame K, Aspartame, Sucralose etc

- **Blends of artificial Sweeteners** – Carbonated waters, Soft drink concentrate, Synthetic syrup for dispensers may contain Aspartame & Acesulfame blend in quantities worked out on the basis of proportion in which sweeteners are combined.

---

#### Table 1: Food Additives – Functional Class

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Functional Classes (for labeling purposes)</th>
<th>Definition</th>
<th>Sub Class (Technological Functions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acid</td>
<td>Increases the acidity and / or impart a sour taste to food</td>
<td>Acidifier</td>
</tr>
<tr>
<td>2</td>
<td>Acidity Regulators</td>
<td>Alters or controls the acidity or alkalinity of a food</td>
<td>Acid, alkali, base, buffer, buffering agent, pH adjusting agents</td>
</tr>
<tr>
<td>3</td>
<td>Anticaking Agents</td>
<td>Reduces the tendency of particles of food to adhere to one other</td>
<td>Anticaking agent, antistickagent, drying agent, dusting powder, release agent</td>
</tr>
<tr>
<td>4</td>
<td>Antifoaming Agents</td>
<td>Prevent or reduces foaming</td>
<td>antifoaming</td>
</tr>
<tr>
<td>5</td>
<td>Antioxidants</td>
<td>Prolongs the shelf life of food by protecting against deterioration caused by oxidatiob, such as fat rancidity and color change</td>
<td>Antioxidant, antioxidant synergist, sequestrant</td>
</tr>
<tr>
<td>6</td>
<td>Bulking Agents</td>
<td>A substance, other than air or water, which contributes to the bulk of food without contributing significantly to its available energy</td>
<td>Bulking agent, filler</td>
</tr>
<tr>
<td>7</td>
<td>Color</td>
<td>Adds or restore color in food</td>
<td>color</td>
</tr>
<tr>
<td>8</td>
<td>Color retention agents</td>
<td>Stabilizes, retains, or intensifies the color of food</td>
<td>Color fixative, color stabilizer</td>
</tr>
<tr>
<td>9</td>
<td>Emulsifier</td>
<td>Forms or maintains a uniform mixture of two or more immiscible phases such as oil and water in food</td>
<td>Emulsifier, plasticizer, dispersing agent, surface active agent, surfactant, wetting agent</td>
</tr>
<tr>
<td>10</td>
<td>Emulsifying Salt</td>
<td>Rearrange cheese proteins in the manufacture of processed cheese, in order to prevent separation</td>
<td>Melding salt, sequestrant</td>
</tr>
<tr>
<td></td>
<td>Term</td>
<td>Description</td>
<td>Other Terms</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>11</td>
<td>Firming Agent</td>
<td>Makes or keep tissues of fruit or vegetables firm &amp; crisp, or interacts with gelling agents to produce or strengthen gel</td>
<td>Firming agent</td>
</tr>
<tr>
<td>12</td>
<td>Flavor Enhancer</td>
<td>Enhances the existing taste and/or odour of a food</td>
<td>Flavor enhancer, flavor modifier, tenderizer</td>
</tr>
<tr>
<td>13</td>
<td>Flour treatment agent</td>
<td>A substance added to flour to improve its baking quality</td>
<td>Bleaching agent, dough improver, flour improver.</td>
</tr>
<tr>
<td>14</td>
<td>Foaming Agent</td>
<td>Make it possible to form or maintain a uniform dispersion of a gaseous phase in liquid or solid food</td>
<td>Whipping agent, aerating agent</td>
</tr>
<tr>
<td>15</td>
<td>Gelling agent</td>
<td>Gives a food texture through formation of gel</td>
<td>Gelling agent</td>
</tr>
<tr>
<td>16</td>
<td>Glazing agent</td>
<td>A substance which, when applied to the external surface of a food, imparts a siny appearance or provides a protective coating.</td>
<td>Coating, sealing agent, polish</td>
</tr>
<tr>
<td>17</td>
<td>Humectant</td>
<td>Prevents food from drying out by countereacting the effect of a wetting agent atmosphere having low degree of humidity</td>
<td>Moisture/water retention agent, wetting agent</td>
</tr>
<tr>
<td>18</td>
<td>Preservative</td>
<td>Prolongs the shelf life of a food by protecting against deteriorating caused by microorganism</td>
<td>Antimicrobial preservatives, bacteriophage control agent, Chemosterilant, disinfection agent</td>
</tr>
<tr>
<td>19</td>
<td>Propellant</td>
<td>A gas, other than air, which expels a food from container</td>
<td>Propellant</td>
</tr>
<tr>
<td>20</td>
<td>Raising agent</td>
<td>A substance or combination of substances which liberate gas and thereby increase volume of a dough</td>
<td>Leavening agent, raising agent</td>
</tr>
<tr>
<td>21</td>
<td>Stabilizer</td>
<td>Makes it possible to maintain a uniform dispersion of two or more immiscible substances in food</td>
<td>Binder, firming agent, moisture retention agent, foam stabilizer</td>
</tr>
<tr>
<td>22</td>
<td>Sweetener</td>
<td>A non sugar substance which imparts a sweet taste to a food</td>
<td>Sweetener, artificial sweetener, nutritive sweetener</td>
</tr>
<tr>
<td>23</td>
<td>Thickener</td>
<td>Increase the viscosity of food</td>
<td>Thickening agent, texturizer, bodying agent</td>
</tr>
</tbody>
</table>
DEVELOPMENTS IN THE MANUFACTURE OF LOW CALORIE TRADITIONAL MILK SWEETS

Dr. Dharam Pal, Ms. Shashi Prabha and Mr. Gajendra Londhe
Dairy Technology Division
NDRI, Karnal

1.0 Introduction

A major part of milk produced in our country is being consumed in form of a wide array of milk sweets and other specialties viz. burfi, peda, kalakand, sandesh, rasogolla, shrikhand etc. These delicacies though very popular in all parts of the country but are quite high in fat and sugar contents (Table 1) and thus do not suit to all categories of consumers particularly the diabetes and obese. So their low fat and low sugar versions are being developed for those consumers who are health conscious and are on restricted diet.

Table 1: Average Fat and Sucrose Content in Traditional Indian Dairy Products

<table>
<thead>
<tr>
<th>Products</th>
<th>Average contents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat (%)</td>
</tr>
<tr>
<td>Rasogolla</td>
<td>5.00</td>
</tr>
<tr>
<td>Sandesh</td>
<td>18.70</td>
</tr>
<tr>
<td>Burfi</td>
<td>18.00</td>
</tr>
<tr>
<td>Peda</td>
<td>19.31</td>
</tr>
<tr>
<td>Kulfi</td>
<td>10.00</td>
</tr>
<tr>
<td>Misti dahi</td>
<td>4.50</td>
</tr>
<tr>
<td>Shrikhand</td>
<td>8.50</td>
</tr>
</tbody>
</table>

In the recent past the diseases like obesity, diabetes and cardiovascular diseases have become major health problems in our country, like the developed western and European countries. Survey conducted by Indian Council of Medical Research (2001) revealed that 49% of women and 36% of men in urban areas are obese. Obesity is associated with many health implications like hyperlipidimia, hypercholesroleemia, diabetes, hypertension, cancer and gallstones (Broomfield et al., 1988; NIH, 1985; Vaselli et al., 1983). Obese people need to achieve a negative energy balance to maintain ideal body weights by cutting down their calorie intake. Reduced fat
formulations need to be developed for people suffering from Cardio Vascular Diseases which account for around 15% deaths in India and this figure is likely to rise to as high as 40% by 2015 (All India Institute of Medical Sciences, 2002). Also India harbors the largest population suffering from diabetes (Pradeep et al, 2002; Sahay and Sahay 2002). According to WHO estimates, the diabetic cases were 19.4 million in 1995, which may increase to about 57.2 million in 2025. The production of sugar free products can enable such people to relish the dairy products without affecting their blood glucose levels. Trend of low fat and low sugar foods is further spurred by calorie conscious section of modern society. So development of low calorie sweets by either reducing or replacing fat and sugar are highly desirable to meet the dietary requirements of obese, persons at risk of CVDs, diabetics and persons on weight management diets.

Food industry has also responded to changed dietary pattern by offering an ever-increasing variety of new ingredient in form of alternative sweeteners and fat replacers with improved safety, stability and functionality. But developing such products does not include just replacing the sugar and fat simply by adding alternative sweeteners and fat replacers. Such products need to be reformulated without significantly affecting the rheological and sensory characteristics, it should comply with the processing conditions of the product and conform to legal requirements. It is very difficult to deliver all the functionality of the sugar as well as fat in the product particularly where sugar and fat ingredients constitute a large proportion of the original formulation, and where processing conditions require more severe heat treatment, for example in heat desiccated dairy products. However, in products that require relatively low heat processing conditions and low temperature storage conditions, the replacement of sugar in particular is not very difficult.

2.0 Sugar Replacement

Artificial sweeteners that are used to replace sugars fall into two basic categories, viz, those which are calorie free (often referred to as high intensity, intense, high potency, artificial or non-nutritive sweeteners), and those which are significantly reduced in calorie or bulk sweeteners (Nabors, 2002). Intense sweeteners are those substances, which on weight basis are substantially sweeter than the common carbohydrate sweeteners such as sucrose. These sweeteners are constituted of compounds that mimic the effects of sugars on the tongue and pass the human intestine unmetabolised without producing any calorie. The examples of some commonly used artificial sweeteners are: aspartame, saccharin, acesulfame-k and sucralose. Until mid 2004, the Prevention of Food Adultration Act (1954) did not allow the use of sugar substitutes in mithais. Now according to the Government of India Gazette Notification of 25th June, 2004 the use of saccharin sodium up to 500 ppm, aspartame up to 200 ppm and acesulfame-k up to 500 ppm is permitted in traditional Indian milk products such as khoa, burfi, peda, gulabjamun, rasogolla and similar milk based sweets sold by any name.

High potential sweeteners alone cannot provide bulk and other functional characteristics of sugar, so a combination approach is always adopted and bulking agents are used for this purpose. But, bulking agents are not needed only in case of formulations where sugar is used at low levels and it does not contribute much for the body and texture of the product. Increasing the proportion of another component of the formulation can also serve the purpose, in such formulations. The bulking agents have fewer calories than sucrose, and possess physical properties similar to sugar. These substances attempt to deliver all functionality of sucrose. The most widely used bulking agents are sugar alcohols such as mannitol, maltitol, lactitol, sorbitol, xylitol, isomals and erythrytol. Also syrup or hydrogenated starch hydrolysates, fructo-oligosaccharide sweeteners, polydextrose and low conversion corn sweeteners or maltodextrin are added as bulking agents in food products.
3.0 Fat Replacement

Fats in a food system provide the following functional characteristics:

- Characteristic rich flavor to the product
- Body and texture and mouthfeel of the product
- Carrier for fat soluble components of the formulation

Fat replacers are divided into 2 main groups, fat mimetics and fat substitutes. Fat substitutes like olestra, salatrim, caprenin, sorbestrin, are compounds physically and chemically similar to fats and replace triglycerides in foods. Fat mimetics are either protein or carbohydrates that have been physically or chemically processed to mimic the properties and functions of fats in foods.

3.1 Carbohydrate Based Fat Replacers

Carbohydrate based fat replacers are derived from cereals, grains and plants, and include both digestible and indigestible complex carbohydrates. They exhibit desirable properties like water binding, which improves rheological characteristics, viz., body, texture, viscosity and consistency (Sharma et al., 1998). Carbohydrate based fat replacers Avicel™ (cellulose based), Paselli SA2® (tapioca dextrin), C’Pur 01906 (potato maltodextrin), stellar® (corn starch), N-oil® (tapioca maltodextrin), oatrim (oat maltodextrin) and Lean Maker® have been reported to be used in dairy products like frozen desserts, salad dressings, cheese spreads, sour cream and yoghurt.

3.2 Protein Based Fat Replacers

Protein based fat replacers are derived from proteins of animal or plant origin (egg, milk, pulses etc.). They bind water and deliver the texture and mouth feel of fat. Protein based fat replacers are designated as GRAS by USFDA. Simplesse, Trailblazer, Lita, Miprodan, and Nutrifat are important among protein based fat replacers. Their use has been reported in frozen desserts, sour cream, yoghurt, butter spreads, cheese spreads, cheeses and ice-cream. Simplesse® is a low calorie protein based fat substitute confirmed as GRAS, produced from milk and / or egg proteins via micro particulation process and its one gram can replace one gram of fat. Trailblazer® as said to be a similar fat replacer but made with a different process. DairyLo® is another milk protein based fat replacer (WP) made by denaturation of ultra filtered whey proteins by controlled heat treatment. It can be used in dairy products (2-5%) to contribute desirable mouthfeel attributes.

3.3 Fat Based Fat Replacers/Synthetic Fat Replacers

These are triglycerides with tailored configuration to reduce their calorie content. Different fat replacers reported under this group are Olestra, Salatrim, Caprenin, EPG (esterified propoxylated glycerols), DDM (dialkyl dihexadecymalonate), TATCA (trialkoxycarboxylate), trialkoxy citrate, and polysiloxane. Olestra is a mixture of hexa, hepta and octa esters of sucrose with long chain fatty acids (Bernhardt, 1988). Salatrim is a family of restructured fats that provide physical properties of fat but with approximately half of calories (5 Cal/g). Caprenin is another reduced calorie tailored fat consisting of 3 fatty acids caprylic, capric and behenic acid.
4.0 Technological Interventions in the Use of Fat Replacers and Artificial Sweeteners

The fat and sugar substitutes and bulking agents are not added as such to dairy products. The following practical difficulties are likely to be encountered in using the fat replacers, bulking agents and artificial sweeteners under the normal technological protocols adopted for manufacture of traditional milk sweets:

4.1 Fat Replacers

- Reduction of fat in milk results in imparting dry and hard texture in khoa and chhana and thus these products become unsuitable for making sweets of good quality.
- The flavour of low fat products is also bland/flat. Invariably various protein based fat mimetics, such as WPC and simplesse are used for these products to overcome the above mentioned defects as well as increase the yield.
- Fat replacers can only be used at certain processing conditions otherwise the texture of the final product will be adversely affected. These conditions are the level of addition, stage of addition and the end use of the product.
- The WPC and simplesse have high water binding property and result in higher water activity in the product containing these fat replacers, which will adversely affect the keeping quality of the product.

In the light of above problems the existing technologies need suitable modifications when these protein based fat replacers replace butterfat.

4.2 Artificial Sweeteners

- 1. Sugar in milk sweets in addition to imparting sweetness helps to develop the desired body and texture of the sweets; hence its replacement with artificial sweeteners will adversely affect these properties.
- In case of sweets having high sugar content the problem of achieving bulk by adding small quantity of artificial sweetener is faced due to which the yield is reduced and so also the profitability. Therefore the addition of bulking agents becomes inevitable along with artificial sweeteners to increase the bulk.
- Artificial sweeteners are not stable at all processing and storage conditions, such as temperature, pH etc. A high heat treatment is employed during manufacture of traditional dairy products and they are generally stored at ambient temperatures. These conditions are unsuitable as far as stability of some of the artificial sweeteners is concerned.
- Some of the artificial sweeteners impart a after taste, particularly at higher concentrations, which is different from the taste of normal sugar.
- As bulking agents like poeyols and artificial sweetener do not participate in maillard and caramalization reactions, these sugar-free products cannot impart the typical brown color and flavour of some of the sweets like milk cake and brown peda.
- The excessive intake of polyols, such as sorbitol have laxative effect. So they need to be used in combination with other bulking agents.
5.0 Low Calorie Traditional Milk Sweets

5.1 Burfi

Burfi is a popular khoa based sweet having smooth and homogenous texture. It is prepared by thorough blending and kneading of hot khoa with sugar. A technology of preparing dietetic burfi wherein fat was reduced to about half of normal burfi and sugar completely replaced by artificial sweetener has been developed (Shashi, 2005). WPC @ 1% was used to replace 3% fat in milk. Sucralose along with a combination of MD and sorbitol produced most acceptable product. The manufacturing conditions in respect of stage of addition of these ingredients have been identified. The consumer studies revealed that more than 90% of respondents graded the dietetic burfi as “very good” to “excellent”.

5.2 Rasogolla

*Rasogolla* is the most popular chhana based Indian sweetmeat. Because of its pleasant and delightful taste, the fame of this sweet has not only spread throughout India but is becoming popular abroad as well. Quite a considerable quantity of this sweet is now being exported to Middle East countries from Bikaner and West Bengal. Because of its high sugar content (32-55%) the people who are suffering from diabetes are not able to enjoy the delicious sweet. Technology has been developed for the manufacture of sugar free *rasogolla* using artificial sweeteners for such a large group of people. Jayaprakash (2003) developed a technology for the production of sugar free *rasogolla*. The important steps involved in preparing sugar free *rasogolla* are: standardization of cow milk (3.5% fat and 8.5% SNF), boiling, filtration, cooling to 60°C, coagulation at this temperature using 1% citric acid, draining of whey for 30-45 min., kneading of prepared chhana, forming balls of 6-7g, cook this balls in 40% sorbitol solution for 20 min. and finally soaking in 40% sorbitol and 0.08% aspartame and packaging.

5.3 Kulfi

Frozen desserts are delicate, delicious and nutritious food liked by all age groups and is popular throughout the world. *Kulfi* is a popular frozen dessert of Indian origin that occupies a privileged position amongst the traditional Indian dairy products. However, because of its high sugar content (13-20%) there are millions of people who are not able to enjoy the taste of this delicious frozen dessert because of being suffering from diabetes.

Bulking agents and artificial sweeteners are used extensively in the production of diabetic products especially frozen desserts. Technology of the production of artificially sweetened *kulfi* using combination of bulking agents mainly maltodextrine, sorbitol and whey protein concentrates and artificial sweeteners such as aspartame, acesulfame-k and sucralose have been developed. Pandit (2004) developed a technology for the manufacture of artificially sweetened kulfi. This involves: standardization of buffalo milk (Fat/SNF ratio 0.66%). Concentrate in open pan to 32% TS, addition of bulking agent (Sorbitol 5.5% and Maltodextrin 4.26%), cool this mixture to room temperature, dissolve aspartem (@ 742 ppm in 5 times water at 35-40°C), add flavouring agent (ground cardamom @ 0.1%), fill in moulds, moulds are kept for freezing in ice and salt mixture in the ratio 4:1 with constant agitation and finally store the *kulfi* in deep freezer before serving.
5.4 Shrikhand

*Shrikhand* is a fermented indigenous dairy product having a typical sweetish-sour taste. It is a popular delicacy in Gujarat, Maharastra and part of Karnataka. Traditionally, *shrikhand* is made from chakka by adding sugar, flavour and milk fat. This product has very high amount of sugar (≥40%). In recent years the acceptance of low-calorie food is getting momentum due to increased awareness of their nutritional and health benefits. Singh and Jha (2005) investigated the effect of sugar replacer on sensory attributes and storage stability of *shrikhand*. They prepared low sugar *shrikhand* using Raftilose as an artificial sweetener. According to them the important steps involved in preparation of such *shrikhand* were: pasteurization of skim milk at 90°C for 16 sec. and cool to 37±1°C, addition of activated yoghurt culture @ 2% and incubating at 42±1°C for 3 ½ hours, draining of whey by hanging curd having 4.3 pH in muslin cloth for 6 hours at ambient temperature, addition of 4% Raftilose and 12.5% sugar to chakka and kneading in shrikhand patra, storage at refrigerated temperature. They observed that the raftilose used at 4% level along with 12.5% sugar resulted in acceptable product with good flavour and overacceptability.

6.0 Suggested Readings


USE OF ADDITIVES IN DEVELOPMENT OF PACKAGING MATERIALS

Dr. G. K. Goyal and Ms. Preeti Singh
Dairy Technology Division
NDRI, Karnal

1.0 Introduction

In modern times packaging has been identified as an integral part of processing in the food industry. Packaging is the science, art and technology of protecting products from the adverse effects of the environment. Packaging serves as a vital link in the long line of production, storage, transport, distribution and marketing. Package is the physical entity that functions as the barrier between the contents and the exterior atmosphere. The package must ensure the same high quality of the product to the consumer, as he is used to getting, in freshly manufactured products. World wide packaging industry is $300 billion. In India around 15,000 units are engaged in packaging industry. Projected growth rate of demand and consumption for packaging in India is 10%.

2.0 Additives in Packaging Materials

Additive may be defined as “a chemical added in minor amount to a host plastic to improve the overall performance of the system”. Additives are categorized by the functions that they perform rather than their chemistry. Additive functions as modifiers (plasticisers, chemical blowing agents, coupling agents, impact modifiers, organic peroxides, nucleating agents), property extenders (flame retardants, heat stabilizers, antioxidants, light stabilizers, antistatic agents) and as processing aids (lubricants, mould release agents, antiblock / slip agents). In food packaging, the role of additives can be seen as antioxidants, pigments, processing aids, lubricants, clarifiers on the issues such as organoleptic properties and migration rates.

Foods are spoiled by a variety of both bacteria and fungi, but only particular microorganism typically attacks each type of food. This is because the chemical properties of foods vary widely, and the indigenous spoilage organisms that are best able to utilize the nutrient colonize different foods. In general, microorganisms grow on food surfaces thereby development of active packaging where the antibacterial agent is immobilized tightly, is the answer to consumers’ demand for food without preservatives. A major line of defence in preventing food-borne illnesses from food products is the package. The primary function of the package — or more specifically the packaging film — is to protect the integrity of the product inside by physically excluding pathogenic and spoilage microorganisms. Packaging equipment and material suppliers are focusing their efforts on developing packaging systems that are more aggressive and proactive in protecting food from microbial contamination.

According to a recent Food Marketing Institute survey, the percentage of consumers who are “completely” or “mostly” confident in the safety of the food supply fell from 83% in 1996 to 74% in 2000. As a result, processors are working hard to reduce contamination of
food products by pathogenic and spoilage microorganisms in order to regain consumer confidence. However, the distribution channel between the processing and packaging plant and the consumer still remains highly vulnerable to microbial contamination.

2.1 Active Packaging

The traditional passive packaging is restricted in protecting food products because of the way that food is distributed and stored. Furthermore, the extended shelf-life of manufactured foods and the consumers demand for minimal addition of preservatives to food products have led to the innovation of active packaging. Major active packaging technologies that exist are based on the use of: oxygen scavengers, moisture regulators, ethylene absorbers, taint removal, ethanol and carbon dioxide emitters as well as antimicrobial systems (Vermeiren et al., 1999). The antimicrobial active packaging technology is based on antimicrobial agents that are immobilized with the polymeric structure or incorporated in plastic resins, before film casting. This technology can be divided into two types: preservatives that are released slowly from the packaged materials to the food surface or preservatives that are firmly fixed and do not migrate into the food products. Both are assumed to control growth of undesirable microorganisms. Among the common microorganisms that contaminate food and drink products are E.coli, Staph. aureus, Campylobacter, Salmonella, Clostridium perfringens and Bacillus cereus. Toxins produced by microorganisms or growth of microorganisms in the human body after the contaminated food has been eaten cause food poisoning. Development of different kinds of active packaging is under intensive progress now (Vermeiren et al., 2002; Van Beest M et al., 2002). One of the key problems of the active packaging technologies resides in the controlled release of the antimicrobial agent from the polymer film (Jin-Ok Choi et al., 2001).

3.0 Chemical Antimicrobial Agents

There are few chemical antimicrobial agents that are used commercially to control microbial growth in foods (Han and Floros, 1997). Many of these chemicals, like sodium propionate, have been used for many years with no indication of human toxicity. Others, like nitrites, ethylene or propylene oxides, are more controversial antimicrobial control because of evidence that these agents are harmful to human health. In recent years there are several reports of using bacteriocins, proteins that possess antimicrobial activity, and are produced by microorganisms, as food preservatives (Ming et al., 1997).

4.0 Synthetic/Vegetable Derived Additives

The trend continues towards use of synthetic or vegetable oil based rather than animal-fat derived additives. In Europe, this trend is due to concern of bovine spongiform encephalopathy (BSE). Since many companies have customers globally, the concern has spread to the U.S. as well.

4.1 Corn-based Plastic Additive Makes Bottles Stronger

A new plastic additive made from corn, designed to make plastic bottles more rigid and stronger than ever before, has been developed. This research was conducted by Iowa Corn Promotion Board (ICPB), which operates the US department of energy's Pacific Northwest national laboratory in Washington State, could help further strengthen the plastic
industry’s ability to counter high oil prices and develop more environmentally friendly packaging materials. The ICPB claims that the compound, isosorbide, has been found to make plastic bottles more rigid and stronger than regular plastic bottles.

4.2 Colorants

Colour and additive concentrates provide flexibility to thermoplastic processors who prefer to create multiple colour effects or enhance the performance of their own base polymers. Colorants are widely used in food packaging materials. FDA allows a variety of organic and inorganic colorants for indirect food contact; other colorants are exempted from FDA regulation based on migration testing in a specific polymer for a specific application. The industry trend towards thinner parts creates a need for a higher colorant loading to maintain colour intensity and opacity. These allow processors and compounders to enhance the functionality of their base resin to meet the specific needs of an application.

4.3 Slips and Antistats

Slip and antistats additives, which function at the surface of the plastic part, are traditionally migratory. The additives are difficult to predict and control because migration occurs over time and depends on part thickness and polymer crystallinity but nowadays non-migratory, surface-functional slip and antistat products that fit a need for controllable, predictable performance in premium films are available. Other advantages of the slip are that it can be used at higher temperatures than conventional slips and has no adverse effect on sealing. The non-migratory antistat does not interact with adhesives, has no effect on sealing or printing and has high thermal stability.

4.4 Masterbatch Additives

The additive could help food companies keep odours within a package or prevent outside ones from affecting their products. Masterbatch additive helps control taste and odour issues in plastics film applications for trash bags and flexible food packaging. The masterbatch, Ampacet 101787™, removes the molecules as they pass through packaging films and other plastic packaging parts. The additive can be used in low-density polyethylene (LDPE), linear LDPE and ethylene vinyl acetate films used for food packaging. The active ingredient in Ampacet 101787™ is highly porous and preferentially adsorbs smaller, odour-causing molecules, especially ammonium-based and polar compounds.

4.5 Antioxidants

These are special compounds such as Vitamin E, which help to protect the plastic under hostile conditions. Antioxidants are used with thermoplastics and adhesives to protect against oxygen degradation and discoloration during processing. GE Specialty Chemicals introduced two new, high performance antioxidants, which perform well under harsh conditions such as gamma irradiation of food packaging. Some companies are using Vitamin E antioxidants for improved organoleptic properties for sensitive applications such as plastic milk and beverage bottles.

Butylated hydroxyanisole (BHA) and the related compound butylated hydroxytoluene (BHT) are phenolic compounds that are often added to foods to preserve fats. BHA and BHT are antioxidants. Oxygen reacts preferentially with BHA or BHT rather
than oxidizing fats or oils, thereby protecting them from spoilage. In addition to being oxidizable, BHA and BHT are fat-soluble. Both molecules are incompatible with ferric salts. In addition to preserving foods, BHA and BHT are also used to preserve fats and oils in cosmetics and pharmaceuticals. BHA is found in butter, meats, cereals, chewing gum, baked goods, snack foods, dehydrated potatoes, and beer. It is also found in animal feed, food packaging, cosmetics, rubber products, and petroleum products. BHT also prevents oxidative rancidity of fats. It is used in packaging materials to preserve food odour, colour, and flavour.

4.6 Antifogs

In fresh-cut produce packaging, antifogs prevent the film from fogging so that the consumer can see the product clearly. The use of antifogs in fresh food packaging is on the increase and will continue in the future as new applications as well as new polymer entrants into the fresh food packaging industry continue to evolve. Antifogs act as a surfactant, so that moisture given off by produce forms a transparent, continuous film on the package surface rather than forming beads of water. Antifogs can be impregnated into the film as an additive or applied as a liquid coating. A new trend towards microwaving of fresh-cut produce packages, such as spinach products, has led to challenges of meeting performance requirements and regulatory requirements, which are stricter at elevated temperatures. For example, the Cryovac (r) Microwaveable Vegetable Bag has an antifog coating which also aids in improving the shelf life of the vegetables. The extended shelf life is due in part to the permeability of the package, but also to the “synergistic effect” of the antifog coating that reduces moisture, which can encourage the growth of spoilage bacteria.

4.7 Other Antimicrobials

Antimicrobial additives used in active packaging systems to slow microbiological degradation of food products have been the subject of much research and publicity, but several obstacles have slowed widespread commercialisation in Europe and the U.S. The use of antimicrobials, or biocides, in packaging is a growing trend in the global food packaging industry. In the U.S., many of the antimicrobials in use protect the packaging or the packaging raw materials, although recent interest has been in antimicrobials to protect the packaged food. The FDA under the Federal Food, Drug and Cosmetic Act (FFDCA) regulates antimicrobials that are incorporated into food packaging. Under the FFDCA, the FDA ensures that such antimicrobial uses are safe with respect to any potential human dietary intake. Unrelated to federal requirements under the FFDCA, antimicrobial products used in food packaging that have no intended antimicrobial effect on the processed food in the package are subject to EPA registration as pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Antimicrobials added to food or delivered to food via the packaging are treated as direct additives and are not subjected to FIFRA. Nisin is produced commercially under the trade name Nisaplin(r) by Aplin-Barrett in the U.K., and can be compounded into the packaging polymer or applied as a powder or a coating. It is widely used in Europe but not extensively in the U.S., although it has FDA approval. Silver compounds are also used in Europe and have FDA approval for some applications. Other research is looking at additives that produce the antimicrobial chlorine dioxide under certain relative humidity or UV light conditions. The advantage of these systems is that the antimicrobial could protect any product within the package, not just what comes in contact with a protective coating. Chlorine dioxide is also less expensive and effective for a broader
range of microorganisms than nisin. The additives are currently being used in a sachet inside the package, but can be compounded into the packaging polymer.

As per the observations of Yang (2000), silver and zinc zeolites are among the most popular compounds for antimicrobial packaging material. These compounds can be incorporated into cloth, paper, and laminated pouches. Zeolites are complex chemical structures that trap ions, such as zinc and silver, and slowly release the ions into the surrounding environment. Zinc- and silver-containing zeolites can also be incorporated into the packaging material. When the film comes in contact with the meat, the zeolites release the zinc and silver ions, which disrupt the normal biochemistry of the microbial cells. In addition to the effect of the zinc and silver ions on microbial growth, some zeolites convert oxygen in the wrapped product into ozone, a reactive form of oxygen, which also inhibits microbial growth. Several years ago Japanese food technologists developed a zinc and silver ion zeolite-containing cloth for home use. Consumers wrap fresh raw meat or poultry in the cloth to prevent the meat from spoiling. “Consumers can wash the cloth 2,000 times without losing antimicrobial activity. He adds that zinc and silver zeolites are FDA- and USDA-approved for food use.

Another natural compound that is effective against mould and yeast is mustard extract. According to Yang (2000), mustard extract is also effective against Gram-negative bacteria, such as E. coli and Salmonella. At the University of Manitoba, researchers discovered that lysozyme-containing whey protein films markedly inhibited the growth of spoilage bacteria. Lysozymes are enzymes that destroy bacterial cells through protein degradation.

Clemson University researchers are working with films produced from chitosan, a carbohydrate extracted from shrimp and crab shells. Chitosan has both anti-bacterial and anti-fungal properties. When the surfaces of raw chicken drumsticks were coated with chitosan, they observed a 90-percent reduction in the total bacterial count on the drumsticks.

4.8 Freshness Indicators

A major trend in the food industry over the past several years has been the consumer’s desire for freshness in taste and appearance and freshness indicators are an upcoming technology. Modified atmosphere packaging controls the flow of carbon dioxide through the food package to extend shelf life, but is dependent on storage conditions in the store or home. Indicators to show when a food has begun to decay are currently being used in bulk packaging, such as the Vistab 8 adhesive labels from Cox Technologies. Current research is focused on making indicators cost-effective for individual packaging. Indicator dyes work by either changing as a function of time and temperature or by reacting with a food degradation product. For example, an indicator in a sensor or in the packaging film could react with an amine given off by fish at the beginning of decay. An integrated time-temperature response is continuously obtained using these indicators. Another example includes Lifelines Freshness Monitor (Lifelines Technologies, USA) and IPOINT Time/Temperature Monitor (IPOINT Biotechnologies A.B., Sweden). The IPOINT Monitor involves two pouches, one containing a lipolytic enzyme and other containing a lipid substrate. Breaking the seal between the two pouches and mixing their contents activates the indicator.
4.9 Oxygen Scavengers

Oxygen scavenger technologies are currently being looked at to meet the need for improved barrier packaging. Increasing barrier needs are driven by the consumer's desire for higher quality convenience foods, increased usage of single-serve packaging because of the smaller packages increased surface volume and exposure to oxygen, and continuing replacement of glass and metal with plastic for weight reduction and increased convenience. The use of oxygen absorbers is a relatively new additive trend in food packaging. Commercial oxygen scavengers include iron oxide powders enclosed in sachets, additives incorporated into the packaging polymer or a polymer layer extruded as part of the package to maintain freshness by absorbing headspace oxygen and oxygen that enters the package. Shelfplus™ O₂ oxygen scavenger is a polymer-based additive that can be incorporated directly into the walls of the package. It can be incorporated into either an existing layer within the package or as a distinct scavenging layer. Another company introduced oxygen scavenging polymer system consisting of an oxidizable resin, ethylene methyl acrylate cyclohexene methyl acrylate (EMCM) and a masterbatch containing a photoinitiator and a cobalt salt catalyst. Other oxygen scavenging polymers based on nylon, polypropylene, polybutadiene and polyisoprene degrade on oxidation into by-products that can migrate into packaged food and cause off-taste or odour. EMCM does not degrade into compounds that cause off-taste or odour and the photoinitiator allows the inactive polymer to be stored and then activated by UV light during the package filling process, says the company.

Cryovac introduced an improved oxygen scavenging film that reportedly removes oxygen 10-20% faster than before. The Cryovac (r) OS Films slow microbial growth and oxidative deterioration of flavours, colour and nutrients. The oxygen scavenging process increases the shelf life of refrigerated pasta by more than 50%.

5.0 Legislations / Regulatory Aspects

The Federal Food, Drug and Cosmetic Act of 1938 (FFDCA) give FDA the authority to regulate food additives. Food additives are any substance or mixture of substances other than the basic foodstuff, which are present in a food as a result of any phase of processing, packaging, or storage. Food additives have different functions. The food industry uses additives to maintain consistency, palatability and wholesomeness, to improve nutritional value, to control acidity or alkalinity, to enhance flavour or impart a desired colour, and to keep food wholesome and appealing while in route to markets. FDA approval of a food additive is not a product license limited to a single sponsor, but is generic in that anyone can manufacture or use an additive consistent with any existing patent protection. In regulating food additives, FDA classifies the additives in four categories: prior-sanctioned substances which were approved either by FDA or the U.S. Department of Agriculture (USDA) before the passage of the 1958 Food Additives Amendments; GRAS additives (generally-recognized-as-safe) which are exempt from regulation because their extensive use has produced no known harmful effects; direct additives which are intentionally added to foods such as lemon flavouring in cookies; and indirect additives which are often trace substances that leach from packaging materials and migrate to food during processing or storage.

In Australia, State Food Acts control the possibilities of such migration by general provisions governing the sale of food: they contain provisions that no person shall sell any article which is adulterated or falsely described, which contains any matter foreign to the
nature of the food or which is packed or enclosed for sale in any manner contrary to any provision of the Act or the Regulations. The Australian Food Standards Code, which complements the Food Acts, sets maximum migration levels for three specific monomers, the "building blocks" of plastics. These are vinyl chloride, acrylonitrile and vinylidene chloride. They are singled out for special attention because of their known potential toxicity. Modern manufacturing methods have reduced monomer residues in food contact plastics to the point where they are no longer measurable.

Numerous other packaging materials are used in contact with food including paper, fibreboard, glass, tinplate, aluminium and various types of plastics. Australian Standards, which are separate from food regulations, are used to define compositional requirements of a number of other plastics for food contact use. However, these standards cover only six of the common plastics used in food packages and refer to the additives which may be used in their manufacture rather than setting limits on migration. The selection and control of these additives has been based on what is permitted in some overseas legislation. Both the United States of America and the European Community either have, or are preparing, complex regulations to control migration from food packaging materials. In some instances these regulations set maximum limits for potentially harmful migrating substances. In other cases where migration presents a minimal hazard, permitted additives may be listed without specific limits being set. Some plastics contain a large number of additives including antioxidants, stabilisers, antistatics and plasticisers. These are included to improve the functional properties of the plastics.

6.0 Conclusion

As consumers demand improved product quality and longer shelf life, there is a continuing need for improved barrier properties and extended shelf life in packaging for food and beverages, cosmetics, and pharmaceuticals. Additives that help create a protective package environment, polymers with good barrier properties, and processes for creating modified atmosphere packaging (MAP) and sensors to measure the package environment can be combined. Within the next decade, active and intelligent packaging options involving use of additives in packaging materials/packages will become key elements in how food processors and manufacturers protect the longevity and nutrient value of their products.

7.0 Suggested Readings


1.0 Introduction

Flavours are used in industrially produced products to ensure that food products have consistent taste and aroma characteristics every time. During processing the natural taste may be lost and/or changed, by adding flavours, a consistent profile can be maintained. In some cases flavour additions are made where food raw materials cannot be applied in the prepared food due to cost, availability etc. Flavours can also be used to mask undesirable attributes or impart new and unusual taste profiles to new product developments. The main reasons of addition of flavours in a food or beverage are:

- Contribute to the taste in food and beverages
- Have a big influence on the consumer's perception of the product
- Ensure the same taste perception every time
- Make products distinguishable from each other

High-quality flavours that satisfy consumer preferences and survive rigorous food processing make the winning difference in successful food and beverages. Cost and availability are prime issues when selecting the raw materials for a food or beverage product. Another is the sensitivity of natural produce and other ingredients, which may lose fragile flavour substances during processing.

2.0 Flavour Classification

2.1 Natural Flavours and Natural Flavouring Substances

"Natural Flavours" and "Natural Flavouring Substances" are flavour preparations and single substance respectively, acceptable for human consumption, obtained exclusively by physical processes from vegetable, for human consumption. They cannot contain any
Nature-identical or artificial flavouring substances. These are further subdivided into three categories:

- **Natural food(s) or food category or source(s) flavouring**: the flavouring part is solely issued from the foods or sources. Also known as FTNF (From The Named Food) e.g., Strawberry Naturome.

- **Natural food(s) or food category or source(s) flavouring with other natural flavourings**: flavour part is mainly or partially derived from the foods or sources. They are also known as WONF (With Other Natural Flavourings). e.g., Natural Banana Flavouring can be fortified with other natural flavouring substances derived from other fruits or spice extracts.

- **Smoke flavouring** is a natural flavouring concentrate obtained by subjecting untreated and uncontaminated hardwood, including sawdust and woody plants, to one or more of the following processes (controlled burning, dry distillation at appropriate temperatures and/or treatment with superheated steam) and obtaining fractions which have the desired flavour potential.

- **Process Flavouring** means a product which is obtained according to Good Manufacturing Practices by heating to a temperature not exceeding 180°C for a period not exceeding 15 minutes, a mixture of ingredients, not necessarily themselves having flavouring properties, of which at least one contains nitrogen(amino) and another a reducing sugar.

### 2.2 Nature-identical Flavouring Substances

Nature-identical flavouring substances are substances chemically isolated from aromatic raw materials or obtained synthetically; they are chemically identical to substances present in natural products intended for human consumption, either processed or not. They cannot contain any artificial flavouring substances.

### 2.3 Artificial Flavouring Substances

Artificial Flavouring substances are those substances which have not been identified in natural products intended for human consumption either processed or not.

**Example**

A Vanilla-flavoured Ice-cream could contain:

- **Natural** flavouring substances, whether derived from Vanilla Beans;

- A **nature-identical** flavouring substance that has been synthesised, but is chemically identical to a substance found in nature, e.g., Vanillin

- An **artificial flavour**, that has been synthesised and has not yet been identified in any natural product. e.g., Ethyl Vanillin.
3.0 Flavour Glossary

In order to understand and communicate various nuances used in describing flavours, a glossary of terms is necessary (Please see Annexure-I). Many more descriptors can be used, however mostly are they duplicates or partly duplicates of the ones listed in the Annexure -I. Too many descriptors tend to create confusion. Everyone with an average capability for smell and taste, and an average memory can learn to use the descriptors, however, it is a matter of training and experience to describe with the given parameters.

4.0 Analytical Tools Used in Flavours Identification

Analysis is one of the important tool for ensuring consistent quality and is a key starting point for the development of a flavour. Understanding the composition of foods, and the reaction during processing it, enables the flavourist to develop a more authentic flavour. Flavor chemists can analyze a product using advance sampling techniques and analytical equipment to determine which chemical compounds a flavour contains. This information is then used by flavourist in the creation of new flavors.

Analytical Equipment used in Flavour Analysis

⇒ High Performance Liquid Chromatography
⇒ Liquid Chromatography/Mass Spectrometry
⇒ Gas Chromatography with olfactometry, sulphur specific detection and multi-dimensional GC to facilitate the identification of chiral compounds
⇒ Gas Chromatography preparative system
⇒ Gas Chromatography/Mass Spectrometry
⇒ Capillary Electrophoresis
⇒ Nuclear Magnetic Resonance
⇒ Fourier Transfer Infra Red
⇒ Ultra Violet Spectroscopy
⇒ Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APCI)
⇒ PTR-MS, which is the latest equipment which monitors, analytically and in real time, i.e. “as it happens”, the aroma volatiles generated by food, in vitro and during eating. The goal is to be able to express aroma perception in quantitative terms.
4.1 Real-time Flavour Release

Flavour and fragrance perception are time dependent phenomena. The ability to monitor the release of aroma molecules from the mouth environment or fragrance compounds from the surface of skin provide a significant challenge to the flavour or fragrance scientist. The requirements of such a measurement capability necessitates the detection of these molecules in ppbv (parts per billion by volume) to ppmv (parts per million by volume) and in real time. The ability to achieve these concentrations and time-scales exclude the utilization of techniques such as gas chromatography since they have a prerequisite of time dependent separation. The development of chemical ionization mass spectrometry technique such as the recently developed Proton Transfer Reaction–Mass Spectrometry (PTR-MS) provides a route to address both the low concentration and complexity of flavours & fragrances and the real time element. The flavor chemistry of food is in constant change during consumption as enzymatic and other reactions influence the flavor molecules. It is this process that the PTR-MS can now observe, producing a real-time picture of the rise and fall of combinations of key flavor chemicals during chewing, swallowing and breathing. Most of the traditional methods of analysis, on the other hand, can only measure the flavor components released at a single point in time.

5.0 Functional Flavours

Health awareness is one of the prime drivers of both the functional and standard food markets. The importance of flavours which meet the expectations of more demanding and health conscious consumers is therefore key. Innovation and distinctiveness need to be combined with consumer appeal and labeling considerations. Following factors are responsible for the increase in Functional Food market:

- Explosive increase in obesity  (See Figure 1). WHO/FAO have stated that globally, overweight is a bigger problem than undernourishment
- Exponential increase in Diabetes - world wide 177 million or 5.2 % of adult population has diabetics. Type 2, or non-insulin-dependent diabetes mellitus up to 95 % of all cases which can be easily controlled through diet modifications
- Health food sales showing increasing trend
- Growing awareness of health problems associated with modern lifestyles
- Greater consumer willingness to take personal responsibility for their own health needs
- Increasing awareness of the nutritional qualities of food
- Increasing consumer sophistication and need for self expression
- Company image (media pressure)
- More consumer awareness of relationship between diet and health
- Strict legislation regarding labelling and claims
Preference for prevention over cure (i.e. functional food instead of tablets)

New market segments/opportunities

Fig: 1 Obesity a global concern
Source: Euromonitor, OECD, International Obesity Taskforce, WHO
Note: Adult obesity measured as Body Mass Index (BMI)>30kg/m²

The above factors are responsible for creation of new market segment with enormous opportunities, which can be encashed by being creative and innovative. Health concerns have also created a boom in low fat, low calorie foods. This has led to strong demand for flavourings which effectively mask the taste of sweeteners and artificially recreate the mouthfeel of fat.

6.0 Masking Flavours

Fortification of convenient food products is a growing demand among health-conscious consumers. But, while the addition of vitamins and minerals may provide nutritional benefits, their taste often leaves much to be desired. Range of masking flavours are available in the market which replace off-tastes with a more appealing flavour sensation. Masking flavours cover taste buds with long-lasting flavour components and even enhance overall mouthfeel. Vanilla and citrus flavours successfully conceal off-tastes created by vitamins, minerals and omega-3 oil. Fishy notes in savoury products and the beany note and powdery mouthfeel common in soya protein drinks are eliminated, along with off-notes from artificial sweeteners, protein hydrolysates and amino acids.
6.1 Sweet Enhancer Flavourings

Low-sugar products can maintain their full-sugar sweetness with new sweetness-enhancing flavours which work very well in dairy products and tea and coffee beverages, the flavours mask artificial sweetener off-notes and boost other flavours. The key benefits for the sweet Enhancer flavours are:

→ Enhance sweetness in low-sugar applications
→ Mask off-notes from artificial sweeteners
→ Boost other flavours Neutral taste

7.0 Flavourings For Low Fat Application

Health concerns have also created a boom in low fat, low calorie foods. This has led to strong demand for flavourings which effectively mask the taste of sweeteners and artificially recreate the mouthfeel of fat.

Low calorie dairy products have a reduced fat and/or sugar content. Due to low fat content following problems may be encountered:

→ More pronounced taste of individual ingredients
→ No masking effect from the fat e.g., oxidised/bitter taste from the dry milk
→ Cardboard notes from the gums
→ Change is sugar/salt balance
→ Pronounced change in Body, texture and mouth feel.

To prevent and optimise these problems, it is important to balance the flavouring and avoid volatile top notes. The flavouring dosages can then be reduced by up to 50 %. In addition, body & mouth feel can be improved by adding flavourings such as milk, cream & butter. Functional flavourings especially developed for low fat and low sugar dairy products are commercially available.

8.0 Conclusion

Greater product diversity and more consumer choice is expected to drive the growth in this health & functional food segment. Flavours viewed as “healthy” include fruit flavours, with cranberry of particular note due to its fashionable image and perceived health benefits. Herbal notes are also important, especially ginseng and guarana which also offer some functional benefit. These flavours are incorporated into a wide range of foods and drinks, not principally because of their taste, but because of their claims to prevent heart disease (green tea), reduce fatigue (ginseng and guarana) and functional attributes (Masking, Sugar enhancing, Cream sensation, etc). Food and drink manufacturers are in constant need of
new flavour ideas to strengthen their position in the market and inspire new consumers to try their products. Therefore it is important to capture the new flavour trends by tracking developments in consumer taste preferences and offering products which can be enjoyed without the feeling of guilt. A number of flavours, such as vanilla, cola, orange and chocolate clearly do have mass appeal. However, food manufacturers must differentiate their products to remain competitive, and using unusual and appealing flavours is an effective way to achieve this.

9.0 Suggested Readings


Fenaroli’s Handbook Of Flavor Ingredients, Fourth edition, G A Burdock, Burdock Group, USA


Flavour Glossary:

- **Acid**: Acetic Acid, Vinegar, Butanoic Acid
  Sharp, Pungent, Irritating, Sour
- **Alliaceous**: Onion or Garlic Slightly burning, Irritating
- **Ammonia-cal**: Ammonia, Certain amines
- **Astringent**: Strong Tea, Red Wine Effect of puckering the mouth Beery
  Fermented Effect of Beer, also Yeasty, Fermented
- **Biting**: Burning, (Very Acid) Hot as in Pepper, Red Chilies
- **Bitter**: Quinine, Narangin, Quassia, Terpenes, Medicines
- **Brown**: Caramelized Sugars, Maple Syrup
- **Burned**: Burned substances, meats, vegetable, paper
- **Buttery**: Fresh Butter,
- **Camphoraceous**: Camphor wood, also medicinal
- **Candish**: Pronounced sweet fruity notes like in candy
- **Caramellic**: See "Brown"
- **Catty**: Sulphur character like in Blackcurrant
- **Chemical**: Unpleasant odour of chemicals, laboratories
- **Citrus**: Orange, lemon, grapefruit notes
- **Cooked**: Refers to processed notes, as distinguished from raw
- **Cocoa**: Cocoa powder
- **Creamy**: Flavour and texture of cream
- **Earthy**: Associated with fresh root vegetable, mushroom
- **Estery**: Light volatile fruity and pungent note, pineapple
- **Fatty**: Associated with fat and oils
• Floral  The fragrance of flowers
• Fresh  Unprocessed, Just plucked
• Fried  Characteristic for foods cooked in oil or fat
• Fruity  Bright and characteristic of fruits
• Green  Characteristic of leaves and cut grass
• Herbal  Characteristic of herbs
• Jammy  Associated with cooked fruits
• Juicy  Mouth watering
• Meaty  Savoury
• Malty  Characteristic of Malt, Dead Beer
• Medicinal  Pharmaceutical products, Traditional Medicins
• Metallic  Canned Foods, Oxidized Iron Smell
• Minty  Reminiscent of Mints
• Mouldy  Like mould, wet forest's soil
• Musky  Characteristic of musk
• Nutty  Characteristic of nuts
• Oily  Strong smell as in old cooking oil
• Peely  Characteristic of fruit / citrus peel
• Peppery  Spicy like pepper
• Perfumy  See Floral
• Phenolic  See smokey, smell of over-heated old coffee
• Rancid  Canned Foods, Oxidized Iron Smell
• Ripe  "At its peak" In between unripe and overripe
• Roasted  Oven baked in the presence of fat
• Rosy  See Floral
• Rubbery  Reminiscent to rubber
• Sharp  Pungent, Acidic. See acid
- **Sour**  
  Lemon, Citric acid, see Acid
- **Smoky**  
  Characteristic of wood smoke
- **Spicy**  
  Characteristic of spices
- **Sweaty**  
  Like sweat
- **Vanilla**  
  Characteristic of vanilla
- **Vinous**  
  Characteristic of grape, wine
- **Waxy**  
  Like Bee-wax, shoe polish
- **Woody**  
  Characteristic of wood or seeds
- **Yeasty**  
  Characteristic of freshly fermented food
1.0 Introduction

Before we start on this subject we have to understand the subject I have been asked to write on.

Functional Food Ingredients: These are (from our perspective) ingredients added to processed food derived from natural sources to enable better & safe production results in terms of shelf life, taste, texture and appearance.

Contemporary Food Industry: This term is relative to the development of the country we live in. Probiotics would be considered contemporary in the West, while here in India, mass produced Dahi is considered contemporary.

However whatever the food, Ingredients made by a company such as ours is key to the success of the food industry not only due to the need during production but due to the research and development for new product, applications, safety and processes that we strive to share with the industry across the world. The interest in India and the need for these products have been relatively recent. We need to briefly examine the reasons for this developing need before we move on to functionalities.

To do this we have to examine what has taken place over the past Years. There was a point when India was a high tax and socialist environment, immensely bureaucratic with laws which had been created during the British Raj. There was an old saying here that “the British invented bureaucracy and the Indians perfected it!”. In the late 90s the government was on the verge of bankruptcy, it is during this time the doors were opened to foreign direct investment. Following this, laws have been further relaxed on conversion of the Rupee and ease of FDI.

The result has been dramatic. The main change owed to Television (this went from 3 channels to over 150 almost overnight), and Banking. The first, (TV) pushed the desire to have better lifestyles and the second (Banking) funded it. As little as 10 Years ago it was not possible to get a bank loan to buy a car or home, if one got it interest rates at up 20-25% did not allow it to be feasible. With the change in law and the entrance of international banking systems (Credit Cards did not exist here 15 Years ago), it is now affordable to own homes at a young age as with cars etc. This is off-course also in relation to salaries increasing, better working environments and more opportunities.
In the past, a young married couple would always live with their parents (housing was not affordable) the wife would traditionally stay at home to help with chores and the men would work. The processed food industry was not trusted and all food was bought fresh on a daily bases. Today most women are as ambitions as the men with opportunities to drive them. Couples are leaving the nucleus of there extended families and living in owned apartments where both husband and wife work. There are restaurants opening on a daily bases in the Metro's and multiplex cinema's and entertainment centers. New Shopping centers are being built on a monthly bases and the upwardly mobile fill them to a bursting point every weekend.

Our own office is a good example. We have a young team with an average age of about 32 all of whom are married and all of whom live in there own apartments. In most offices there is a balance of 30% women. In some areas like Hospitality (Hotels etc), Call-centers etc the balance would be 70%. The women of today in the metros are no longer a top-up for their husbands’ salaries but a real contributor to the home. As a result their position is taken very seriously and therefore the importance of there careers is recognised. This means there is less time for them to spend in kitchen preparing food, particularly Indian food, which takes a while to cook. As a result they are looking to convenience, healthy and safe solutions for themselves and their families. The awareness of Brands and trusted Companies is part and parcel of daily discussions, as is any new convenience food, which becomes available. After all one of the key reasons of not eating out or buying ready meals was not just that they were not readily available but also the concern of cleanliness and safety. It is due to this the Demand has risen for convenient, safe and functional food.

2.0 Functional Food Ingredients categories

The broad categories of functional food ingredients which are derived from natural sources are as follows:

➔ **Emulsifiers:** e.g., Mono-diglycerides (GMS), Distilled Monoglycerides (DGMS) Ensure flexible processing and high quality functionality in numerous products.

➔ **Hydrocolloids:** e.g., Pectin, Locust Bean Gum, Guar Gum. These are texturants and impart the right mouth-feel to the product.

➔ **Cultures:** These are the basis of manufacturing different types of cheese, fine meats (Sausages/Salami), yoghurts etc.

**Functional Systems:** In modern food production, any one ingredient rarely provides all the functionalities, the producer wants in a food product. Instead of applying three individual ingredients, the producer can use one functional system (ingredients blends) premixed to meet the requirements. Therefore, functional system serve as optimised all-in-one solutions designed to meet specific functional needs

➔ **Enzymes:** e.g., Bakery enzymes, lipases, proteases, etc. used in bakeries, the Beer Industry and others for increased shelf-life standardisation and the basic manufacturing.
Antimicrobials: e.g., Nisin, natamycin. Protect food and beverages against undesirable bacteria, yeasts and moulds.

Antioxidants: e.g., Ascorbyl palmitate, BHA, Rosemary Extracts. Delay the onset of rancidity in margarine, snacks, dressings, meat and other applications.

Flavours: Vanilla, Strawberry, Pineapple, Smoke, Cooked Chicken. Added in concentrate form in manufacturing enable ease of processing and tailor the product to the taste of the market.

Sweeteners: Fructose, Xylitol, Litesse. Enhance the nutritional profile, sweetness and quality of food and beverages without adding harmful calories allowing sufferers of diseases such as diabetes and heart diseases to enjoy taste sensations without fear.

3.0 Growth in Functional Food Market

For food manufacturers, the functional food market is an attractive area offering good growth potential, but developing successful functional food products means understanding what motivates consumers to purchase these foods. This is not easy in a market where consumers are unsure about what is healthy and which product best addresses their needs. For manufacturers to succeed, it is important to have effective products with credible claims that are simple for the average person to understand. Clarity of message and credibility of product are crucial. As consumers are becoming more and more health conscious, the demand for more indulgent foods that have reduced fat, no sugar added and are reduced in calories is likely to increase. With the advancement in functional ingredients e.g., emulsifier/stabilisers blends, Probiotic cultures, sugar and fat replacers, masking flavours, etc., it is now possible to manufacture dairy products, which are sugar-free, low calorie, low fat and have specific health benefit without compromising the taste & texture.

Functional foods have been born due to consumer awareness. These impart not only a satisfaction in taste but also perform a health related function.
4.0 Health & Wellness Trend

The health & wellness concept has added a new dimension to this scenario due to the following reasons:

→ **Alarming Increase in Obesity:** According to FAO/WHO release 1.2 billion people are overweight and app 250 Million people are obese. World over overweight is a bigger problem than under-nourishment.

→ **Diabetics:** World wide 177 million or 5.2% of adult population are affected. The majority of this population is in Asia. Type 2 or non–insulin dependent diabetes mellitus account for 95% of all cases. These can be managed through diet modifications especially the type of carbohydrates.

→ **Osteoporosis:** As per the WHO study the lifetime risk for Osteoporosis for women is between 30 and 40%. Calcium, Vitamin D and exercise are essential for prevention of Osteoporosis. Health benefits and claims such as "calcium leads to bone health" are associated with dairy products and are easily recognized by the consumers.

→ **Healthy foods sales:** There is an increasing consumer awareness of relationship between diet and health. Further, consumers are willing to pay a premium for foods that can give some health benefits.

→ **Consumer demand for low calories Foods:** Low calorie, reduced calorie foods and diabetic foods are becoming increasingly popular in all segments of food industry. This is seen by the launch of many low calorie ice-cream and milk variants.

The above factors are responsible for creation of new market segments with many opportunities. Real Health concerns have also created a need for low fat, low calorie foods. As a result functional food ingredients play a role to create mouth-feel taste and sweetness without the negative effects of the high calorie and high fat in the food products. Greater product diversity and more consumer choice is driving the growth in health & functional food segment.

A survey carried out recently establishes the Key Health Drivers and below is results for the responses for peoples Main Health Concerns Many consumers feel that functional foods improve general health and well-being. Some of the major trends in functional foods are as follows:
**Probiotic:** This category includes products like Dahi, Yoghurt, Lassi, Buttermilk, etc. Probiotics have been linked with a number of health benefits, including:

- Enhanced gut function and stability
- Improved protection against infection and cancers/mutagens
- Immune system modulation
- Alleviated lactose intolerance
- Improved digestion and nutrient absorption
- Reduced blood cholesterol
- Reduced allergy risk

Companies such as Danone, Nestle, and Yakult have been very active in this area launching probiotic milk and yogurt drinks.

- **Addition of different bio-actives:** A number of whey, casein and dairy protein derived peptides and hydrolysates which act as bio active peptides are already commercially available. These bio-active peptides can be used to position the products as anti-hypertensive, regulation of fat metabolism, etc. Ice-cream and frozen desserts can also be used as vehicles to deliver bioactive dairy peptides.

- **Vitamin and calcium fortification:** Many dairy products can be adapted to nutrient fortification and inclusion of nutraceuticals. Vitamin & calcium fortification is often carried out in infant foods & dairy products targeting women. Omega-3 and omega-6 fatty acids can also be incorporated in the fat phase of the dairy products to provide functional benefits. Therefore, many dairy products can be used successfully to deliver unique nutritional benefits to consumers beyond the basic nutrition of current products.

- **Fibre-enrichment:** Inadequate intake of dietary fibre in the human diet has been implicated in many diseases e.g., constipation, obesity, diabetes, gallstones, lipid metabolism, appendicitis, etc. To increase fibre intake through enjoyable, tasty and safe foods these can be added. Polydextrose is a good choice as an economical fiber source for use in ice cream and frozen desserts.

Polydextrose is not digested in the upper gastrointestinal (GI) tract and is partially fermented in the lower GI tract, making it a beneficial ingredient for digestive health. The physiological benefits of polydextrose include increased fecal bulk, reduced transit time, lower fecal pH and reduced concentration of putrefactive substances in the colon. Polydextrose’s prebiotic effects help promote growth of beneficial intestinal bacteria, while fermentation in the large intestine yields short-chain fatty acids, including butyrate. Improved GI function has been demonstrated with a daily intake of 4-12g of polydextrose without adverse effects. In addition to the health benefits, polydextrose has multiple functional benefits in ice-cream & frozen desserts. The freezing point depression factor is 0.6 vs. sucrose at 1.0; therefore, it can protect the structure of ice cream as it inhibits sugar recrystallization and starch retrogradation. It also improves storage stability by narrowing the difference between the storage temperature and the composite glass transition.
temperature of maximally frozen concentrated solutions for frozen desserts (Tg'). The relative sweetness of polydextrose is practically zero so the sweetness of the finished product can be adjusted by using high-intensity sweeteners.

Fortification can be as simple as adding protein, vitamins, minerals or complex carbohydrate. It can be a bit more complex through the addition of a variety of biologically active "nutraceutical" compounds.

5.0 Conclusion

While it is clear that there is no simple formula for successful functional food development, some basic criteria must be fulfilled. Successful functional foods tend to have tangible benefits are clearly documented through sound, scientific investigation; the associated claims must be sensible and credible.

Key Success Criteria

→ Products address a widespread and widely publicised health issue.
→ Product claims are backed by scientific evidence.
→ Benefits are clear to the consumer.
→ Product is genuinely effective, and results are noticeable to the consumer.
→ Products are tasteful and have the right appearance.
→ Products are affordable with strong branding, communication of benefits to the consumer must be strong and distribution.

Consumers are becoming more and more health conscious. As a result of this, the demand for more indulgent foods that have reduced fat, no sugar added and are reduced in calories is likely to increase. Therefore, functional dairy products should address the widespread health issues to have significant consumer appeal. In order to be successful, the functional dairy products should give tangible benefits to the consumers which are supported by documented sound scientific investigations and associated claims must be sensible and credible.
1.0 Introduction

Milk and dairy products represent an excellent source of nutrients. They provide high-quality protein in addition to energy, minerals, and fat-soluble and water-soluble vitamins. Thus milk and/or products derived therefrom constitute an integral component of human diet in most parts of the world. However, on account of the high digestibility of milk protein, milk fat as well as lactose contained in milk, dairy products are essentially “low-residue” foods with regard to the human alimentary canal, i.e. foods with very little residual (undigested) portion passing from the small intestine into the large intestine. Therefore, when consumed along with other foods which are also lacking in indigestible material i.e. ‘dietary fiber’ (capable of withstanding the digestive enzymes in the gastrointestinal tract, thereby contributing to the bulk of food residue passing into the large bowel), dairy products without any fruits and/or vegetables added to them could lead to problems associated with fiber-lacking diet.

The major problems that could be traced to a low-fiber or low-‘roughage’ diet include poor fecal bulking, leading to constipation and several other intestinal disorders. Studies relating non-infectious diseases and diet among people in East Africa and South Africa revealed about five decades back that the pattern of disease incidence was dependent on the diet and it was different in those parts of the World as compared to that in the Western countries. A few years later it was reported that the incidence of disorders such as diverticular disease, appendicitis, colorectal cancer, obesity, diabetes and coronary disease were all related to the intake of dietary fiber as evidenced by the fact that the incidence was much less in the Africans who consumed larger amounts of fiber-rich foods as compared to their Western counterparts with a relatively low consumption of dietary fiber. These findings led to the ‘dietary fiber hypothesis’ and triggered considerable research on the role of dietary fiber in human health.

Realization of the significance of fiber in diet has also encouraged research on various means of enhancing dietary-fiber intake including development of new food formulations with increased fiber content. Thus, fiber-fortified dairy foods are among the functional foods, the demand for which is growing the world over for a variety of reasons. For example, the actual dietary fiber consumption in the USA is 11-13 g daily as against the recommended level of 20-30 g per day. The present discussion pertains to what dietary fiber is and how it can be incorporated into dairy foods.
2.0 Definition and Classification of Dietary Fiber

The early definition of dietary fiber as “the skeletal remains of the plant cell wall that were indigestible” has vastly changed as greater understanding of the role and mechanism of dietary fiber developed through continuing studies. While there may still not be unanimity on the definition, the American Association of Cereal Chemists (AACC) recommendation in this regard is widely accepted. Accordingly, dietary fiber is the remnants of the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. It includes non-starch polysaccharides such as celluloses, some hemi-celluloses, gums and pectins as well as resistant (or ‘unavailable’) starch, oligosaccharides, lignin, and ‘associated’ plant substances. Dietary fiber exhibits one or more of the desired physiological effects viz. laxation (as indicated by fecal bulking and softening with increased frequency and/or regularity), blood cholesterol attenuation, and blood glucose attenuation.

Dietary fiber materials may be classified in different ways. A common classification is based on solubility in water which identifies two types of dietary fiber: soluble fiber and insoluble fiber. Soluble dietary fiber (SDF) includes beta-glucans, pectins, gums, mucilages and some hemi-celluloses. Foods rich in soluble fiber include fruits, oats, barley and beans. Insoluble dietary fiber (IDF) includes celluloses, some hemi-celluloses and lignin, which are found in vegetables and whole grains (especially bran) of wheat and most other cereals.

3.0 Dietary Fiber in Dairy Foods

While milk is inherently free from dietary fiber, only those milk-based products which contain fruits, vegetables or gums have dietary fiber in them. Examples of fruit-containing dairy products include fruit-and-nut ice cream, fruit custard, fruit yoghurt, fruit cream and fruit shrikhand. The fruits used include banana, mango, pineapple, strawberry, apricot etc. which greatly enhance the mouthfeel and palatability of the product. Many traditional Indian milk sweets containing a variety of vegetables and/or nuts can be considered to be a good source of dietary fiber. These include khoa-based sweets containing carrots, bottlegourd (Lauki), cabbage, coconut, cashewnut, almond, pistachio etc. The milk-and-cereal based Doda Burfi is among the sweets rich in dry fruits, and hence, in dietary fiber. Raita, a traditional fermented dairy product prepared in homes may contain a variety of vegetables such as onion, cucumber, tomatoes, carrots, pumpkin etc., or fresh fruits such as grapes, mango, etc. Cereal-based desserts such as kheer and dalia (cracked-wheat dessert) may contain varying amounts of dietary fiber depending on the level of the bran portion of grains and the extent to which they are garnished with dry fruits. Sapota (chikoo) is also used in a Halwa-type dairy dessert.

In preparation of the above products, pretreatment of the fiber ingredient is important. While little needs to be done with fruits and nuts except for washing, removing inedible portions and chopping or pulping, extensive pretreatment of vegetables is required. Depending on the vegetable used, it may necessitate peeling, cutting, precooking etc. However, some traditional preparations such as (carrot-based) Gajar-ka-Halwa or Gajarpaak, Lauki-ka-Halwa or Lauki-kheer, etc. may involve cooking of the washed and cut vegetable in milk itself while atmospheric evaporation causes concentration of milk solids leading to the desired finished product consistency. The physical form (or degree of comminution) and extent of cooking together with the vegetable-to-milk solids ratio will usually determine the products texture. The fruit-based products have the characteristic fruit flavour often aided by the additional synthetic flavoring whereas vegetables being generally
bland in taste, much of the flavour appeal of the vegetable-based sweets results from the added flavouring, often of natural origin e.g. cardamom, saffron, nutmeg, cinnamon, etc.

Sweet-potato and yam are among the tubers and roots that are fairly good sources of dietary fiber. Non-conventional milk-based desserts and yogurts have been prepared using sweet-potato and yam. Bamboo shoots and aloe-vera have also come to be known as food ingredients with considerable health value presumably on account of their dietary fiber content. Their potential is beginning to be appreciated as is evident from the fact that very recently an aluvera-based dairy drink has been commercially marketed.

4.0 Commercial Fiber Preparations as Food Ingredients

The bran portion of wheat grains has been a very well-recognized source of primarily IDF. Consumption of whole grains in different forms has for long been recommended by medical practitioners to patients of constipation and related disorders. Extensive studies have proven wheat bran as an excellent source of dietary fiber. It is, therefore, no wonder that packaged wheat-bran preparations are commercially available on the Western markets for use as fiber-supplement in suitable culinary dishes. Rice bran and oat bran have also been found to serve as valuable sources of dietary fiber and are commercially available, now even in India.

Whole-grain based breakfast cereals marketed in a variety of physical forms and tastes are indeed an attractive way to enrich the normal diet with fiber. Special breakfast formulations such as ‘Muesli’ containing rolled oats and dried fruits and nuts and consumed along with milk as is a popular practice in Europe represents another example of fiber-enriched dairy foods.

The increasing trend towards enrichment of conventional foods with dietary fiber has recently led to development of a range of fiber preparations which are commercially available to the food industry in Europe and North America. Among such preparations are Fibre-Hi (Pro-fibre Nutrition, U.K.), Oatrim (Golden Jersey Products, U.S.A.), Primacel (NutraSweet Kelco, USA), carrageenan (Grindsted Products, Denmark), Mercel (Meer Corp. New Zealand), cellulose and marine colloids (FMC Corporation, USA), Soluline IDA and ID Tex (ID Foods France), Exafine (Cosucra SA, Belgium) and a fiber topping (Crissy Food International, Denmark). Some of these products are currently offered by certain importing agencies in India.

4.1 Some Technological Properties of Selected Soluble Fibers

The most extensively used SDF, inulin, an oligosaccharide, is manufactured by Orafti (Belgium) and marketed under the trade-name of ‘Raftiline’. Obtained from chicory roots, Raftiline is a fructan (a glucosyl fructose) consisting of glucosyl and fructosyl units. While Raftiline ST/GR/ST-Gel are based on native chicory inulin (90-94% inulin in dry matter) with an average degree of polymerization (DP) of 10, the modified inulin preparations, viz. Raftiline HP/HPX/HP-Gel (at least 99.5% inulin in DM) have an average DP higher than 23. The latter is twice as much effective as the former in improving the mouth-feel of the food. As per the supplier’s recommendations 1-8% of Raftiline HP/HPX/HP-Gel or 2-12% of Raftiline ST/GR/ST-Gel could be used in yoghurt, cheese, dairy drinks and dairy desserts. Available as a white odourless spray dried powder, Raftiline is neutral to slightly sweet in taste and has a moderate solubility (Raftiline HP, 2% and Raftiline ST/GR/ST-Gel 12% in water at 30°C) and a very low reducing ability (2 DE for ST/GR/ST-Gel and 0 for HP/HPX/HP-Gel). It has a low viscosity (less than 1 mPa.s) at 10-
80°C, but forms a gel at high concentrations. Heat treatment (e.g. 90°C for 5 min) at pH 3.5 can hydrolyze about 1% (HP) to 3% (ST/GR/ST-Gel) of inulin. It is stable at pH higher than 4 and less than 5% inulin is hydrolyzed at a pH above 5 even at a temperature of up to 150°C. In India, Raftiline is supplied by S. A. Pharmachem, Mumbai. Inulin is also available as 'Fibruline' (Cosucra SA, Belgium).

Another SDF, oligofructose (or, fructooligosaccharides) of plant origin is manufactured as Raftilose by Orafti (Belgium) or Fibrulose by Cosucra (Belgium). This partial hydrolysate of inulin has a sweet neutral taste, high solubility, glucose syrup-like viscosity and moderate reducing power. It is recommended as a sugar replacer in a variety of products including ice cream, sherbets, dairy desserts, yoghurt and dry mixes. It acts as a humectant on account of its ability to lower the water activity. Its bifidogenic effect has been confirmed in human subjects.

Polydextrose with an average DP of about 12 is another soluble fiber with proven health value in humans. Marketed by Danisco India, this oligosaccharide preparation is sweetish in taste and has a good solubility in water. Among other resistant (or, non-digestible) oligosaccharides are Neosugar and Actilight (glucosyl fructoses), Oligomate (galactooligosaccharides), Cup-oligo (galactosyl galactose or, transgalactooligosaccharide), Isomalto (glucosyl glucose or, isomaltooligosaccharides), Soya-oligo and Soytrim (sololigosaccharides viz. raffinose and stachyose), pyrodextrins (complex mixture of corn/potato-starch pyrolysis), and Xylooligo (xylosyl xylose or, xyloooligosaccharides).

Many of the gums used as hydrocolloids or thickeners in dairy and food products also represent a valuable group of soluble gums which are resistant polysaccharides. These include gum arabic, gum acacia, guar gum, locust bean gum, tragacanth gum etc. of plant origin. Other similar polysaccharides are alginic acid, alginates, agar-agar and carrageenan of seaweed origin, and gellan and xanthan gums of microbial origin. Pectin and carboxy methyl cellulose are also important hydrocolloids serving as SDF. The viscosity of these gums vary from product to product, but most gums give viscos solutions which may often limit the level of their incorporation into a food. Also, flavourwise most of these products are not bland or neutral, which again, places a serious restriction on their use in any substantial quantitites. A few gums may also impart some colour or dark appearance to the product. Some of these gums e.g. locust bean and guar gum, may be favoured for their emulsifying ability in addition to their water holding capacity.

Psyllium gum (Isabgul or Ispaghula) obtained from the husk of the tropical plant (herb) Plantago ovata seed is an arabinoxylan. A water-soluble fiber, psyllium husk also has a large viscolysing or thickening ability. Its value as a dietary fiber has been established in several studies and is one of the most frequently suggested remedies for constipation in humans. It has been used as a stabilizer in ice cream.

4.2 Properties of Certain Insoluble Fibers

Among the insoluble fiber preparations besides wheat bran, oat bran etc. are Vitacel fiber preparations (Clarico-FPC India, Mumbai) such as wheat fiber (WF-200 etc.), oat fiber (HF-101 etc.), tomato fiber (TF-200), orange fiber (OF-10 etc.), and Vivapur microcrystalline cellulose (Vivapur-101 etc.). These preparations are available in a powder form ranging in appearance from white to light yellow. While their thickening effect is usually low, they may have a considerable influence on the taste and mouthfeel of the product. Grittiness is caused by gums added in excess of a certain level depending on other constituents and their concentrations in the fiber-added formulation. Higher milk solids level together with use of mucilagenous gums may help retard the coarseness attributable to some of the
insoluble fibers. Of course, the particle size of the fiber preparation is also an important determinant of grittiness or coarseness.

The fibers’ insolubility leads to sedimentation in low-viscosity systems. These fibers have appreciable water absorption capacity (300-1100%) and oil absorption ability (200-500%). Some of these preparations are recommended for use in cheese foods.

5.0 Current Research in Fiber Fortification of Dairy Foods

Attempts have been made to enhance, via incorporation of external dietary fiber, the healthfulness of dairy products which otherwise do not contain any fiber. Considerable work along these lines is afoot at this Institute too. In one such study various combinations of soluble and insoluble fibers have been examined for their suitability in a milk base which could be converted into a dairy dessert (custard-type), rice-kheer or flavoured yoghurt. Selected fiber formulations have shown a definite potential in preparing a fiber-rich packaged kheer with extended shelf life under refrigeration.

Fiber-fortified paneer has been developed using inulin and soya fiber. A research programme is being initiated to produce extended shelf life paneer with added health attributes via incorporation of dietary fiber among other health factors such as calcium, phytosterol, etc. Low-fat spreads of both dairy and non-dairy (or, mixed-fat) types are being developed using soluble dietary fibers such as inulin. A special milk-food formulation containing commercial dietary fiber preparations along with other compositional modifications in terms of monounsaturated and omega-fat contents has been developed for patients of cardiovascular diseases.

Another health-food formulation currently being developed at this Institute is low-fat ice cream containing a fat replacer as also added soluble dietary fiber. Fiber enrichment of certain fermented foods by using whole cereal grains in various forms is also being investigated. Malted milk food which is prepared from malted barley and milk besides other ingredients is not only an easily digestible, nutritious formulation but also carries substantial quantities of oligosaccharides. A study is being undertaken to enhance the dietary-fiber content of this product by including oats in the powdered food. Recent reports suggest that oats used in various forms can serve to produce a variety of milk foods with improved health as well as nutritional attributes for children and adults alike.

6.0 Conclusion

Milk and most dairy products are essentially ‘low-residue’ foods which may, under certain circumstances, be considered undesirable from the health point of view. Dietary fiber has now been very well recognized as an essential component of a healthy diet, and hence, incorporation of external dietary fiber can greatly raise the health status of the otherwise highly nutritious food items that milk products are. Fruits and nuts have often been used in certain dairy products such as ice cream, yoghurt, shrikhand, and dairy desserts, with appreciable fiber contribution from these ingredients. Certain traditional dairy delicacies are prepared using vegetables and fruits which are rich in dietary fiber. Khoya-based sweets e.g. Carrot-Halwa, Lauki-kheer or Lauki-Halwa, Chikoo-Halwa, etc. are a few examples. Newer products such as those containing fiber sources like sweet-potato and yam have been reported in recent times. Further, specialized fiber preparations, often representing purified fibers, are increasingly available as commercial ingredients for more healthful dairy foods. Both soluble and insoluble dietary fibers have been studied for their suitability in certain dairy foods such as spreads and ice cream as well as traditional milk
products including kheer and paneer. The current work at this Institute is expected to lead to a number of such foods which would be of a considerable interest to the dairy industry looking for more value addition in their product line. Consumers would also be greatly benefited by such ‘designer’ dairy foods which are more healthy than those having been offered thus far.

7.0 Suggested Readings


CURRENT TRENDS IN USE OF MINOR INGREDIENTS IN LOW FAT SPREAD

Dr. R. R. B. Singh and Dr. D. D. Patange
Dairy Technology Division
NDRI, Karnal

1.0 Introduction

Butter is very rich in fat content. It is therefore not only expensive but also very rich in calories. The conventional butter also suffers from poor spreadability at low temperature. Butter stored under refrigeration becomes hard and brittle and loses its spreadability at ambient temperature. Low fat spread opens up a new avenue for dairy manufacturers to introduce low-energy, low-priced fat products with improved functional properties. These spreads not only offer healthy nutrition but also have good taste and flavour as well as very good spreadability at refrigeration temperature, and retain its stand up property even at high ambient temperatures. Spreads blend easily with other foods for convenience in cookery and serving.

Low fat dairy spread contains about 40 per cent moisture. It is therefore difficult to achieve a stable emulsion, which is critical for its keeping quality and spreadability. Thus the manufacture of low-fat spread requires technological innovation to create both a stable emulsion and good spreadability at refrigeration temperature. Development of such products however requires a complex assortment of stabilizers, emulsifiers, preservatives and other additives to increase quality and freshness. Without these additives, manufacturer simply can not formulate, store and transport low-fat dairy spread in a form that is convenient to use.

2.0 Stabilizer

The traditional butter contains 80 per cent fat, with oil as the continuous phase. When the fat content is reduced it becomes increasingly difficult to maintain the original water-in-oil emulsion and therefore stabilizers must be used. A stabilizing agent performs the function by providing stability to such hetero-phase system and prevents phase separation. In a low-fat spread, the aqueous phase tends to be structured or strengthened usually by the addition of protein or carbohydrate. These hydrocolloids have very large molecular weight and can form a three dimensional network in water, thereby increasing the viscosity of the system. At a certain hydrocolloid concentration, the water becomes immobilized and a gel is formed. Whatever the blend of hydrocolloids employed to structure the aqueous phase, the main functions are two fold: firstly, to inhibit the water droplets’ aggregation, thereby making the product stable to processing and storage conditions, and secondly, to produce an emulsion that gives good meltdown in the mouth and consequently rapid flavour release with satisfying cooling effect. Some of the structuring systems developed for aqueous phase in reduced fat spread are: a) viscous gels (involves use of milk proteins and polysaccharides such as alginate); b) homogenous gels (using gelatin, carrageenan or maltodextrin) and c) filled gels (gelatin with starch or maltodextrin). Various hydrocolloids used in the low-fat spread and their functions are given in Table 1.
Table 1: Stabilizers Used in Low-fat Spreads and Their Functions

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Stabilizer</th>
<th>Function*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carrageenan</td>
<td>S, T, E, GA, PB</td>
</tr>
<tr>
<td>2</td>
<td>CMC</td>
<td>S, T, PB</td>
</tr>
<tr>
<td>3</td>
<td>Pectin</td>
<td>S, T, E, GA</td>
</tr>
<tr>
<td>4</td>
<td>Starch</td>
<td>S, T, BA</td>
</tr>
<tr>
<td>5</td>
<td>Corn starch</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Sod. alginate</td>
<td>S, T, E, GA, PB</td>
</tr>
<tr>
<td>7</td>
<td>Gelatin</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Locust bean gum</td>
<td>S, T, E</td>
</tr>
<tr>
<td>9</td>
<td>Guar gum</td>
<td>S, T, E</td>
</tr>
<tr>
<td>10</td>
<td>Pectin + Alginate</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Xanthangum + gelatin</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Gelatin + maltodextrin</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Gelatin + CMC</td>
<td>-</td>
</tr>
</tbody>
</table>


Carrageenan has found wide application in the dairy industry due to its merits such as better reactivity with protein, diversity of the texture according to the type of fraction used (kappa, iota, lambda), and smaller quantities that are to be added. Pectin, locust bean gum, and guar gum are also used as viscosity enhancer of aqueous phase in low-fat spreads. The increase in firmness is believed to be due to increased affinity between hydrocolloid molecules in more concentrated dispersion. These interactions increase viscosity in the continuous phase thereby enhancing stability.

When sodium alginate is hydrated in aqueous phase, the viscosity increases. This is partly due to viscosity of alginate itself, and partly as a result of the reaction of alginate and calcium from the milk solids in the formulation. Few workers have however stated that carrageenan induced gelation in aqueous phase is not through modification of solution rheology but by binding of casein micelles in milk even in the absence of soluble calcium. Whey protein has good functional properties (emulsifying and stabilizing) and can be used as stabilizer in the spread. Besides these, inulin or fructan mixtures have also been used as stabilizers (1-15 per cent by weight) in spread. Use of more than one stabilizer is sometime recommended in the literature.

3.0 Emulsifiers

Low-fat spreads are generally a water-in-oil emulsion. Formation of an emulsion requires the presence of surface-active agents (called emulsifiers or surfactants) to facilitate the formation of dispersion of one immiscible liquid (water) in the other (oil).
Emulsifiers reduce the interfacial tension between the aqueous phase and oil phase thereby improving the stability of w/o (water-in-oil) and o/w (oil-in-water) emulsions. The emulsifiers’ functionality is the direct result of their chemical structure, which consists of two parts, one hydrophilic and the other lipophilic. The type and level of emulsifier to be added in the low-fat spread is governed by the type of emulsion, nature of other ingredients and processing conditions. The level of emulsifiers in spreads is reported to vary from 0.1 to 1.5 per cent; generally the level of emulsifier decreases with increasing level of fat.

Commercial mono and diglycerides are the common emulsifiers used in w/o type low-fat-spread. These mono/ diglycerides also contribute to the initial nucleation of high melting triglyceride crystals during the crystallization process. Such crystals can preserve the emulsion structure by forming solid shell around the aqueous phase droplets and are the main stabilizing system in the product.

Use of GMS substantially enhances flowability of the spread during processing and reduces oiling off. However, higher levels of emulsifier have destabilizing effect on emulsion. In the presence of a low polarity emulsifier like monoglycerides, the protein in cold stored emulsion is desorbed to a large extent, from the lipid phase, to which they had adsorbed previously, at high temperature.

Sorbitan esters are well known emulsifiers which are derived from simple reaction between sorbitol and fatty acid (stearic, palmitic, oleic and lauric) to form 1.4 and 1.5 sorbitan monoesters. These products are known as Span 60, 40, 80 and 20, respectively. More hydrophilic derivatives are obtained by ethoxylation with Ca. These complex mixtures are known as Tweens and are widely used in food industry. Tweens can better adsorb on to the fat particles and more easily dissolve in molten fat (prior to cooling process). Liquid surfactants such as sorbitan monolaurate significantly accelerate the transformation from $\alpha$ to $\beta$ form of fat crystals. The $\beta$ form leads to formation of relatively small crystals resulting in good plasticity and thereby imparting proper softness to the fat product. Other emulsifiers tried in the spreads are lecithin, phosphatides, centrophil, polynol-A and hymono-8803 (90%) monoglyceride.

Consumers have however shown a certain degree of concern over the use of emulsifiers and other additives, and willingness to pay more for additive-free dairy foods. In spreads the use of added emulsifiers can be eliminated or at least reduced in part by using residual levels of natural emulsifiers in food systems such as dairy cream which contains sufficient amount of natural emulsifiers to stabilize the fat spread in which it is an ingredient.

4.0 Emulsifying Salts

Emulsifying or chelating salt plays an important role in production of emulsion. These salts are believed to bring about emulsification of fat, stabilization of the interface between fat, protein and water and shortening of body i.e. creaming in products like cheese spread. Emulsifying salts play these roles via their buffering or chelating action and altering the protein function and thereby improving the texture of spread.

Common emulsifying salts used in low-fat spreads and similar products include trisodium citrate, citrate in combination with trisodium phosphate, mixtures of sodium citrate and disodium hydrogen orthophosphate and trisodium phosphate alone. Generally, these are added at rates varying from 1 to 4 per cent. Research reports suggests that citrate is more effective than other salts in o/w low-fat spread. Also, citrates have slight firming effect on the spread but spreadability and mouthfeel are not appreciably influenced by any of the examined emulsifying salts viz., trisodium citrate, sodium monophosphate, sodium tripolyphosphate, and sodium hexametaphosphate.
5.0 Plasticizers

Plasticizer or softening agents are generally humectants. These compounds are used to maintain the soft texture and plastic nature of spread over an extended temperature range. Most plasticizers work by holding water within spread. Glycerol, sorbitol, glycol etc. may be used in spreadable products. There are also technological advantages such as workability and shelf life. Shelf life of the product can be extended as humectants have an ability to depress the water activity of aqueous phase.

Glycerol and sorbitol (0.5 - 1.0 %) have been found to improve the mouthfeel of soy-based o/w spread, without adversely affecting its firmness. Use of glycerol, sorbitol and polydextrose in combination has been suggested in reduced-fat margarine suitable for bakery products.

6.0 Acidifying Agents

Spreads in general have relatively low storage stability owing to their high moisture content. A low pH in the system retards bacterial growth and thus helps in extending the product’s shelf life. A pH lower than 4 would result in syneresis and higher pH would lead to the formation of o/w emulsion. Spreads with best body have been obtained with pH in the range of 5.7 to 5.9. Several pH correcting agents have been permitted in spreads by IDF and IFMA (International Foodservice Manufacturer Association). These include acetic acid; lactic acid; sodium, potassium and calcium lactates; citric acid; sodium, potassium and calcium citrates; tartaric acid and its sodium salts; calcium orthophosphate; sodium carbonate; sodium hydrogen carbonate; sodium hydroxide and calcium hydroxide. The acids are usually added after pasteurization and before homogenization (if followed during the processing sequence). Acidification of spread with lactic acid results in improvement in body and tends to impart slightly tart flavour to a low-fat spread product.

The most widely used organic acid in food industry is citric acid which accounts for more than 60 per cent of all acidulants consumed. Citric acid has been found to be useful as an acidifying agent in low-fat spreads. Its major advantages include high solubility in water, appealing effects on flavour, and strong metal chelating properties. In processed cheese and cheese foods, citric acid plays a role in emulsification, buffering, flavour enhancement and texture development. In products like margarine, citric acid enhances the performance of microbial inhibitors. Another important property of citric acid in spread is that it acts as an antioxidant synergist. Addition of ascorbic acid to lower the pH to 6.5 would also help improve the nutritional value of spread.

7.0 Colourants

Colour is more important than flavour as it influences the first impression of the consumer about the product. Spread appears to be creamy white in colour unless the colour is added. Without proper colouring, the product appears lifeless and unattractive. To match the colour of butter, two types of colour, namely annatto and β-carotene are added in low-fat spreads. Other approved colours for use in the edible table spreads are turmeric, β-apo-8’ carotenal and its esters. Generally, oil soluble colouring material is used for w/o emulsion and water soluble colouring material for o/w emulsion. Correct amount of butter colour needs to be incorporated, as the presence of MSNF (milk solids-not-fat) complicates the attainment of the desirable shade.

Annatto colour is used in low-fat spreads at the rate of less than 0.01 to 0.3 per cent. A salient feature of β-carotene as a colourant is that besides being nutritionally important (as provitamin A), it enhances the oxidative stability of the fat products. The recommended level of addition of β-carotene is 4 ppm. Among several oil-soluble and
water-soluble colourings, a mixture of oil soluble butter annatto (0.15%) and \( \beta \)-carotene (10 mg/kg) impart an acceptable yellow shade in an o/w spread. The light reflectance characteristic of spread is different from that of a regular margarine because of the increased number of water droplets present. Therefore, it has been suggested that to obtain the same colour intensity in low-fat spreads the amount of colour to be added should be twice that of the quantities added in margarine.

8.0 Flavourings

In conjunction with texture or mouth-feel, flavour is another most important aspect of food. As low-fat dairy spread is a blend of different dairy and/or non-dairy ingredients, it may or may not have the desired flavour. It is thus essential that external flavourings are added to develop or impart the desired flavour. Several attempts have been made to simulate the butter-like flavour or cheese-like flavour in spreads. Cultured milks, cultured butter, ripened cream, starter distillate (1.0%), diacetyl (15 ppm) and Butterbuds 8X and Buterdol (0.23%) have been used in order to simulate the butter flavour in spreads.

Cultured milk if used as a source of desirable flavour is usually added at the end of pasteurization to reduce the loss of volatile flavour and aroma materials. A suitable dairy culture can be added at the level of 1-10 per cent in the spread. Flavour starter have been prepared by cultivation of mixed culture of lactic acid bacteria in a milk product enriched with whey protein and then added to a low-fat spread. Synthetic flavour blend like water-soluble ‘sweet cream buttery’ and fat soluble ‘lactic buttery’ flavours have also been added in low calorie spread.

A large number of butter flavour formulations have been developed which can successfully be used for flavouring butter-type spreads including margarine. Diacetyl is the most important of all compounds recognized to contribute to the butter flavour. Therefore, every flavouring composition meant for margarine and similar products invariably consists of diacetyl besides other components. Numerous studies have been conducted on the use of diacetyl as flavouring agent and all conclude that diacetyl alone imparts definite culture flavour, but gives an undesirable harsh flavour at higher concentration. Diacetyl has been used at the rate of 0.5-15 ppm

Various other forms of flavouring materials have been incorporated into spreads to enhance their acceptability. Due to consumers’ inclination towards natural flavouring materials, use of black pepper, garlic, onion and ginger etc. as flavouring materials have increased tremendously in recent years. They are being incorporated into spreads and other foods to which the Indian customer is accustomed. These flavouring agents include cocoa powder, vanillin, apricot, strawberry, banana, lemon or fruit juice and mint. Care should be however taken to ensure that flavour content and types are defined to produce oral response similar to full fat products because the higher emulsifier levels used for spreads can produce tighter emulsion, and gelling or thickening agents can affect how the flavour is perceived.

9.0 Salt

Sodium chloride added to spread not only provides taste and palatability but also helps retard the growth of bacteria and fungi in the spread, thereby acting as a preservative. Addition of 1-1.5 percent salt to spread results in inhibition of microorganisms, although the salt content in spread may vary widely. It has been suggested that low calorie spread must have higher salt content than normal margarine to obtain the same taste. The emulsion in the low calorie product is bound more strongly than in margarine and the salt percentage in the aqueous phase of the spread is three
to four times lower than in margarine with the same salt content.

Sodium chloride also plays a significant role in increasing the viscosity of the spread containing caseins. The probability exists that swelling and partial unfolding of caseinate aggregates accrue when NaCl concentration is high, thus increasing the voluminosity of the protein and hence the viscosity of the system. Studies in the past have suggested that as the salt content in the spread is increased from 1.2 to 2.8 per cent, the stability of emulsion in the product increases. At higher salt content (3%), fat and water separation in the product results while at 1.7 per cent salt level a stable emulsion with acceptable taste and spreadability is obtained.

10.0 Preservatives

The presence of protein and lactose (in butter serum or milk powders) in the aqueous phase of the high-moisture spreads, promotes bacterial growth thereby reducing shelf life. It is for this reason that preservatives are added to spreads and the cold chain maintained at <7°C to limit the microbial growth. Although heat treatment surely is adequate to kill all molds, these are observed not only in opened pack due to recontamination, but also in unopened packs. Use of bacterial and mold inhibitors such as preservatives in low-fat spread is therefore always necessary. Various preservatives are used in low-fat spread include sorbic acid; benzoic acid; salts of sodium, potassium and calcium; nisin; propionate etc., among which sorbic acid and potassium sorbate are well known chemical bench mark preservative and these are mostly used in w/o emulsion.

Sorbic acid is the only organic acid permitted as a food preservative and is most effective fat-soluble preservative. It has been used in the range of 0.03 – 0.1 per cent in various spread formulations. The salts of sorbic acid, especially the potassium salts have an important application due to their high solubility in water. Potassium sorbate is classified as non-toxic. The World Health Organization stipulates for sorbate an acceptable daily intake (ADI) at 25 mg/kg-body weight. Potassium sorbate acts not only as mold inhibitor but also as flavour releasing agent. Addition of potassium sorbate to the aqueous phase though less effective at pH 6.4 than at pH below 6 provides a significant measure of protection to the product. When sorbate is combined with sodium chloride or orthophosphate in acidic medium they inhibit the growth and toxin production by clostridium botulinum. Benzoic acid imparts maximum antimicrobial activity in the pH range 2.5 to 4 and is most effective against yeast and bacteria. The optimum level of sodium benzoate/propionate recommended in some studies is 0.1 per cent in low-fat spread.

11.0 Other Additives

11.1 Antioxidants

Antioxidants are not commonly employed in low-fat spreads for short term storage. However, use of certain antioxidants in spread making has been suggested to prevent fat oxidation. The antioxidant approved for use in edible table spreads (but not butter) are L-ascorbic acid, sodium and calcium ascorbate, ascorbyl palmitate, natural and synthetic tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Addition of non dihydroguaiaretic acid (NDGA) in a low -fat spread was found to help in maintaining its original flavour. BHA and BHT are readily soluble in fats and oil and insoluble in water. BHT is more effective than BHA in suppressing oxidation of animal fat. Addition of BHA significantly affects the pH, free fatty acids and peroxide value of low-fat spread.
11.2 Vitamins

Vitamins are organic compounds that are essential in very small amounts for the maintenance of life in both humans and animals. Vitamins A, C, D and E are permitted in table spreads. Addition of vitamin A and D is most common. Vitamin A (β-carotene) is added to edible table spreads primarily as a colouring agent. Vitamin D may be added to table spreads primarily to enhance the nutritional content of product for infants, children, pregnant and lactating women and elders. These are added in low-fat spread to enhance its nutritional and physiological value. Some studies have specified the level of addition of vitamin A and D at 2700 and 900 units, respectively.

12.0 Suggested Readings


1.0 Introduction

The use of enzymes is one of the oldest applications in food processing. For many thousands of years, man has used naturally occurring micro-organisms - bacteria, yeasts and molds - and the enzymes they produce to make foods such as bread, cheese, beer and wine. Today, enzymes are used for an increasing range of applications: bakery, cheese making, starch processing and production of fruit juices and other drinks. They can improve texture, appearance and nutritional value and may generate desirable flavours and aromas. Currently-used food enzymes sometimes originate in animals and plants (for example, a starch-digesting enzyme, amylase, can be obtained from germinating barley seeds) but most come from a range of beneficial micro-organisms.

Enzymes are catalytic proteins that offer several advantages over chemicals including the ability to tolerate a wide range of raw materials, variations in processing conditions, pH, temperature and cost. They are specific in their action on substrates. They safely and effectively enable food technologists to selectively modify carbohydrates, proteins and lipids in complex food systems to create foods that taste good, improve health and are safe to consume. Processes which use enzymes therefore have fewer side reactions and waste by-products, giving higher quality products and reducing the likelihood of pollution. They also allow some processes to be carried out which would otherwise be impossible. An example is the production of clear apple juice concentrate, which relies on the use of the enzyme, pectinase.

Since the early 1980s, companies which produce enzymes have been using genetic engineering techniques to improve production efficiency and quality and to develop new products. The first commercial food product produced by biotechnology was an enzyme used in cheese making. There are clear advantages here for both industry and consumers, with major improvements in enzyme production giving better products and processes. However, progress is being slowed down because the debate on some other, more controversial applications of biotechnology - such as genetic engineering in animals - is continuing throughout Europe.

At present, modern biotechnology can be used to give a range of advances in enzymatic production technology:

- Improved productivity and cost-effectiveness in existing processes. By producing enzymes more efficiently, the amount of raw materials, energy and water needed to make a product can be reduced by as much as one-half by changing from a traditional strain of microbe to a genetically modified one.
Companies can tailor their enzymes more precisely to customer demands for products with specific properties.

Manufacturers can supply enzymes which otherwise could not be produced in large enough quantities, giving the consumer access to a wider variety of products.

Table 1 and 2 detail the respective outlines of a short history of the evolution of enzymes and their applications in food processing.

2.0 Enzymes in Baking

Enzymes are rapidly becoming very important to the baking industry. They are used in baking to make consistently high-quality products by enabling better dough handling, providing anti-staling properties, and allowing control over crumb texture and colour, taste, moisture, and volume.

Most bread is made of wheat, which has naturally occurring enzymes that, when water is added, modify the starch, protein and fiber in wheat. The yeast that is added produces carbon dioxide from simple sugars, which makes the bread rise. However, the quality of wheat flour varies due to natural variation, the time of year it is picked, disparities in milling and many other inconsistencies.

Gluten is the viscous and elastic network of wheat protein that gives bread its unique consistency and holds the carbon dioxide that makes the bread rise. The reason wheat is the primary grain used in baking is because of the special gluten that is not found in other grain such as barley and rye, which produce denser and harder breads. Often, enzymes are added to these other grains to weaken their gluten and produce biscuits, crackers, cookies and other crisp bread dough.

Enzymes are now replacing chemical supplements that were used in the baking industry to account for these discrepancies. For instance, enzymes can replace chemical oxidants, such as bromates, used to strengthen gluten.

3.0 Enzymes in Brewing

Enzymes have proved to be useful for the brewing industry in many areas of beer production. Beer brewing involves the production of alcohol by allowing yeast to act on plant materials such as barley, maize, sorghum, hops and rice. Yeast converts simple sugars into alcohol and carbon dioxide. However, the sugars found in the plant materials are most often complex polysaccharides that yeast is unable to convert. The traditional method for breaking down these complex polysaccharides is called malting. This is the process whereby barley, for example, is allowed to partially germinate, producing enzymes that break down the complex polysaccharides into simple sugars that the yeast can utilize. However, the process of malting can be expensive and often difficult to control.

By adding enzymes to unmalted barley the complex polysaccharides can be broken down to simple sugars and reduce or eliminate the costly and complicated process of malting. They can be added to the beer after its fermentation to induce faster maturation. Enzymes also work as filtration improvers, reducing the presence of viscous polysaccharides such as xylans and glucans. Enzymes are often used to remove carbohydrates in the production of light beer and to induce chill proofing. But most importantly, enzyme supplementation is simpler and less expensive than the malting process.
4.0 Enzymes in Dairy Processing

Not only are enzymes naturally present in milk, but they are also used to enhance numerous aspects of dairy production. Enzymes can substitute animal-derived microbial rennets, enhance the dairy flavour in various foods and accelerate the ripening of naturally aged cheeses.

Microbial rennets used in cheese production are effective substitute for calf/cow, goat, sheep or swine-derived rennets. When milk is treated with milk-clotting proteases or microbial proteases, it causes casein to precipitate in the form of curd. The curd is separated from other milk ingredients (whey) and further processed to produce soft and hard cheeses. Microbial lipases and proteases can be cost-effectively added to fresh milk or cheese curd to produce products with enhanced dairy and cheese flavours. Certain fungal lipases are used to enhance the dairy flavour in coffee whiteners, margarine and snack foods. These enzyme blends also accelerate the ripening of naturally aged cheeses and produce intensely flavoured, enzyme-modified cheeses (EMC) used as a flavour base in snack foods, soups, frozen dinners and cheese-sauces.

Protease enzymes also play an important role in cheese ripening and flavour development. The production of strong cheese flavours is enhanced when fungal lipases and proteases are added to fresh cheese curd. It is possible develop a variety of intense cheese flavour profiles in a few hours or days when using these protease and lipase enzymes together.

Milk and whey ultra-filtration systems become increasingly more costly as more time is spent on cleaning the filter. In addition the detergents used to clean the filter often include highly alkaline solutions that add to plant waste effluent load and treatment costs. Certain specialty enzymes are commercially available, which are to be added with mild detergents and pH-adjusting agents. These solutions will help remove milk protein and fat deposits from the filter, thereby increasing filtration rates without using the harsh chemicals that add to waste effluent treatment costs.

5.0 Enzymes in Juice Industry

Enzymes increase processing capacity and improve economy in the fruit juice and wine industries. The most commonly used enzymes in these industries are pectinases. Pectinases increase juice yields and accelerate juice clarification. They produce clear and stable single-strength juices, juice concentrates and wines, from not only core-fruits such as apples and pears, but also stone fruits, berries, grapes, citrusfruits, tropical fruits and vegetables like carrots, beets and green peppers.

Some pectinases that contain hemicellulase enzyme activities not only increase juice yields, but also increase the colour and health-promoting antioxidants in fruit and vegetable juices extracted by pressing or decanter centrifuge. By reducing fruit and vegetable mash viscosity and improving solid/liquid separation, they increase colour extraction and juice volume by as much as 15 per cent.

Certain other enzymatic preparations speed up filtration and prevent storage or post-packaging haze formation by depectinizing and reducing starch in raw juices. Pectin and starch must be removed from freshly extracted juices prior to filtration, fining and concentration. Unripe fruits and vegetables can contain as much as 15 per cent starch. Pectinases and amylases can reduce starch and pectin in raw fruits and juices, thus achieving clear and stable juices and juice concentrates.
6.0 Enzymes in Wine Making

Many of the biochemical reactions involved in wine production are enzyme-catalyzed. They begin during the ripening and harvesting of grapes and continue through alcoholic and malolactic fermentation, clarification and ageing. Winemakers often supplement naturally occurring grape enzymes with commercial enzymes to increase production capacity of clear and stable wines with enhanced body, flavour and bouquet.

When added to grapes or musts, these products increase free-run juice volume and extraction of colour, fermentable sugars and flavour components, as well as reduce pressing and fermentation time. These pectinase or pectinase-containing hemicellulase products can increase free-run juice volume by 20 to 30 per cent and lower fermentation time by 30 to 50 per cent by reducing grape-pectin viscosity.

Rapid clarification and well-separated lees have a positive effect on finished wine flavour, texture and colour. Pectinases that depectinize grape-musts during fermentation or young wines prior to fining and filtration are commercially available. Grape musts and wines treated with these enzyme preparations are less viscous. They ferment, settle and mature more quickly.

A beta-glucanase-containing pectinase, is also used to degrade Botrytis-glucan. Wines made from overripe grapes infected with Botrytis cinerea mold are often difficult to clarify and filter due to high concentrations of Botrytis-produced glucan polysaccharides. The use of the enzyme can speed up clarification and filtration.

Some acid proteases clarify and stabilize certain wines by reducing or removing naturally occurring and yeast-synthesized, heat-labile proteins.

7.0 Enzymes as Food Processing Aids

Enzymes have many applications in modern food processing to improve the physical and functional properties of a variety of processed foods. A wide range of protease enzyme products used to produce food-protein hydrolysates with reduced viscosity, improved solubility, flavour, nutritional and emulsifying characteristics are now available in the market. Certain proteases rapidly hydrolyze both animal and plant proteins to reduce or eliminate gel formation on heating. They are used to hydrolyze soy, pea, yeast, maize and wheat-flour proteins to produce non-bitter, protein hydrolysates. Pre-processed or separated plant and milk proteins such as soy isolates and concentrates, casein and whey can be easily hydrolyzed to produce non-bitter protein hydrolysates suitable for use as active ingredients in dietary supplements, functional beverages, fermented, baked and other foods. Certain other food processing protease enzyme products are also available as meat tenderizers and to hydrolyze meat and fish proteins to produce non-bitter, protein hydrolysates.

Some other proteases break down and increase the solubility, dispersibility and digestibility of proteins. They also hydrolyze vegetable and animal proteins to peptides, polypeptides and small amounts of amino acids.

Enzymes are used for extraction and liquefaction of plant materials and for downstream processing in the food, starch, alcohol and brewing industries. In the food industry, these products are used to improve the processing and extraction of wheat and corn gluten, soybean protein, cereal starch, seed and nut oils, flavours and colours, coffee, tea and other botanical extracts.
8.0 Miscellaneous Applications in Foods

A World Health Organization study in 2004 reported that 300 million people across the globe are clinically obese. Experts suggest that for such consumers, the food industry should concentrate more on food products and beverages that are less energy-dense. Fueled by the success of the Atkins and other related low-carbohydrate diets, millions of people are now restricting their intake of carbohydrates. The food industry is also rapidly reformulating its products to better address the needs of these consumers. Enzyme preparations that reduce the carbohydrate levels are now marketed as processing aids. One example is that of glucoamylases, that can be used to produce low calorie and low carbohydrate beers.

Another focus in the future will be on developing products that will enhance and preserve the quality and safety of processed foods. Enzymes and various antimicrobial peptides have been effectively used to reduce pathogens in foods and to extend the shelf life of many perishable foods. For example, glucose oxidase and catalase function together as a very powerful oxygen-scavenging system in packaged foods and beverages. When sufficient glucose levels are present, glucose oxidase and catalase remove all of the available oxygen from an enclosed system, thereby preventing the flavour and colour defects associated with oxidation and retarding spoilage from mold growth.

9.0 Conclusion

Enzyme technology and utilization is an expensive venture. Very often it becomes difficult to justify the costs involved in enzymatic processes. The need to contain the cost is much more important in the food industry than, for example, in the pharmaceutical industry. This becomes manifest when we compare the added value per kilogram of a food product with the added value of a drug. The basis for the production and commercialization of an enzyme at competitive price thus lies in achieving a product of the desired quality at acceptable cost of the final product. This is the direction the search for source organisms for the production of industrial enzymes is taking. As the application of industrial enzymes in food processing requires minimum cost at maximum safety, this search will probably remain restricted to already producing GRAS (Generally Recognized As Safe) organisms. On the other hand, if the purpose were only to find an enzyme with new properties with no concern for the cost, the search should be as extensive as possible.

10.0 Suggested Readings


Valyasevi, R. and Rolle, R.S. 2002. An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: scope for their improvement. 75(3): 231-239


www.anbio.org.br

www.eufic.org/it/tech/tech01e.htm

www.pro-bio-faraday.com
Table 1: A Short History of Enzymes

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 BC</td>
<td>The Egyptians and Sumerians developed fermentation for use in brewing, bread-baking and cheese-making.</td>
</tr>
<tr>
<td>800 BC</td>
<td>Calves’ stomachs and the enzyme, chymosin, were used for cheese-making.</td>
</tr>
<tr>
<td>1878</td>
<td>The components of yeast cells which cause fermentation were identified and the term &quot;enzyme&quot; was first used, derived from the Greek term meaning &quot;in yeast&quot;.</td>
</tr>
<tr>
<td>1926</td>
<td>Enzymes were first shown to be proteins.</td>
</tr>
<tr>
<td>1980s</td>
<td>Enzyme preparations were developed to improve the digestibility and nutrient-availability of certain animal feeds.</td>
</tr>
<tr>
<td>1982</td>
<td>The first food application of a product of gene technology, alpha-amylase, took place.</td>
</tr>
<tr>
<td>1988</td>
<td>Recombinant chymosin was approved and introduced in Switzerland, marking an early approval of a product of gene technology for a food use.</td>
</tr>
<tr>
<td>1990</td>
<td>Two food processing aids obtained using gene technology: an enzyme for use in cheese-making in the US, and a yeast used in baking in the UK.</td>
</tr>
</tbody>
</table>

Adapted from: [http://www.eufic.org/it](http://www.eufic.org/it)
<table>
<thead>
<tr>
<th>Area</th>
<th>Enzyme</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>Rennet (protease)</td>
<td>Coagulant in cheese production</td>
</tr>
<tr>
<td></td>
<td>Lactase</td>
<td>Hydrolysis of lactose to give lactose-free milk products</td>
</tr>
<tr>
<td></td>
<td>Protease</td>
<td>Hydrolysis of whey proteins</td>
</tr>
<tr>
<td></td>
<td>Catalases</td>
<td>Removal of hydrogen peroxide</td>
</tr>
<tr>
<td>Brewing</td>
<td>Cellulases, beta-glucanases, alpha amylases, proteases, maltogenic amylases</td>
<td>For liquefaction, clarification and to supplement malt enzymes</td>
</tr>
<tr>
<td>Alcohol production</td>
<td>Amyloglucosidase</td>
<td>Conversion of starch to sugar</td>
</tr>
<tr>
<td>Baking</td>
<td>Alpha-amylases</td>
<td>Breakdown of starch, maltose production</td>
</tr>
<tr>
<td></td>
<td>Amyloglycosidases</td>
<td>Saccharification</td>
</tr>
<tr>
<td></td>
<td>Maltogen amylase (Novamyl)</td>
<td>Delays process by which bread becomes stale</td>
</tr>
<tr>
<td></td>
<td>Protease</td>
<td>Breakdown of proteins</td>
</tr>
<tr>
<td>Wine and fruit juice</td>
<td>Pectinase</td>
<td>Increase of yield and juice clarification</td>
</tr>
<tr>
<td></td>
<td>Glucose oxidase</td>
<td>Oxygen removal</td>
</tr>
<tr>
<td></td>
<td>Beta-glucanases</td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>Protease</td>
<td>Meat tenderising</td>
</tr>
<tr>
<td></td>
<td>Papain</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Proteases, trypsin, aminopeptidases</td>
<td>Breakdown of various components</td>
</tr>
<tr>
<td>Starch</td>
<td>Alpha amylase, glucoamylases, hemicellulases, maltogenic amylases, glucose isomerases</td>
<td>Modification and conversion (eg. to dextrose or high fructose syrups)</td>
</tr>
<tr>
<td></td>
<td>dextranases, beta-glucanases</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>Inulinases</td>
<td>Production of fructose syrups</td>
</tr>
</tbody>
</table>

Adapted from: http://www.eufic.org/it
Table 3. Some Enzymes that are Permitted Food Additives

<table>
<thead>
<tr>
<th>No.</th>
<th>Additive</th>
<th>Permitted source</th>
<th>Permitted in/ upon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetolactate decarboxylase</td>
<td><em>Bacillus subtilis</em> ToC46 (pUW235)</td>
<td>Brewers' Mash, Distillers' Mash</td>
</tr>
<tr>
<td>2</td>
<td>Aminopeptidase</td>
<td><em>Lactococcus lactis</em></td>
<td>Cheese, Dairy based flavouring preparations, Hydrolyzed animal, milk and vegetable protein</td>
</tr>
<tr>
<td>3</td>
<td>Amylase</td>
<td><em>Aspergillus niger</em> var.; <em>Aspergillus oryzae</em> var.; <em>Bacillus subtilis</em> var.; <em>Rhizopus oryzae</em> var.; Barley Malt</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout Bread, Flour, whole wheat flour, Cider; Wine, Chocolate syrups, Distillers' Mash, Malt-flavoured dry breakfast cereals, Single-strength fruit juices, Precooked (instant) cereals, Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup) or glucose solids (dried glucose syrup), Unstandardized bakery products</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus licheniformis</em>; <em>Bacillus licheniformis</em> LA 57 (pDN1981); <em>Bacillus licheniformis</em> PL 1303 (pPL1117)</td>
<td>Distillers' Mash, Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup) or glucose solids (dried glucose syrup), Brewers' mash</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus stearothermophilus</em></td>
<td>Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup) or glucose solids (dried glucose syrup), Distillers' Mash, Brewers' Mash, Bread, Flour, Whole wheat flour, Unstandardized bakery products</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus subtilis</em> B1.109 (pCPC720) (ATCC 39, 705)</td>
<td>Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup) or glucose solids (dried glucose syrup)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus subtilis</em> B1.109 (pCPC800)</td>
<td>Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup) or glucose solids (dried glucose syrup), Distillers' Mash, Brewers' Mash, Bakery Products</td>
</tr>
<tr>
<td>4</td>
<td>Amylase (maltogenic)</td>
<td><em>Bacillus subtilis</em> DN1413 (pDN1413)</td>
<td>Starch used in the production of dextrins, maltose, dextrose, glucose, (glucose syrup) or glucose solids (dried glucose syrup), Bread, Flour, Whole wheat flour, Unstandardized bakery products</td>
</tr>
<tr>
<td>No.</td>
<td>Enzyme</td>
<td>Source and Description</td>
<td>Uses</td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>5</td>
<td>Bovine Rennet</td>
<td>Aqueous extracts from the fourth stomach of adult bovine animals, sheep and goats</td>
<td>Cheese, cottage cheese, cream cheese, cream cheese with (named added ingredients), cream cheese spread, cream cheese spread with (named added ingredients)</td>
</tr>
<tr>
<td>6</td>
<td>Bromelain</td>
<td>The pineapples <em>Ananas comosus</em> and <em>Ananas bracteatus</em></td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Bread, Flour, Whole wheat flour, Sausage casings, Hydrolyzed animal, milk and vegetable protein, Meat cuts, Meat tenderizing preparations, Pumping pickle for the curing of beef cuts, Sugar wafers, waffles, pancakes</td>
</tr>
<tr>
<td>7</td>
<td>Catalase</td>
<td><em>Aspergillus niger</em> var.; <em>Micrococcus lysodeikticus</em>; Bovine (<em>Bos taurus</em>) liver</td>
<td>Soft drinks, Egg albumen, Liquid whey treated with hydrogen peroxide</td>
</tr>
<tr>
<td>8</td>
<td>Cellulase</td>
<td><em>Aspergillus niger</em> var.</td>
<td>Distillers' Mash, Liquid coffee concentrate, Spice extracts, Natural flavour and colour extractives</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Trichoderma reesei</em> QM 9414</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Chymosin A</td>
<td><em>Escherichia coli</em> K-12, GE81 (pPFZ87A)</td>
<td>Cheeses, Unstandardized milk-based dessert preparations</td>
</tr>
<tr>
<td></td>
<td>Chymosin B</td>
<td><em>Aspergillus niger</em> var. <em>awamori</em>, GCC0349 (pGAMpR); <em>Kluyveromyces marxianus</em> var. lactis, DS1182 (pKS105)</td>
<td>Cheeses, Cream cheese spread, Sour cream, Unstandardized milk-based dessert preparations</td>
</tr>
<tr>
<td>10</td>
<td>Ficin</td>
<td>Latex of fig tree (<em>Ficus</em> sp.)</td>
<td>Ale; Beer, Light beer, Malt liquor, Porter, Stout, Sausage casings, Hydrolyzed animal, milk and vegetable protein, Meat cuts, Meat tenderizing preparations, Pumping pickle for the curing of beef cuts</td>
</tr>
<tr>
<td>11</td>
<td>Glucoamylase</td>
<td><em>Aspergillus niger</em> var.; <em>Aspergillus oryzae</em> var.; <em>Rhizopus oryzae</em> var.</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Bread, Flour, Whole wheat flour, Chocolate syrups, Distillers' Mash, Precooked (instant) cereals, Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup), or glucose solids (dried glucose syrup), Unstandardized bakery products</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Strain and Species</td>
<td>Applications</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Glucanase</td>
<td>Aspergillus niger var.; Bacillus subtilis var.</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Distillers’ Mash, Mash destined for vinegar manufacture, Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup), or glucose solids (dried glucose syrup)</td>
<td></td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>Humicola insolens var.</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Distillers’ Mash</td>
<td></td>
</tr>
<tr>
<td>Glucose Isomerase</td>
<td>Bacillus coagulans var.; Streptomyces olivochromogenes var.; Acinoplanes missouriensis var.; Streptomyces olivaceus var.; Microbacterium arborescens NRRL B-11022; Streptomyces murinus DSM 3252; Streptomyces rubiginosus ATCC No. 21,175</td>
<td>Glucose (glucose syrup) to be partially or completely isomerized to fructose</td>
<td></td>
</tr>
<tr>
<td>Hemicellulase</td>
<td>Bacillus subtilis var.</td>
<td>Distillers’ Mash, Liquid coffee concentrate, Mash destined for vinegar manufacture</td>
<td></td>
</tr>
<tr>
<td>Inulinase</td>
<td>Aspergillus niger var. Tieghem</td>
<td>Inulin</td>
<td></td>
</tr>
<tr>
<td>Invertase</td>
<td>Saccharomycetes sp.</td>
<td>Soft-centered and liquid-centered confections, Unstandardized bakery foods</td>
<td></td>
</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>Products</td>
<td>Applications</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>----------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>Lipase</strong></td>
<td>Aspergillus niger var.; Aspergillus oryzae var.; Edible forestomach tissue of calves, kids or lambs; Animal pancreatic tissue; Rhizopus oryzae var.</td>
<td>Lactose-reducing enzyme preparations, Milk destined for use in ice cream mix, Flour, whole wheat flour, bread, flavoured (naming the flavour) milk, skim milk, partly skimmed milk, malted milk, skim milk with added milk solids, partly skimmed milk with added milk solids</td>
<td>Dairy based flavouring preparations, Liquid and dried egg white (liquid and dried albumen), Ripened cheeses, Bread, Flour, Unstandardized bakery products, Hydrolyzed animal, milk and vegetable protein</td>
</tr>
<tr>
<td></td>
<td>Aspergillus oryzae (MLT-2) (pRML 787) (p3SR2); Mucor miehei (Cooney and Emerson); Rhizopus niveus</td>
<td>Modified triglycerides, Cheese, Dairy based flavouring preparations, Hydrolyzed animal, milk and vegetable protein</td>
<td></td>
</tr>
<tr>
<td><strong>Lipoxidase</strong></td>
<td>Soybean whey or meal</td>
<td>Bread, Flour, Whole wheat flour</td>
<td></td>
</tr>
<tr>
<td><strong>Lysozyme</strong></td>
<td>Egg white</td>
<td>Cheese</td>
<td></td>
</tr>
<tr>
<td><strong>Milk coagulating enzyme</strong></td>
<td>Mucor miehei (Cooney and Emerson) or Mucor pusillus Lindt by pure culture fermentation process or Aspergillus oryzae RET-1 (pBoel777)</td>
<td>Cheeses, Sour cream, Dairy based flavouring preparations, Hydrolyzed animal, milk and vegetable protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endothia parasitica by pure culture fermentation processes</td>
<td>Cheeses (Swiss, Parmesan, Romano, Mozzarella)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Pancreatin</td>
<td>Pancreas of the hog (<em>Sus scrofa</em>) or ox (<em>Bos taurus</em>)</td>
<td>Liquid and dried egg white (liquid and dried albumen), Precooked (instant) cereals, Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup), or glucose solids (dried glucose syrup), Hydrolyzed animal, milk and vegetable proteins</td>
</tr>
<tr>
<td>23</td>
<td>Papain</td>
<td>Fruit of the papaya <em>Carica papaya</em> L. (Fam. <em>Caricaceae</em>)</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Beef before slaughter, Sausage casings; Water-soluble edible collagen films, Hydrolyzed animal, milk and vegetable protein, Meat cuts, Meat tenderizing preparations, Precooked (instant) cereals, Pumping pickle for the curing of beef cuts, Unstandardized bakery products,</td>
</tr>
<tr>
<td>24</td>
<td>Pectinase</td>
<td><em>Aspergillus niger</em> var.; <em>Rhizopus oryzae</em> var.</td>
<td>Cider, Wine, Distillers' Mash, Single-strength fruit juices, Natural flavour and colour extractives, Skins of citrus fruits destined for jam, marmalade and candied fruit production, Vegetable stock for use in soups, Tea leaves for the production of tea solids</td>
</tr>
<tr>
<td>25</td>
<td>Pentosanase</td>
<td><em>Aspergillus niger</em> var.; <em>Bacillus subtilis</em> var.</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Corn for degeneration, Distillers' Mash, Mash destined for vinegar manufacture, Unstandardized bakery products, Bread; Flour; Whole wheat flour</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Trichoderma reesei</em> (QM9414)</td>
<td>Bread; Flour; Whole wheat flour, Distiller's Mash, Unstandardized bakery products</td>
</tr>
<tr>
<td>26</td>
<td>Pepsin</td>
<td>Glandular layer of porcine stomach</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Cheeses, Defatted soy flour, Precooked (instant) cereals, Hydrolyzed animal, milk and vegetable proteins</td>
</tr>
<tr>
<td>27</td>
<td>Protease</td>
<td><em>Aspergillus oryzae</em> var.; <em>Aspergillus niger</em> var.; <em>Bacillus subtilis</em> var.</td>
<td>Ale, Beer, Light beer, Malt liquor, Porter, Stout, Bread, Flour, Whole wheat flour, Dairy based flavouring, preparations, Distillers' Mash, Sausage casings, Hydrolyzed animal, milk and vegetable protein, Industrial spray-dried cheese powder, Meat cuts, Meat tenderizing preparations, Precooked (instant) cereals, Unstandardized bakery foods, cheeses</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Micrococcus caseolyticus</em> var.</td>
<td>Hard or semihard, pressed and soft variety of cheeses</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus licheniformis</em> (Cx)</td>
<td>Hydrolyzed animal, milk and vegetable protein</td>
</tr>
<tr>
<td>No.</td>
<td>Enzyme</td>
<td>Organism/Strain</td>
<td>Applications</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>28</td>
<td>Pullulanase</td>
<td><em>Bacillus acidopullulyticus</em> NCIB 11647; <em>Bacillus licheniformis</em> SE2-Pul-int211 (pUBCDEBRA11DNSI)</td>
<td>Starch used in the production of dextrins, maltose, dextrose, glucose (glucose syrup), glucose solids (dried glucose syrup or fructose syrups and solids, Bread; Flour; Whole wheat flour, Unstandardized bakery products</td>
</tr>
<tr>
<td>29</td>
<td>Rennet #</td>
<td>Aqueous extracts from fourth stomach of calves, kids, or lambs</td>
<td>Cheeses, sour cream, Unstandardized milk based dessert preparations</td>
</tr>
<tr>
<td>30</td>
<td>Transglutaminase</td>
<td><em>Streptoverticillium mobaraense</em> strain S-8112</td>
<td>Prepared fish products (except for any of these products for which standards are set out in these Regulations), Simulated meat products, Cheese products, Processed cheese products, Cream cheese products, Yogurt, Frozen dairy desserts</td>
</tr>
<tr>
<td>31</td>
<td>Trypsin</td>
<td>Pancreas of the hog (<em>Sus scrofa</em>)</td>
<td>Hydrolyzed animal, milk and vegetable proteins</td>
</tr>
<tr>
<td>32</td>
<td>Xylanase</td>
<td><em>Aspergillus oryzae</em> Fa 1-1 (pA2X1TI)</td>
<td>Bread; Flour; Whole wheat flour</td>
</tr>
</tbody>
</table>


* Maximum level of use as per Good Manufacturing Practices

# Not legally permitted in India.
1.0 Introduction

Essential for the manufacture of ice cream of highest quality are ingredients of excellent quality, a mix that is formulated and balanced to provide proper function of each component, and intelligent processing, freezing and hardening of the product. However, the selection of excellent ingredients is, without doubt, the most important factor in successful manufacture of frozen desserts. Ice cream contains 35-42 percent total solids (TS), which comprises 10-16 percent milk fat, 9-12 percent milk solid not fat (MSNF), 14-20 percent sugars and 0.3-0.5 percent stabilizers and emulsifiers. The final product is a complex structured mixture comprising ~ 25 percent ice, ~ 50 percent air, 5 percent fat and ~20 percent unfrozen serum.

2.0 Role of Ingredients in Ice Cream

The properties and stability of ice cream are influenced by the characteristics of the ingredients and their interaction with other components in the mix. Some of the common dairy ingredients traditionally used in ice creams include whole, concentrated or evaporated milk, cream, anhydrous milk fat, butter, sweetened condensed milk, skim milk powder, and whey protein. Many newer specialized ingredients for ice cream are now being used. A range of sugars and sweeteners, stabilizers, emulsifiers and mineral salts are also used in ice cream formulations.

Although the ingredients of ice cream must supply minimal amount of milk solids and food solids per unit of volume, the source of milk solids can vary widely. For example, ice cream can be made using highly perishable cream and concentrated milk or from highly stable butter or anhydrous milk fat plus non-fat dry milk. Of course, these and other forms of milk solids can be mixed in numerous combinations. Likewise, numerous types of sweeteners are available to suit specific product profiles. Ingredients for stabilization and emulsification are several, and formulas vary in proportions for each as well as in total concentrations.

2.1 Milk Fat

Milk fat contributes significantly to the rich, full and creamy flavour and to the smooth texture of the ice cream. The best source of milk fat for ice cream mix is fresh cream from fresh milk. If the quality is good, free from rancidity and oxidation cream
provides the best flavour of all the milk fat – based ingredients. Milk fat influences the textural and flavour properties of ice cream. The content of fat, its solid fat content, the fat globule size and the properties of its surface all affect the properties of ice cream. A network of fat is required for the formation of structured ice cream and is crucial for the resistance of the ice cream to heat shock and melting. This network is formed by partially coalesced fat which results from the destabilization of fat globules. The fat network is generally considered to be essential for maintaining the structure of ice cream as it stabilizes air bubbles and the foam structure of the ice cream (Udabage and Augustin, 2003). The melt resistance of ice cream increases with increasing level of fat in the formulation (Campbell and Pelan, 1997). In addition, an increase in the fat content improved the resistance of the ice cream to heat shock, an effect that might have been expected considering the contribution of fat in the development of the structure of ice cream (Prindiville et. al. 1999).

2.2 Milk, Skim Milk and Buttermilk

Fresh milk or skim milk should be mixed in the mix whenever available at reasonable prices, because it is an economical and high quality source of NMS/MSNF and provides the source of water in the mix necessary for balancing the formulation. Fat present in whole milk must be accounted for when determining the required amount of concentrated milk fat source. Fresh milk or skim milk must have a low titrable acidity, a low bacterial count and a clean flavour.

Sweet cream butter milk (SCBM) is obtained by churning cream that has no developed detectable acidity, i.e. cream that is of quality suitable for use in any retail product. Buttermilk solids can be a substitute for NMS, especially in mixes made with butter, butter oil, or anhydrous milk fat as a source of fat. Buttermilk contains a high concentration of fat globule membrane phospholipids than does skim milk. Thus, it reduces the need for emulsifiers in regular mixes and is desired in mixes containing no emulsifiers. Sweet cream buttermilk has the beneficial effects on the whipping ability of mixes and contributes to richness of flavour. Buttermilk is especially desirable in ice cream made without added emulsifiers, low in fat content or with any form of de-emulsified milk fat.

2.3 Concentrated Milks

Nonfat milk solids (NMS) contributes significantly to the flavour and texture of ice cream. All formulations must include a source of NMS, usually either concentrated or dried milk sources, to reach sufficient levels in NMS have an indirect effect on flavour. The non-fat portion of milk is composed of approximately 55 percent lactose, 37 percent protein, 8 percent minerals, and small amount of vitamins, acids and enzymes. The amount of NMS in ice cream mixes ranges from 6 to 14 percent, and usually varies inversely with the fat content. The proteins help to give body and a smooth texture to the ice cream. Lactose displaces water and adds to the sweetness, produced largely by the added sugars. The mineral salts carry a slightly salty flavour that rounds out the finished flavour of ice cream (Marshall et. al., 2003).

2.4 Whey Products

There is a growing interest in the replacement of traditional dairy ingredients, such as fresh milk or whole milk, with milk powders and whey based ingredients with varying degree of lactose hydrolysis in ice cream formulations. The ratio of major
components is significantly affected by the addition of whey solids or their substitution for NMS in formula. Whey solids (WS) characteristically contain about 77 percent lactose, 13.4 percent protein, 8.6 percent minerals, and 1 percent fat. Compared with NMS in the formula, the higher lactose content in whey solids increases the potential for crystallization of this sugar in the frozen product. Furthermore, the comparatively high concentration of lactose and minerals cause the freezing point depression. Finally, protein content is decreased. These are major reasons for the limitation of only 25 percent substitution of whey solids for NMS. These problems can be solved by using de-lactosed and de-mineralized whey solids, as found in whey protein isolates. These ingredient substitutions are aimed at reducing the cost of production and increasing the ease of handling and storage of raw materials, while maintaining and improving the characteristics of ice cream that is produced (Westerbeek, 1996). The limitations for incorporation of high levels of whey powders in ice cream stem from their higher level of lactose compared to skim milk powder and flavour of whey. Huse et al. (1984) demonstrated that acceptable ice creams could be made with 50 per cent substitution of skim milk with whey solids and that hydrolysis of the lactose in whey solids improved the texture of ice cream.

2.5 Mineral Salts

Various mineral salts have been used to help controlled churning and separation of fat in mixes during freezing and increase the stiffness and smoothness of finished ice cream. For example, calcium sulphate, added at the rate of 0.1 percent before pasteurization, produces a dry, stiff ice cream from the freezer and reduces the rate of melting.

The mineral salts, used at concentrations of 0.1 and 0.2 percent, were blended with the stabilizer before mix preparation. When ice cream samples containing mineral salts were compared to the controlled ice cream, it was found that ice cream containing one of the salt shows less fat destabilization than the control sample drawn after the same length of time in the freezer (Marshall et al., 2003).

2.6 Non-Dairy Fats

Vegetables fat are used extensively as fat source in ice cream in United Kingdom, parts of Europe, Asia and Latin America. As far as Indian market is concerned, under the PFA rule no fat except milk fat is permitted in ice cream. Five factors of great interest in selection of fat source are: the crystal structure of fat; the rate at which fat crystallizes; the temperature dependent melting profile of the fat, especially at refrigerator and freezer temperatures; the content of high melting triglycerides, which can produce a waxy, greasy mouth feel; and the flavour and purity of the oil.

For optimal partial coalescence during freezing it is important that the fat droplets contain an intermediate ratio of liquid : solid fat at the time of freezing. Blends of oil often used in ice cream manufacture are selected to take into account such physical characteristics such as flavour, availability, stability during storage, and of course cost of the final product. Hydrogenation is often necessary to achieve the appropriate melting characteristics. Palm kernel oil, peanut oil, and fractions thereof, with varying degrees of hydrogenation, are all used to some extent.
2.7 Fat Replacers

Concerns about the impact of diet on health have led consumers to reduce consumption of high fat foods, and "lite" food products have entered the lifestyle of most people. There has been great interest in the market place for the development of lower fat alternatives to traditional ice cream products. As a result, a large amount of product development time has been used in searching for a combination of ingredients that will replace the texture and flavor characteristics of fat in the ice cream (Ohmes et. al., 1998, Aime et. al., 2001) and these often involved the use of fat replacers. High quality frozen desserts containing 5-6 per cent fat have been produced without fat replacers, but mixes containing less than 4-5 per cent fat usually require additional ingredients specifically chosen for their fat replacing properties. Fat replacers are mostly carbohydrate, protein or lipid based. Their main requirements are to provide fewer calories to the product than do traditional fat sources, either through reduction of required weight in the mix or through caloric reductions per unit weight of ingredient. Ice cream products are very complex systems, both in structure and flavour, and fat contributes greatly to both. In creating products that are meant to deliver to the consumer the same attributes as in ice cream but with less fat or calories, it is imperative that structural contribution of fat be considered to the same extent, as is flavour. The goal is to deliver high quality products and develop lasting market share for these products.

Fat replacers could be lipid based, protein based or carbohydrate based. The fat based replacers currently available are monoglycerides, structured lipids and sugar fatty acidpolyesters. Protein based fat replacers contain microparticulated protein particles that stimulates colloidal fat. There are wide range of carbohydrate - based fat replacers including those derived from maltodextrins of various sources, microcrystalline cellulose (MCC), methylcellulose, hydroxypropyl methul cellulose, polydextrose, pectin and inulin.

2.8 Sugars

Sugars contribute to the sensory properties of ice cream by imparting sweetness to the finished product. Sugar have a functional role, as they contribute to the depression of the freezing point and the development of the viscosity of the ice cream mix and, hence, help to improve the body and texture of the ice cream. The type and amount of sugar used affects the freezing point of an ice cream mix, as well as the crystallization rate (Wittinger and Smith, 1986; Miller and Hartel, 1997). Hydrolysis of lactose with lactase, or sucrose with invrtase, decreased the freezing point of the ice cream mix, reduce the firmness and increase the sweetness of the product (Lindamood et. al., 1999). The increase in sweetness allows a reduction in concentration of added sugar in the formulation. The organoleptic properties and meltdown characteristics of the ice cream were not affected when the extent of hydrolysis of the total saccharides was less than ~ 59 percent.

The lactose, which is present in the MSNF component of the ice cream mix, can cause a grainy texture if lactose crystallization is not controlled. Lactose crystallization can be minimized by rapid cooling of the pasteurized ice cream mix, ageing the mix at ~ 3 °C with occasional stirring and storage at ~ -25 °C.

2.8.1 Sugar Replacers

The calorie content of ice cream and frozen desserts is often declared in terms of Kcal/100 g. The major contributors of calorie in these products are fat and carbohydrate. So, in order to produce ice cream and frozen desserts low in calorie, it is necessary to
reduce or remove the sugar content in addition to reduction of fat content. Low calorie and non calorie sweeteners are also available in the market which provide energy between 1 and 3 calories per gram but only very small amounts are needed because of its intense sweetness (130-200 times) e.g. aspartame, saccharin, acesulfame-k, cyclamate etc. Bulking agents are needed to replace the loss of dry solids from sugar and retain an acceptable texture. To compensate for the lack of sweetness, it is often necessary to apply an intensive sweetener. A number of bulking agents for use in ice cream/frozen desserts e.g., Polydextrose (1 Kcal/g), Lactitol (2 Kcal/g), and Maltodextrin (4Kcal/g) are available in the market. The applications of sugars with a high level of sweeteners make it possible to reduce the total sugar content (Kumar, 2004).

2.9 Stabilizers

Most ice cream manufacturers use commercial stabilizer/emulsifier blends formulated by specialized firms with ingredients that they purchased from primary suppliers. These blends usually are combinations of stabilizers and emulsifiers but are sometimes referred to in short of stabilizers. The most frequently used ingredients in the mixtures for regular ice cream are guar gum and locust bean gum, cellulose gum (the primary hydrocolloids), carrageenan (the secondary hydrocolloid), monoglycerides/di-glycerides and polysorbate 80 (the emulsifiers).

The amount and kind of stabilizer/emulsifier blend in ice cream varies with mix composition; ingredients used; processing times, temperatures and pressures; storage temperatures and many other factors. Stabilizers are used to build viscosity and their ability to do so dependent on their inherent hydration properties, as well as on the temperature and time allowed for their hydration (Goff et. al., 1994). Stabilizers influence the rate of cooling and the rate of whipping of the ice cream during freezing and have a marketed influence on the body and texture of the ice cream. Over stabilization due to use of too high levels of stabilizers can result in ice cream that do not melt, especially in the formulation of low fat ice creams, in which fat is replaced by polysaccharides.

2.10 Emulsifiers

The main emulsifying components in the ice cream mix are the proteins and the low molecular weight emulsifiers. They influence the structure of the interfacial membrane in the ice cream mix during processing and in the final ice cream. Emulsifiers used in ice cream manufacture these days are of two main types: the mono and di-glycerides and the sorbitan esters. Blends of each are common as the mono- and di-glyceride component may be more functional at the fat interface, giving rise to a smaller distribution of air cells and providing a measure of control over the action of polysorbate 80, which is more functional at the fat interface promoting partial coalescence of the fat (Marshall et. al., 2003). Typical concentrations in use are 0.1-0.2 percent mono- and di-glycerides and 0.02-0.04 percent Polysorbate 80.

2.11 Fortifying Ingredients

The fortification of ice cream is a growing practice. In late 2002 what was a by no means exhaustive search of new frozen dessert products introduced globally since August 2001 revealed 17 products with some degree of fortification. Of those, feature the addition of calcium either on the package or in the marketing material. The venue for these products included France, the USA, Singapore, and Indonesia (Tharp, 2003).
2.11.1 Fortification of different bioactive peptides

A number of whey protein and casein derived peptides and hydrolysates, which act, as bioactive peptides are already commercially available. These bioactive peptides can be used to position the products as anti hypertensive, regulation of fat metabolism etc. Ice cream and frozen desserts can be used as a vehicle to deliver bioactive peptides.

2.11.2 Vitamin and calcium fortification

Ice cream and frozen desserts can be most readily adaptable to nutrient fortification and inclusion of nutraceuticals, vitamins and calcium fortification is often necessary as the nutrient density in ice cream is often lower than milk due to increased fat and sugar in ice cream. Omega 3 and omega 6 fatty acids can also be incorporated in the fat phase of ice cream to provide functional benefits. Therefore, ice cream and frozen desserts can be used successfully to deliver unique nutritional benefits to consumers beyond the basic nutrition of current products (Kumar, 2004).

2.11.3 Fibre fortification

Ice cream is a good source of important nutrients like fat, protein, carbohydrate, minerals and vitamins. However, like any other dairy products ice cream too, lacks in dietary fibre content. Fibre is the new magic word in nutrition and with good reason! A diet rich in fiber has numerous health benefits such as its effectiveness in controlling obesity, stroke, diabetes, cancer and gastrointestinal disorders. Fibre is a substance found only in plants, such as fruits, vegetables, and grains. Dietary fiber is made up of two main types—insoluble and soluble. Soluble fiber forms a gel when mixed with liquid, while insoluble fiber does not. Insoluble fiber passes through digestive tract largely intact and more quickly than soluble fiber, preventing or relieving constipation. It may prevent colon cancer by moving cancer-causing substances through digestive tract more quickly. Both types of fiber are important in the diet and provide benefits to the digestive system by helping to maintain regularity.

Polydextrose could be the good choice as an economical source of fibre for incorporation into ice cream and frozen desserts. As polydextrose is not digested in the upper gastro intestinal (GI) tract and is primarily fermented in the lower GI tract, making it beneficial ingredient for digestive health. Improved GI function has been demonstrated with the daily a daily intake of 4-12 g of polydextrose without adverse effects (Kumar, 2004).

Inulin also acts as a dietary fiber, as we consume roughly two third of our recommended daily allowance (RDA) of dietary fibre, using raftiline could help us to achieve a more balanced diet (Frank, 1995). Raftiline is not hydrolysed in the digestive tract thus it doesn't increase insulin levels, making it an ideal for diabetic patients, besides it has a low calorific value (1 Kcal / gm) and also acts as a prebiotic thus increases the beneficial bifido bacteria (5-10 times) in the intestine (Coussement, 1995).

3.0 Conclusion

Ice cream is expected to remain a popular product among consumers. Both ice cream manufacturers and suppliers of ingredients for ice cream industry need to keep abreast of developments in ice cream formulations and processing. For the dairy industry, this means the continued development of cost effective ingredients that offer improved convenience and enhanced functionality in ice cream. As the ice cream market grows, and
as more large manufacturers of ice cream move towards the use of powdered dairy ingredients in place of fresh dairy ingredients, because of ease of handling and storage, there is likely to be increased interest in examining the functionality and consistency of dried dairy ingredients in ice cream.

Further research will be required to determine how factors such as composition of milk and whey ingredient, the history of milk or whey before dehydration, and the effects of the storage conditions of dried ingredients impact on their suitability for use in a range of ice cream formulations.

### 4.0 Suggested Readings


LACTIC ACID BACTERIA AS NUTRACEUTICAL INGREDIENTS

Dr. Sudhir Kumar Tomar
Sr. Scientist
Dairy Microbiology Division
NDRI, Karnal

1.0 Introduction

There is no accepted international definition of functional foods. The terms functional foods and nutraceuticals are often used interchangeably and are variably defined. Functional foods are generally characterized as foods similar in appearance to conventional foods, consumed as part of a usual diet, and providing health-related benefits beyond meeting basic nutritional needs. The term "Nutraceuticals", launched by Stephen DeFelice in the 1980's, defines a wide range of foods and food components with a claimed medical or health benefit. Such nutraceuticals can be categorised into following groups:

- No- or low-calorie sugars such as mannitol and trehalose
- Dairy products or dairy components with low lactose and/or galactose content and bacterial (starter) cultures with high lactose/galactose utilising activities.
- Soy products with low raffinose content and bacterial (starter) cultures with high raffinose-converting activity
- Oligosaccharides as stimulants for the digestive system
- B-vitamins such as folic acid and riboflavin

A food can be considered naturally “functional” if it contains a food component that affects one or more targeted functions in a beneficial way. For example, dairy foods can be said to be functional because of their content of calcium. Foods can also be made functional by either adding certain functional components (e.g., antioxidants, probiotics) or replacing components with more desirable ones. While functional foods are generally presented as “food,” nutraceuticals are often considered to be the products produced from foods but sold in other forms (e.g., pills, powders) and demonstrated to have physiological benefits. Of interest, Japan is the only country worldwide where a legal definition and regulatory approval process for functional foods as Foods for Specified Health Use (FOSHU) exist.

2.0 Growth of Functional Food/Nutraceutical

These foods are considered to elicit benefits to health and well-being or to have disease-preventing properties beyond their inherent nutritional value. Recent growth in the functional foods market stems from a variety of factor:
Identification of physiologically active components in foods (e.g., phytochemicals, omega-3-fatty acids, conjugated linoleic acid, probiotic bacteria cultures)

End users seeking minimally processed food with extra nutritional benefits and organoleptic value

The number of people who cook complete meals at home is on the wane in developed countries

An aging population

Rising health care costs which are leading consumers to take more responsibility for their own health.

An increasing number of end users who are attempting to self treat medical issues such as joint problems, insomnia, and menopause.

3.0 Lactic Acid Bacteria as Nutraceutical Ingredient

From times immemorial, Lactic acid Bacteria (LAB) have been used for the production of fermented foods and have the GRAS ('generally recognized as safe') status. There are 11 genera, and starter bacteria are found in five namely, the Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, and Streptococcus groups. The bacteria used in the manufacture of fermented dairy products are generally LAB; however, Propionibacterium shermanii, Bifidobacterium spp. and, Bifidobacterium spp which are not LAB are also used. Besides fermented dairy products, they are also used for pickling of vegetables, baking, winemaking, curing fish, meats and sausages. A new application of food-grade lactic acid bacteria is their use of 'cell factories' for the production of 'nutraceutical'. During the production of foods the microorganisms can be added so that the health-nutraceuticals accumulate in the food during fermentation. Nutraceuticals can also be produced in a fermentor and subsequently added to the foods as ingredients. In both cases the nutritional value of the foods increases. Developments in the genetic engineering of food-grade microorganisms means that the production of certain nutraceuticals can be enhanced or newly induced through overexpression and/or disruption of relevant metabolic genes. During fermentation processes, microbial growth and metabolism result in the production of a diversity of metabolites. These metabolites include enzymes which are capable of breaking down carbohydrates, proteins and lipids present within the substrate and/or fermentation medium; vitamins; antimicrobial compounds (e.g. bacteriocins); texture-forming agents (e.g. exopolysaccharide); amino acids; organic acids (e.g. citric acid, lactic acid) and flavour compounds (e.g. esters and aldehydes). Endowed with these versatile metabolic abilities, LAB have found a number of multifunctional roles:

- Digest food, especially fats, proteins and carbohydrates;
- Manufacture natural antibiotics;
- Control excess LDL cholesterol levels;
- Prevent the formation of cancer-causing substances;
- Actively fight certain types of tumours;
- Hinder pathogenic bacteria and virus growth;
- Increase nutrient absorption;
Produce antibodies and anticarcinogens;
Break down toxins;
Bolster the immune system;
Improve digestive processes by eliminating gas and putrefying wastes;
Prevent urinary tract and vaginal infections, inflammatory bowel disease;
Control yeast infections such as athlete's foot, thrush and candidiasis;
Maintain regularity, fight diarrhea and food poisoning;
Balance sex hormones, enhancing fertility; and
Maintain radiant, healthy skin.

4.0 Nutraceuticals Produced by LAB

A number of metabolites of LAB which have been amply researched and documented and have the potential to be used as bioingredient are discussed in following sections.

4.1 Probiotics

Probiotic (derived from the Greek word meaning “for life”) generally refers to live bacteria that beneficially affect the host by improving its intestinal microbial balance. Dairy foods appear to be the preferred medium for introducing probiotic bacteria such as human-derived species of LABia (e.g., L. acidophilus, L. casei, L. gasseri, L. rhamnosus, L. reuteri, Bifidobacterium bifidum, B. breve, B. infantis, and B. longum). Lactobacillus spp. (naturally found in the human small intestine) and various Bifidobacterium spp. (a major organism in the human large intestine) are the most commonly used probiotic cultures. The list of LAB and related microorganisms commonly used as probiotic is provided in Table 1.

4.1.1 Potential and established health benefits

With respect to health benefits of probiotics, research studies indicate the following:

→ Improved intestinal health
→ Modulation of the immune response
→ Reduced risk of cancer
→ Reduced risk of heart disease
→ Improved tolerance to milk

Dosage—Around the world there is increasing scrutiny of food and supplement claims. Both scientific and regulatory authorities are looking for assurance that a probiotic product can deliver live strains at sufficient concentration to the large intestine to provide a benefit to the individual. The current opinion is that levels of $10^6-10^7$ cfu/g should be present at the time of consumption and thus $10^7-10^8$ at time of manufacture for a claim, stated or implied, to be made.
4.2 Vitamins

4.2.1 Folic acid

Folic acid is an essential component in the human diet. It is involved, as cofactor, in many metabolic reactions, including the biosynthesis of the building blocks of DNA and RNA, the nucleotides. Low folic acid in the diet is associated with high homocysteine levels in the blood and, subsequently, with coronary diseases. Fermented milk products, especially yoghurt, are reported to, sometimes, contain, even higher amounts of folate. Folate has been found in yoghurt. This high level is a direct result of the production of additional folate by the lactic acid bacteria in the yoghurt. Of the two lactic acid bacterial species in yoghurt, Lactobacillus bulgaricus and Streptococcus thermophilus, only the latter is reported to produce folate. Recently, some other food-grade bacteria were observed to produce folate during milk fermentation.

4.2.2 Riboflavin

Vegetables and dairy products are rich sources of riboflavin. Riboflavin is a building stone for important co-enzymes involved in energy metabolism. The nutrient is produced by several microorganisms including L. lactis and P. freudenreichii spp. shermanii.

In a joint project coordinated by NIZO food research (http://www.nutracells.com/), Smid and his Irish colleagues of University College Cork have increased the production of riboflavin by these bacteria by means of metabolic engineering and strain selection.

---

Table 1: Microorganisms in Probiotic products

<table>
<thead>
<tr>
<th>Lactobacilli</th>
<th>Bifidobacteria</th>
<th>Other LAB</th>
<th>Non-LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>B. adolescentis</td>
<td>Ent. faecalis</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>L. casei</td>
<td>B. animalis</td>
<td>Ent. faecium</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>L. crispatus</td>
<td>B. bifidum</td>
<td>Lactoc. lactis</td>
<td>Propionibacterium</td>
</tr>
<tr>
<td>L. gallinarum(^a)</td>
<td>B. breve</td>
<td>Leuconostoc mesenteroides</td>
<td>freudenreichii</td>
</tr>
<tr>
<td>L. gasseri</td>
<td>B. infantis</td>
<td>Ped. acidilactici</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>L. johnsonii</td>
<td>B. lactis</td>
<td>Sporolactobacillus</td>
<td>('boulardii')</td>
</tr>
<tr>
<td>L. paracasei</td>
<td>B. longum</td>
<td>inulinus</td>
<td></td>
</tr>
<tr>
<td>L. plantarum</td>
<td></td>
<td>Strep. thermophilus</td>
<td></td>
</tr>
<tr>
<td>L. reuteri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Strain available from DSM, Germany.
4.3 Low Calorie Sugar

Because of the ever-increasing prevalence of obesity in Western countries the market for low-energy sugars is promising. This group includes the polyols mannitol and sorbitol, and trehalose which have the same sweetening capacity as sucrose, glucose and fructose but provide only half of the energy delivered by sucrose because they are poorly absorbed. *Lactobacillus plantarum* is being extensively used for production of mannitol and sorbitol. Trehalose is a well-known non-reducing disaccharide synthesized by a wide variety of organisms especially Propionibacteria. It is only partially digested in humans, and therefore it is considered a dietetic sugar. It is also poorly metabolized by many other organisms, including lactic acid bacteria. The oral bacteria, *Streptococcus mutans* and *Streptococcus salivarius*, for instance, are not able to perform acidification with trehalose as only carbohydrate. Other (biological) activities than can be attributed to trehalose are protection of proteins and whole cells against denaturation under different stress conditions such as heating, drying and freezing.

4.4 Enzymes

Some fermented dairy products, especially yogurt and yogurt-like products, contain significant amounts of galactose. This is a result of incomplete lactose utilization by the lactic acid bacteria, which prefer the glucose moiety of lactose over the galactose moiety. Consumption of (too much) galactose, especially in combination with alcohol, can create serious health problems in individuals. Therefore, the ability to specifically remove lactose and/or galactose from dairy products is of major interest. The LAB are being explored as source of two important enzymes in milk processing: autolysins and lactases. While autolytic LAB can be used for cheese maturation, thermophilic β-galactosidase-producing strains can be potentially exploited to produce lactase-rich extracts, which could then be used as bioingredients for lactose hydrolysis in dairy products.

4.5 Exopolysaccharide

Several strains display the capacity to produce and secrete sugar polymers or exopolysaccharides. Both homopolysaccharides and heteropolysaccharides are produced by LAB. Homopolysaccharides like glucans and fructans are synthesized extracellularly from sucrose; they are mainly produced by *Leuconostoc* (dextrins) and *Streptococcus* (mutans, fructans). Heteropolysaccharides are synthesized intracellularly and secreted as slime in the environment. They are produced by both mesophilic (e.g. *Lactococcus lactis* and *Lactobacillus sakei*) and thermophilic (e.g. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) strains. The EPS producing LAB can be used for improving the rheology, body and texture, and mouthfeel of fermented milk drinks and for development of low-fat and low milk solids fermented milk products and low moisture cheeses.

4.6 Bacteriocin

In recent years, numerous food poisoning outbreaks, involving various pathogens and food products, and the increasing concern over the preservation of minimally processed foods have spurred growing awareness of the importance of food safety. This has prompted new approaches to inhibit foodborne pathogens. In particular, there has been a renewed interest in the antimicrobial activity of LAB, which is the bacteriocin production.
As a result of intense investigations on LAB bacteriocins, considerable progress has been made by both basic and applied research disciplines towards a better understanding of these substances and a large number of chemically diverse bacteriocins have been identified. Four classes of bacteriocins have then been defined based on observed common characteristics, mainly structural. New bacteriocins are still being discovered and regularly reviewed and documented in books and reviews, with class I (lantibiotics) and class II (small heatstable non-lanthionine-containing peptides) bacteriocins being the most abundant and thoroughly studied.

5.0 Biotechnology in the Production of Food Ingredients

As described in earlier sections, flavouring agents, organic acids, food additives and amino acids are all metabolites of microorganisms during fermentation processes. Microbial fermentation processes are therefore commercially exploited for production of these food ingredients. Metabolic engineering, a new approach involving the targeted and purposeful manipulation of the metabolic pathways of an organism, is being widely researched to improve the quality and yields of these food ingredients. It typically involves alteration of cellular activities by the manipulation of the enzymatic, transport and regulatory functions of the cell using recombinant DNA and other genetic techniques. Understanding the metabolic pathways associated with these fermentation processes, and the ability to redirect metabolic pathways, can increase production of these metabolites and lead to production of novel metabolites and a diversified product base. Although several studies indicate that specific strains of probiotic cultures alleviate lactose maldigestion symptoms and reduce the duration of diarrhea, additional research is needed to scientifically substantiate many other alleged beneficial health effects of probiotic bacteria, as well as the mechanisms underlying these effects. To better understand the potential beneficial properties of probiotics such as *Lactobacillus* and *Bifidobacterium*, researchers are identifying the genetic characteristics of these probiotics. Genetic analysis and modification of probiotics can lead to the development of new probiotics with beneficial health effects, improved effectiveness of existing properties of probiotic strains in vitro and in vivo, and development of probiotic products for specific functional characteristics.

6.0 Future of Functional Foods

Though future for functional foods appears promising, it ultimately depends on scientific evidence of their efficacy, safety, and organoleptic quality. Whether or not functional foods will be successful long-term depends on several factors as follows:

- Linking the consumption of functional foods or food ingredients with health claims should be based on sound scientific evidence, with the "gold standard" being replicated, randomized, placebo-controlled, intervention trials in human subjects. The Food and Drug Administration of USA has approved only certain health claims.
- Interactions among components in functional foods, the bioavailability of nutrients, and the effect of processing on the health-enhancing potential of functional foods all must be considered in labeling functional foods.
- Individual strains of the same bacterial species often differ in their physiological properties and effects.
Biomarkers are needed to adequately assess the physiological impact of functional foods.

Importantly, consumers must become aware of the beneficial health effects of functional foods.

Functional foods represent one of the most intensively investigated and widely promoted areas in the food and nutrition sciences today. However, it must be emphasized that these foods and ingredients are not magic bullets or panaceas for poor health habits. Diet is only one aspect of a comprehensive approach to good health.

7.0 Suggested Readings


BIOTHERAPEUTIC VALUE OF PROBIOTIC YEASTS IN DAIRY PRODUCTS

Dr. D. N. Gandhi
Principal Scientist
Dairy Microbiology Division
NDRI, Karnal

1.0 Introduction

Probiotics are friendly and non-pathogenic microorganisms, that when ingested in certain number in the form of fermented dairy products or in pharmaceutical preparation, lead to benefit the microbial balance of intestine to perform desirable physiological activities and suppress the growth of various pathogenic and putrefactive organisms. Lactic acid bacteria such as Lactobacilli and Bifidobacterium had been explored as major probiotics worldwide. However, in recent years Yeast cultures in particularly *Saccharomyces* spp has been recognized as one of the potential probiotic genus since it is a non-pathogenic yeast, and can grow well at human body temperature (37°C). It is a unique probiotic organism as it can survive under gastric acidity and is not adversely affected by antibiotic(s). Further, it does not alter the normal flora of the host bowel. Among the genus *Saccharomyces*, *S.cerevisiae* and *S. boulardii* are being used as a commercial probiotic by pharmaceuticals. *S.cerevisiae* and *S. boulardii* inhibit the pathogenic bacteria of gastrointestinal tract such as *Vibrio cholerae, Salmonella enteritidis, Clostridium difficile* etc without affecting the natural microflora (Czerucka et al., 2002). Probiotic *Saccharomyces* spp is the yeast of choice because it can, bypass the side effect like acidosis, and bacteraemia, produced by lactic acid bacteria. In addition, probiotic *Saccharomyces* spp show direct antagonistic effect, antisecretory effect by acting specifically on the binding of toxins to intestinal receptors and trophic effect with stimulation of enzymatic expression and the intestinal defense mechanism. Probiotic *Saccharomyces* spp releases polyamines, which helps in repairing the damage caused due to diarrhoea (Costalos et al. 2003). Above all, incorporation of *Saccharomyces* spp enhances the growth of lactic culture in fermented dairy products such as in yoghurt, acidophilus yeast milk, and kefir with an increase in shelf life. The metabolic products of milk sugar fermented by lactic acid bacteria and the presence of organic acids, which can be utilized easily by these yeasts, can benefit *Saccharomyces* spp. (Lourens and Viljeon. 2001)

2.0 Biotherapeutic Significance of Yeast

Living microorganisms are widely used for several therapeutic purposes and their beneficial effects are rooted in history. Usually, certain strains of lactic acid bacteria and bifidobacteria (probiotics) are applied in pharmaceutical preparations, feed additives and so-called functional foods. Besides bacteria, yeasts also possess some medicinal efficiency, and the beneficial properties of strains of *Saccharomyces* spp are well
documented. In addition to their nutritive value (e.g. provision of vitamins of the B group), yeasts offer the advantage of exerting pronounced resistance during gastrointestinal passage as well as of modulating a disturbed gastrointestinal micro ecology. Yeast preparations have also been successfully applied in combination with antibiotics to treat *Clostridium difficile*-related diarrhoea and to re-establish a normal gut function after long-term antibiotic therapy. Furthermore, they also offer some capacity in the prevention of traveler’s diarrhoea. *Saccharomyces spp* also have a protective effect and specific activities against various enteric pathogens. *Saccharomyces spp* stimulate immune response with IgA production and phagocyte system in gnotobiotic mice.

Being not in accordance with the current nomenclature, strains of so-called *Saccharomyces boulardii* can be regarded as the most prominent representatives of ‘probiotic yeast’ within the community of biotherapeutic *S. cerevisiae* strains. Now a days, a considerable number of pharmaceutical preparations (capsules, powders, tablets, pellets) containing probiotic yeast (*Saccharomyces spp*) cells are commercially available, and are marketed mainly via pharmacies and health stores .It has been suggested that biotherapeutic agents must be given in sufficient concentration to exert therapeutic properties, remain stable and viable before use and survive in the intestinal ecosystem of the host to develop their therapeutic properties.

### 2.1 Probiotic Attributes of Saccharomyces Spp

Genera *Saccharomyces* have 16 spp like *S. cerevisiae*, *S. boulardii*, *S.burnetii*, *S.kluveri* *S.byarus*, and *S.rosinii*. Among all spp of this genus, *S.cerevisiae* and *S. boulardii* are described in the clinical literature as biotherapeutic agent and reported to be efficacious in the prevention or recurrence of different type of diarrhoea and colitis in human *S.cerevisiae* and *S.boulardii* is a non pathogenic yeast, it has been shown effective in the treatment of acute diarrhoea in children and has been reported to exhibit useful effects in the prevention of diarrhoea in critically ill tube fed patients.*S.cerevisiae* and *S.boulardii* releases polyamines to help repair mucous membrane. These polyamine increase the activity of short chain fatty acids and disaccharide enzymes (lactase, maltase, sucrase), these enzymes helps to prevent different types of diarrhoea. Polyamine stimulate the colonic mucosa in growing, multiplying, and repairing of intestinal cells *S.boulardii* is generally administered in lyophilized powder (Periti and Tanelli, 2001) and application as a food additive has only been reported in a limited number of cases such as in the fermentation of vegetable raw materials and incorporation into commercial yogurts (Lourens and VJljeon, 2001). *S.cerevisiae* and *S.boulardii* is a unique organism that has ability to survive in gastric acidity and not adversely affected or inhibited by antibiotics. It does not alter or adversely affect the normal flora of bowel. Because of this reason it has been recognized as good friendly probiotic organisms (including Lactobacillus and Bifidobacterium) can be taken at the same time as *S.cerevisiae* and *S.boulardii*. Combination of standard treatment for *Clostridium difficile* infection combined with *S.boulardii* and *S.cerevisiae* has been shown to reduce the risk of recurrence in patients experiencing renewed infection (Aloysius et al., 2005).

### 3.0 Mechanism of Probiotic Activity of Yeast

*S.cerevisiae* and *S.boulardii* share a common mechanism of action against pathogenic bacteria. These are as given below:
3.1 Pharmacokinetics

S.cerevisiae and S. boulardii resist to gastric acidity and proteolysis and are able to achieve quickly high concentrations in the gastrointestinal tract and to maintain constant levels in a viable form. They are not permanently colonizing the colon and not easily translocate out of the intestinal tract. Further it can be detected alive throughout the digestive system, if they are given daily in its freeze-dried form. In gnotobiotic mice, a single dose of S. boulardii leads to colonization of the intestinal tract, the yeast being detectable at a constant level (10^7 c.f.u./ml) for 60 days. In healthy human volunteers, which received a single oral dose of 1 g S. cerevisiae or S. boulardii, the time to reach maximum stool concentration was found within 36 to 60 hours, and the time to decrease below detectable levels was 2 to 5 days later.

3.2 Pharmacodynamics

The pharmacodynamics of S. cerevisiae and S. boulardii involves 3 different aspects.

3.2.1 Direct antagonism

S. boulardii reduces the growth of Clostridium albicans, E. coli, Salmonella typh, Shigella dysenteriae, Vibrio cholerae, Salmonella enteritidis and Clostridium difficile. S. cerevisiae reduces the growth of E. coli, Shigella flexnerii, Clostridium difficile, Vibrio cholerae. The mechanism of action of S. cerevisiae and S. boulardii in experimental infections has been extensively studied. Both animals studies and in vitro assays have shown that S. cerevisiae and S. boulardii have a protective effect and specific activities against various enteric pathogens and members of family enterobacteriaceae (Czerucka and Rampal., 2002). They show antagonistic effect mainly because of the competition for adhesion site in gastrointestinal tract.

3.2.2 Antisecretory effect on the binding of toxins to intestinal receptors

Pathogenic strain of C. difficile produces two well-characterized toxins A and B that cause mucosa damage and inflammation of colon. S. cerevisiae and S. boulardii significantly reduces the liquid secretion and mannitol permeability caused by inhibition of Clostridium difficile toxin A in rat ileum, compared with control group. Chromatographic fraction of S. boalrdii filtered supernatant led to the identification of an active fraction that decrease toxin A-induced rat ileal secretion by 46%, and intestinal permeability by 74% and prevent toxin A-mediated inflammation and villous damage. It was demonstrated that this fraction was enriched in a protease that acts both by proteolysis of the toxin. This protease was identified as a 54-kDa serine protease, which exerts a proteolytic activity against toxin B molecule. S. cerevisiae and S. boulardii protease diminishes the ability of toxin A and B mediated inhibition of protein synthesis and reduction of colonic resistance.

3.2.3 Trophic effect

It has been reported that rats treated with Saccharomyces spp showed significant increases in sucrase, isomaltase, lactase and maltase activities. In their study on human volunteers used an in situ technique to measure brush border enzyme activities in snap-frozen biopsies. After treatment with S. cerevisiae and S. boulardii, an increase in lactase, -glycosidase and alkaline phosphatase activity was detected both at the basal part and
apical part of the villi, which increases ranging from 22 to 55% compared to the basal activities measured before treatment. Thus in humans as in rats, *S. cerevisiae* and *S. boulardii* enhance the expression of disaccharidases and alkaline phosphatase that may improve the absorption of carbohydrates, usually in acute and chronic diarrhea disorders. *Saccharomyces cerevisiae* and *S. boulardii* contain polyamine (spermine and spermidine) which have the same trophic effect on the intestinal mucosa, with an increase in diisaccharidase activity, as an equivalent amount of spermine and spermidine given to test animals.

4.0 Effect of Combination of Lactobacillus Strains and *S. Boulardii* on Persistent Diarrhoea

Children receiving *S. boulardii* and lactobacilli had a gradual reduction in the number of daily stools, more noticeable after the first day treatment compared to those in placebo group. Patient treated with *S. boulardii* and *Lactobacillus* had a significant faster recovery compared with placebo control. *S. boulardii* and *Lactobacillus* were found to be similarly effective to decrease the duration of diarrhoea. Lactobacillus in addition to well known trophic effect of *Saccharomyces boulardii* on intestinal mucosa, mediated by the release of polyamine. The meta-analysis suggest that probiotics can be used to prevent antibiotic associated diarrhoea, *S. boulardii* and *Lactobacilli* have the potential to be used in this situation. *S. cerevisiae* also help in *C. difficile* associated diarrhoea. *S. cerevisiae* deliver vitamin B and other nutrients like selenium and chromium. Selenium have been reported to have an anti-carcinogenic activity while chromium have an anti-diabetic activity.

5.0 Occurrence of Saccharomyces Spp in Milk and Milk Products

Saccharomyces spp such as *S. burnetii*, *S. kluyveri*, *S. byanus*, *S. rosinii* *S. cerevisiae* and *S. boulardii* may be isolated from a variety of dairy products, including milk, yogurts, cream, dahi, cheeses and kefir. Yeasts rarely grow in milk stored at refrigeration temperature because they are out-grown by psychotropic bacteria. However, in sterilized milk in the absence of competition, *Saccharomyces spp* exhibits weak lipolytic and proteolytic activity and is capable of growth to reach populations of $10^8$-$10^9$ c.f.u/ml. *Saccharomyces spp* often present in soft mold ripened cheeses. It has been reported in Italian Stretching cheese at 3.1% of the yeast population present. They may be present in semi-hard cheese including cheddar cheese. Growth of *Saccharomyces spp* in cheeses is thought to be related to its ability to use lipid and protein products form other species and possibly its ability to utilize lactic acid present in the cheese (Robinson et al 2000).

6.0 Survival of Saccharomyces SPP in Fermented Milk Products and Preparation of Yeast Based Fermented Milk Products

The frequent occurrence of yeast in dairy related products indicate the ability of yeasts to survive and metabolized milk constituents. *Saccharomyces spp* can’t ferment lactose so it develops in milk as secondary flora after bacterial growth. Lactic acid bacteria (LAB) ferments about 35% of the lactose in milk through hydrolysis of glucose and galactose. Glucose moiety is changed into lactic acid. Lactic acid cause low pH of yoghurt and the ability of yeast to utilize organic acid create a selective environment for yeast growth in fermented milk products. Milk products that include yeasts in their starter culture are acidophilus-yeast milk, Kefir, Koumiss and Leben. Method for the preparation of acidophilus yeast milk has been standardized milk at National Dairy Research Institute. For
this preparation, skim milk with 8% sugar content is given a heat treatment to 90C /15 mts and cooled to 37C. A combination of Lactobacillus acidophilus and Saccharomyces cerevisiae starter cultures is added at the level of 1% each and fermentation is carried out in crown corked bottles at 39C /20-22 hrs. It is then stored under refrigeration temperature and consumed as chilled beverage.

7.0 Conclusion

Although there are several reports on the probiotic attributes of lactic cultures in the recent years, but keeping in view potential biotherapeutic properties of new emerging probiotic yeast, there is a need to generate more data on isolation, product development and therapeutic properties of Saccharomyces sp in vitro and in vivo alone or in co-fermentation with lactic acid bacteria in dairy products.

8.0 Suggested Readings


1.0 Introduction

Dietary proteins are known to carry a wide range of nutritional, functional and biological properties. Nutritionally, the proteins are a source of energy and amino acids, which are essential for growth and maintenance. Functionally, the proteins contribute to the physicochemical and sensory properties of various protein-rich foods. Furthermore, many dietary proteins possess specific biological properties, which make these components potential ingredients of functional or health-promoting foods. Many of these properties are attributed to physiologically active peptides encrypted in protein molecules. Bioactive peptides are breakdown products of proteins by proteases present in the gastrointestinal tract, which have specific biofunction only after release from the parent protein source. Bioactive peptides have been defined, therefore, as specific protein fragments that have a positive impact on body function or condition and which ultimately may influence health. During the last twenty years, the knowledge of bioactive peptides has steadily increased, and at present numerous peptides exhibiting various activities - such as opiate, antithrombotic or anti-hypertension, immunomodulatory, antioxidant, antimicrobial and mineral binding have been isolated from various food proteins. These activities are based on the inherent amino acid composition and sequence. Bioactive peptides usually contain 3 – 20 amino acid residues per molecule, possessing hydrophobic amino acid residues in addition to proline, lysine or arginine groups and are resistant to the action of digestion peptidases. These peptides are likely to be produced industrially in the future in the form of hydrolysates or peptide mixtures which can be used as ingredients for various dietary or pharmaceutical products. In this article the current knowledge about bioactive peptides derived from various proteins, with emphasis on their potential applications for designing health-promoting functional foods have been reviewed.

2.0 Mechanisms of Physiological Activities of Different Bioactive Peptides

2.1 Antihypertensive Properties

Biologically active peptides derived from food proteins with an affinity to modulate blood pressure have been thoroughly studied. Angiotensin I converting enzyme (ACE; kinases II peptidyldepeptide hydrolase, EC 3.4.15.1) is important for blood pressure regulation. In the event where decreased blood volume or decreased blood flow to the kidneys is sensed, renin acts on angiotensinogen to formangiotensin I. ACE then catalyses the hydrolysis of the inactive prohormone angiotensin I (decapeptide) to angiotensin II
(octapeptide). The result is an increase in blood pressure through vasoconstriction, via increase systemic resistance and stimulated secretion of aldosterone resulting in increased sodium and water resorption in the kidneys. ACE also inactivates the vasodilating peptide bradykinin (nonapeptide) and endogenous opioid peptide Met-enkephalin. Many bioactive peptides have been isolated from various food sources, which have been shown to have an inhibitory affect on angiotensin I and thereby exhibit antihypertensive activity. ACE inhibitors function by cleaving the C-terminal dipeptide of angiotensin I, thereby preventing angiotensin I conversion to angiotensin II by ACE.

2.2 Opioid Activities

Opioid peptides derived from food proteins have affinities to bind to opiate receptors and express similar opiate activity, which in turn can be reversed by an opioid antagonist, such as naloxone. Naloxone crosses the bloodbrain barrier and blocks opioid activity, thus being a useful tool to determine specific effects of agonist opioid peptides. Peptides with opioid activities are regarded as ligands of opioid receptors. Structurally, both exogenous and endogenous opioid peptides vary in the N-terminal sequence of the peptide, such as having a tyrosine residue at the amino terminal (the bioactive) site. Endogenous opioid peptides originate from proopiomelancortin, proenkephalin (yields enkephalin), and prodynorphin (yields endorphin and dynorphin). Specific organ tissues that exhibit opioid activity include the spinal cord, adrenal gland and the digestive tract, via both δ and μ receptors and the pituitary gland and hypothalamus through the μ, δ and ε receptors. The m-type receptors mediate primarily neuroendocrine function and relate to pain sensation and analgesia. The d-type receptor is associated with emotions and reward behavior. Other responses of opioid peptides reported include, stress response, analgesia, sedation, dullness, respiratory depression, hypotension, changes of body temperature, as well as an influence on satiation, decline of gastric secretion and changes in sexual behavior.

2.3 Immunomodulatory Activity

Milk protein hydrolysates and peptides derived from caseins and major whey proteins can enhance immune cell functions, measured as lymphocyte proliferation, antibody synthesis and cytokine regulation. The peptides released during milk fermentation with lactic acid bacteria, as these peptides have been found to modulate the proliferation of human lymphocytes, to down-regulate the production of certain cytokines and to stimulate the phagocytic activities of macrophages. The protective effect of a casein-derived immunopeptide on resistance to microbial infection by Klebsiella pneumoniae has been demonstrated in mice. The immunomodulatory milk peptides may alleviate allergic reactions in atopic humans and enhance mucosal immunity in the gastrointestinal tract (Korhonen & Pihlanto, 2003a). In this way immunomodulatory peptides may regulate the development of the immune system in newborn infants.

2.4 Mineral Sequestering Activity

Caseinophosphopeptides (CCPs) are bioactive peptides derived from tryptic digestion of casein and have been used in confectionary products such as breakfast foods, breads, pastry, bean curds, chocolate, caramel, juices, boiled fish, tea and mayonnaise. The CPPs have divalent metal ion sequestering activity and with binding will solubilize different ions such as Ca, Mg and Fe, along with trace elements that include Zn, Ba, Cr, Ni, Co and Se. Calcium is a mineral of special concern because it has many important
functions in the human body including bone development and recalcification and the prevention of hypertension and colon cancer. Calcium absorption occurs in the human body by both active vitamin D-dependent transport in the duodenum and jejunum and passive vitamin D-independent transport in the distal ileum. The affinity of CPPs to inhibit amorphous calcium phosphate precipitation by linking the seryl phosphate groups to calcium phosphate in a nanometer-sized particle results in the stabilization of the amorphous di-calcium phosphate.

2.5 Antioxidant Activity

It has been reported that His-containing peptides acted as metal-ion chelators, active-oxygenquenchers and hydroxy-radical scavengers. Each of these actions alone could not be correlated with the antioxidative activity of the peptides; thus, it was proposed that the overall antioxidative activity must be attributed to the collective effects of these actions. Antioxidative properties of another His-containing peptide, carnosine, were reviewed by Chan et al. Carnosine is a dipeptide composed of b-alanine and L-histidine and is found in high concentrations in muscle cells including fish. It was reported that the cellular actions of carnosine had extensive antioxidative and hydrogen buffering properties.

2.6 Antimicrobial Activity

Numerous peptides with antimicrobial activity have been isolated from dairy products; amphibian sources, marine fish sources and cereals. Antimicrobial peptide activity is usually expressed by the disintegration of cell membrane, whereby the lipid bilayer of the cell membrane is the principle target. Interaction between the antimicrobial peptide and the cell membrane is an important requirement for antimicrobial activity. Majority of antimicrobial peptides that contain a-helical structures are cationic and amphipathic, while others are also hydrophobic a-helicalpeptides. The cationic properties of the peptide enables binding with the anionic phospholipid-rich membrane which initiates cell lysis.

3.0 Food Sources of Bioactive Peptides

3.1 Animal Food Products

Numerous important bioactive proteins exist in milk like immunoglobulins (Igs), lactoferrin, and lactoperoxidase and have either anti-microbial activity, are involved in the transfer of passive immunity, or have hormonal and growth factors for the needs of the developing neonate. In addition, there exist different bioactive peptides that have been derived from the action of digestive proteases on milk proteins. The enzymatic hydrolysis of milk proteins has led to the production of a variety of biologically active peptides, such as opioid peptides, immunostimulating peptides, and angiotensin I-converting enzyme (ACE), antimicrobial and antioxidant. In addition, caseinphoshopeptides (CPP) derived from enzymatic digestion of casein have been found to enhance calcium solubility and potentially intestinal calcium absorption . Two vasorelaxing peptides, namely ovokinin I and ovokinin II, have been obtained from egg ovalbumin . Fujita et al., isolated a vasorelaxing peptide with an amino acid sequence of Phe-Arg-Ala-Asp-His-Pro-Phe-Leu from an egg ovalbumin peptic digest. The peptide, termed ovokinin, was the first discovery of an egg derived bioactive peptide. A second vasorelaxing peptide, with the structure Arg-Ala-Asp-His-Pro-Phe termed, ovokinin was later obtained from a chymotryptic digest of egg ovalbumin. Kohama et al., discovered and isolated a novel ACE-inhibitor from the acid extract of tuna.
muscle by column chromatography. Another marine source of ACE-inhibitory peptides was generated from a thermolysin digest of dried bonito; a Japanese traditional seasoning ("Katsuo-bushi"), which is made from the muscle of the bonito fish. Additional antihypertensive bioactive peptides derived from sardine, tuna and bonito fishes. Fish protein hydrolysates are also sources of immunomodulatory peptides and antioxidative peptides, such as carnosine. Acid peptide fractions from a cod stomach hydrolysate have been reported to include medium size peptides (500 Da–3000 Da) with immuno-stimulatory activity. The mudfish (Misgurnus anguillicaudatus), produces a strongly basic, 2502 Da protein that contains 5 arginine and 4 lysine residues. The protein, termed misgurin, has antimicrobial activity against a broad spectrum of microorganisms without significant hemolytic activity. Liver tissue from Atlantic salmon (Salmosalar) contains an antimicrobial peptide with a molecular mass of 20,734 Da. Potent antibacterial peptides originating from the peptide Pardaxin, have been isolated from Moses sole fish (Pardachirus marmoratus).

3.2 Plant Food Products

Three strong ACE inhibitors isolated after hydrolysis of a zein and characterized as Leu-Arg-Pro; Leu-Ser-Pro; and Leu-Gln-Pro contained a common C-terminal praline residue. Wheat gluten is a well-known source of opioid peptides among food proteins, in addition to milk proteins and hemoglobin. Zioudrou et al., [1], first tested opioid activity in wheat gluten after reports regarding wheat gluten with mental disorders. Hydrolysis of wheat gluten by the microbial protease, thermolysin produced stronger opioid activity. Opioid peptides with amino acid compositions of Gly-Tyr-Tyr-Pro-Thr; Gly-Tyr-Tyr-Pro; Tyr-Gly-Gly-Trp-Leu; and Tyr-Gly-Gly-Trp were isolated from pepsin and thermolysin digests and named gluten exorphins.

A5, A4, B5, and B4, respectively. A peptide with immunomodulatory activity causing smooth muscle contraction named oryzatensin (Gly-Tyr-Pro-Met-Tyr-Pro-Leu-Pro-Arg) has also been obtained from arice tryptic hydrolysate. Shorter, C-terminal fragments of oryzatensin demonstrating similar activity were also reported. Phagocytosis by leukocytes from the human blood was induced in vitro by oryzatensin and the production of superoxide anions in leukocytes was also stimulated. Recently six peptide fragments with antioxidant activity were isolated from digests of a soybean protein, b-conglycinin. Many plants produce anti-microbial peptides which have a role in the defense of the plant against infection or natural invaders. A class of small, basic peptides, referred to as thionins, has been identified in seeds of wheat, barley, rye and oats. Thionins are cysteine- and lysine-rich polypeptides that contain 43-46 amino acid residues and have considerable homology among different species. Several thionins have been shown to exhibit anti-microbial activity against plant pathogenic fungi and bacteria in vitro. Duvick et al., reported small, acid soluble, basic peptides derived from maize kernels with anti-microbial properties which were both physically and chemically similar to thionins, with the exception that they did not have free cysteine groups. A purified peptide, termed MBP-1, contained 33 residues and had a molecular weight of 4127 Da as well as forming mainly \( \alpha \)-helical structures. Japanese soysauce has also been shown to contain ACE inhibitors. ACE-inhibitors derived from soy sauce contain nicotinamine as a principle bioactive component. This compound, which has powerful chelating activity, also possesses a proline analogue, azetidic acid 2-carboxylic acid, a potential contributing to the ACE-inhibitory function.
4.0 Production of Bioactive Peptides

There are a number of methods by which peptides with biological activity can be produced from precursor proteins. The most common ones are (a) enzymatic hydrolysis with digestive enzymes, (b) by means of the microbial activity of fermented foods or (c) through the action of enzymes derived from proteolytic micro-organisms. Once the structure of bioactive peptides is known, it is also possible to synthesize peptides. Three main approaches are available at present: (1) chemical synthesis; (2) recombinant DNA technology; and (3) enzymatic synthesis.

4.1 Enzymatic Hydrolysis

Up to this point, enzymatic hydrolysis of proteins has been the most common way to produce bioactive peptides. Pancreatic enzymes, preferably trypsin, have been used for the chemical characterisation and identification of many known bioactive peptides. For example, ACE-inhibitory peptides as well as CPPs are most commonly produced by trypsin. On the other hand, other enzymes and different enzyme combinations of proteinases—including alcalase, chymotrypsin, pancreatin and pepsin as well as enzymes from bacterial and fungal sources—have been utilised to generate bioactive peptides. Microbial enzymes have also been successfully used to generate ACE-inhibitory peptides. Hydrolysis can be performed by conventional batch hydrolysis or by continuous hydrolysis using ultrafiltration membranes. The traditional batch method has several disadvantages, such as the relatively high cost of the enzymes and their inefficiency compared to a continuous process, as noted in numerous studies. The enzymatic membrane reactor, which integrates enzymatic hydrolysis, product separation and catalyst recovery into a single operation, is an attractive configuration for this purpose. It has already been widely applied to total conversion of food proteins of various origins, in order to produce hydrolysates with improved nutritional and/or functional properties.

4.2 Microbial Fermentation

Many industrially used dairy starter cultures are highly proteolytic. This property is traditionally exploited by the dairy industry, as the peptides and amino acids degraded from milk proteins during fermentation contribute to the typical flavour, aroma and texture of the products. The proteolytic system of lactic acid bacteria, such as Lactococcus lactis, Lactobacillus helveticus and Lactobacillus delbrueckii var. bulgaricus, is already well known. This system consists of a cell wall-bound proteinase and several intracellular peptidases. In recent years, rapid progress has been made in the elucidation of the biochemical and genetic characterization of these enzymes. At least 16 peptidases responsible for the conversion of the released peptides into free amino acids have been characterized from lactic acid bacteria. Williams et al. showed that the activities of peptidases are affected by growth conditions. The proteolytic system of lactic acid bacteria provides transport systems specific for amino acids, di- and tripeptides and oligopeptides of up to 18 amino acids. Longer oligopeptides which are not transported into the cells can be a source for the liberation of bioactive peptides in fermented milk products when further degraded by intracellular peptidases after cell lysis. In the gastrointestinal tract, digestive enzymes may further degrade long oligopeptides, leading to a possible release of bioactive peptides. Once liberated in the intestine, bioactive peptides may act locally or pass through the intestinal wall into blood circulation ending up in a target organ, with subsequent regulation of physiological conditions through, e.g., nerve, immune, vascular or endocrine systems.
4.3 Peptide Synthesis

The selection of the most suitable method for peptide synthesis depends mainly on the length and quantity of the desired peptide. The total enzymatic synthesis is currently limited to relatively short sequences. On the other hand, recombinant DNA technology is the preferred choice for relatively large peptides and is suited to products consisting of up to several hundred amino acids. Currently, the most widely used approach to the synthesis of peptides on the laboratory scale is the chemical one. Two variants of this methodology exist, namely liquid-phase and solid-phase synthesis. The solid-phase approach is still the most powerful method for synthesis of peptides composed of about 10 to over 100 residues on a small scale, as well as for the rapid production of peptide libraries for screening purposes. The application of recombinant DNA technology typically requires a long and expensive research and development phase, but once the system is established the product can be obtained in large quantities from very inexpensive starting materials via fermentation. Attempts to extend this approach to the preparation of short peptides have not yet been truly successful. For example, the expression of the opioid pentapeptide sequence L-[Leu 5]-enkephalin (Tyr-Gly-Gly-Phe-Leu) in Arabidopsis thaliana and Brassica napus albumin seed storage proteins, followed by proteolytic digestion of the fusion proteins with trypsin, provided the desired pentapeptide with a yield of only 50-200 nmol (28-112 mg) gram⁻¹ seed. Feeney et al. reported that the construction of glutenin genes and their expression in E.coli is a viable method for producing peptides. Despite significant advances, the synthesis of short sequences using genetic engineering methods often remains impractical, due to the low expression efficiencies obtained and difficulties encountered in product extraction and recovery.

5.0 Fractionation and Enrichment of Bioactive Peptides

Commercial production of bioactive peptides from food proteins has been limited by a lack of suitable large-scale technologies. Until now, membrane separation techniques have provided the best technology available for the enrichment of peptides with a specific molecular weight range (Korhonen & Pihlanto, 2003b). Ultrafiltration is routinely employed to enrich bioactive peptides from protein hydrolysates. Enzymatic hydrolysis can be performed through conventional batch hydrolysis or continuous hydrolysis using ultrafiltration membranes. Ultrafiltration membrane reactors have been shown to improve the efficiency of enzyme-catalysed bioconversion and to increase product yields, and they can be easily scaled up. Furthermore, ultrafiltration membrane reactors yield a consistently uniform product with desired molecular mass characteristics. Continuous extraction of bioactive peptides in membrane reactors has been mainly applied to milk proteins. Stepwise ultrafiltration using cut-off membranes of low molecular mass have been found useful for separating out small peptides from high molecular mass residues and remaining enzymes. Two-step ultrafiltration process and were able to produce a mixture of polypeptides and a fraction rich in small peptides with a molecular mass below 2000 Da. Several ion exchange chromatographic methods have been developed for the enrichment of CPPs from casein hydrolysates, but the production costs of this technique were prohibitive for large-scale operation. Recently, ion exchange membrane chromatography has emerged as a promising technique for the enrichment of peptide fractions from protein hydrolysates.
6.0 Occurrence of Bioactive Peptides in Foods

It is now well documented that bioactive peptides can be generated during milk fermentation with the starter cultures traditionally employed by the dairy industry. As a result, peptides with various bioactivities can be found in the end-products, such as various cheese varieties and fermented milks (Korhonen & Pihlanto-Leppä, 2001; Korhonen & Pihlan-to, 2003a, b, c; Korhonen & Pihlanto-Leppä, 2004). These traditional dairy products may under certain conditions carry specific health effects when ingested as part of the daily diet. An increasing number of ingredients containing specific bioactive peptides based on casein or whey protein hydrolysates have been launched on the market within the past few years or are currently under development by international food companies. Such peptides possess, e.g., anticariogenic, antihypertensive, mineral-binding and stress-relieving properties.

7.0 Future Applications of Bioactive Peptides

The occurrence of many biologically active peptides in dietary proteins is now well-established. Numerous scientific, technological and regulatory issues have, however, to be resolved before these substances can be optimally exploited for human nutrition and health. Firstly, there is a need to develop novel technologies, e.g., chromatographic and membrane separation techniques by means of which active peptide fractions can be produced and enriched. Secondly, it is important to study the technological properties of the active peptide fractions and to develop model foods which contain these peptides and retain their activity for a certain period. It is recognized that peptides can be more reactive than proteins, due to their lower molecular weight, and the peptides that are present in the food matrix may react with other food components. The interaction of peptides with carbohydrates and lipids as well as the influence of the processing conditions (especially heating) on peptide activity and bioavailability should also be investigated. In particular, possible formation of toxic, allergenic or carcinogenic substances, such as acrylamide or biogenic amines, warrants intensive research. To this end, modern methods need to be developed to study the safety of food stuffs containing biologically active peptides. In this context, microbial fermentation seems to be a potential natural technology applicable to the production of bioactive peptides either from animal or plant proteins. This potential is already well-demonstrated by the presence of bioactive peptides in fermented milks and cheese varieties. Controlled fermentation of raw materials rich in proteins by known lactic acid bacteria strains may be developed on the commercial scale with respect to continuously operating fermentors or bioreactors. Alternatively, commercial production of specific peptide sequences could be enabled through recombinant enzyme technology utilizing certain production strains, or through the use of purified proteolytic enzymes isolated from suitable microorganisms. Furthermore, molecular studies are needed to assess the mechanisms by which the bioactive peptides exert their activities. This research area is currently considered as the most challenging one, due to the understanding that most known bioactive peptides are not absorbed from the gastrointestinal tract into the blood circulation. Their effects, therefore, are likely to be mediated directly in the gut lumen or through receptors on the intestinal cell wall. In this respect, the target function of the peptide concerned is of utmost importance. It is anticipated that in the near future such targets shall be the following lifestyle-related disease groups: a) cardiovascular diseases, b) cancers, c) osteoporosis, d) stress and d) obesity. Peptides derived from dietary proteins offer a promising approach to prevent, control and even treat such disease conditions through a regulated diet.
8.0 Suggested Readings


1.0 Introduction

Biotechnology contrary to its name is not a single technology. Rather it is a group of technologies that share two features – working with living cells and their molecules and having a wide range of applications that can improve quality of our lives and raise the economic status of our population and alleviate poverty. Biotechnology can be broadly defined as ‘using organisms or their products for commercial purposes’. Recent developments in molecular biology have given biotechnology new meaning, new dimensions and prominence, as well as new potential. It is modern biotechnology that has captured the attention of the public. Modern biotechnology can have a dramatic impact on the world economy and society. One example of modern biotechnology is genetic engineering. Genetic engineering is the process of transferring individual genes between organisms or modifying the genes in an organism to remove or add a desired trait or characteristic.

2.0 How Does Modern Biotechnology Work?

In order to recognize the immense potentials of Biotechnology, we need to know how does it work at cellular and molecular level to bring about transformation of new material into a useful product at large scale. All organisms are made up of cells that are programmed by the same basic genetic material, called DNA (deoxyribonucleic acid). Because all organisms are made up of the same type of genetic material (nucleotides A, T, G, and C), biotechnologists use enzymes to cut and remove DNA segments from one organism and recombine it with DNA in another organism. This is called recombinant DNA (rDNA) technology and it is one of the basic tools of modern biotechnology. rDNA technology is the laboratory manipulation of DNA in which DNA, or fragments of DNA from different sources, are cut and recombined using enzymes. This recombinant DNA is then inserted into a living organism. rDNA technology is usually used synonymously with genetic engineering. This technology allows researchers to move genetic information between unrelated organisms to produce desired products or characteristics or to eliminate undesirable characteristics. Genetic engineering is used in the production of drugs, human gene therapy, and the development of improved agriculture and animal productivity including milk yield and composition.

Modern biotechnology also offers effective techniques to address food safety concerns. Biotechnical methods may be used to decrease the time necessary to detect food borne pathogens, toxins, and chemical contaminants, as well as to increase detection
sensitivity. Enzymes, antibodies, and microorganisms produced by using rDNA techniques are currently being explored to monitor food production and processing systems for quality control.

3.0 Industrial Biotechnology

Industrial biotechnology applies the techniques of modern molecular biology to improve the efficiency and reduce the environmental impacts of industrial processes. For example, industrial biotechnology companies develop biocatalysts, such as enzymes, to synthesize chemicals. Enzymes are proteins produced by all organisms. Using biotechnology, the desired enzyme can be manufactured in commercial quantities.

Traditional chemical synthesis involves large amounts of energy and often undesirable products, such as HCl. Using biocatalysts, the same chemicals can be produced more economically and more environmentally friendly. An example would be the substitution of protease in detergents for other cleaning compounds. Detergent proteases, which remove protein impurities, are essential components of modern detergents. They are used to break down protein, starch, and fatty acids present on items being washed. Protease production results in a biomass that in turn yields a useful byproduct – an organic fertilizer.

4.0 Human Applications

Biotechnical methods are now used to produce many proteins for pharmaceutical and other specialized purposes. A harmless strain of *Escherichia coli* bacteria, given a copy of the gene for human insulin, can make insulin. As these genetically modified (GM) bacterial cells age, they produce human insulin, which can be purified and used to treat diabetes in humans. Microorganisms can also be modified to produce digestive enzymes. Gene therapy – altering DNA within cells in an organism to treat or cure a disease – is one of the most promising areas of biotechnology research. New genetic therapies are being developed to treat diseases such as cystic fibrosis, AIDS and cancer.

5.0 Application of Biotechnology in Food Sector

5.1 Probiotics

Microorganisms are naturally present in the digestive system of the animals. Some microbes aid digestion and others can potentially cause pathogenesis. Use of antibiotics disturb the microbiological balance of gut flora eliminating most of the beneficial flora. Using probiotics can help build up the beneficial bacteria in the intestine and competitively exclude the pathogenic bacteria. Probiotic bacteria are sold mainly in fermented foods, and dairy products. These foods are well suited in promoting the positive health image of probiotics for following reasons:

- Fermented foods, and dairy products in particular, already have a positive health image.
- Consumers are familiar with the fact that fermented foods contain living microorganisms (bacteria)
Probiotics used as starter organisms combine the positive images of fermentation and probiotic cultures.

When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product and become active when entering the consumer’s gastrointestinal tract. These factors include:

- The physiologic state of the probiotic organisms added (whether the cells are from the logarithmic or the stationary growth phase).
- The physical conditions of product storage (Temperature),
- The chemical composition of the product to which the probiotics are added (Acidity, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content)
- Possible interactions of the probiotics with the starter cultures (Bacteriocin production, antagonism, and synergism). The interactions of probiotics with either the food matrix or the starter culture may be even more intensive when probiotics are used as a component of the starter cultures.

An unappreciated problem faced by the dairy industry is the lack of species identification among the food grade organisms. Conventional phenotypic methods, however, are not suitable for identification of probiotic LAB, and are often ambiguous. Genetic methods and genetic analysis of genome sequences have paved the way for recent molecular techniques for identification. The commonly used molecular techniques for identification of these organisms include Polymerase Chain Reaction with group specific primers, Dot Blot Hybridization, Terminal Restriction Fragment Length Polymorphism (TRFLP), Fluorescent in situ Hybridization (FISH), Density Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE) and so on. Genomic sequences and bioinformatics present volumes of information for rational selection of genes for identification, confirmation and characterization of functional roles of lactobacilli. Some of the important genes that are encoded in genome of *L. acidophilus* NCFM are S layer protein (Slp-A and –B), Mub-9 (Mucus binding protein), BSH A and B (Bile salt hydrolases), PrLA-I and PrLA-II (phage ruminants), EPS cluster (endopolysaccharide) and Lactocin –B (bacteriocin). So, by targeting the gene responsible for particular probiotic functions, appropriate specific primers can be designed to explore and develop suitable PCR techniques for identification of probiotic lactobacilli. This strategy could be extremely valuable in the search for new and promising indigenous probiotic cultures for application in production of health foods, nutraceuticals and other value added products.

Most of the probiotic cultures available are of foreign origin and cannot be used for commercial application in India. Hence, there is a need for having our own indigenous cultures. In this regard, a DBT network project has been recently sanctioned with NDRI as the co-ordinating center to develop a repository of indigenous probiotic cultures from different regions and their characterization at strain level by molecular approaches.

5.2 Recombinant Enzymes

For many thousands of years, man has used naturally occurring microorganisms like bacterial, yeasts and moulds – and the enzymes they produce to make foods such as bread, cheese, beer and wine. For example in bread-making the enzyme, amylase, is used
to break down flour into soluble sugars, which are transformed by yeast into alcohol and carbon dioxide (makes the bread rise). Other applications of biotechnology include production of milk that can be consumed by lactose intolerant people, improved fermented products, production of natural preservatives in milk, and methods for treating and processing waste products for further use or non-damaging disposal.

### Table 1: Marketed Enzymes Produced Using Gene Technology

<table>
<thead>
<tr>
<th>Principal enzyme activity</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-acetolactate decarboxylase</td>
<td>Brewing</td>
</tr>
<tr>
<td>Alpha-amylase</td>
<td>Baking, brewing, distilling, starch</td>
</tr>
<tr>
<td>Chymosin</td>
<td>Cheese</td>
</tr>
<tr>
<td>Beta-glucanase</td>
<td>Brewing</td>
</tr>
<tr>
<td>Glucose isomerase</td>
<td>Starch</td>
</tr>
<tr>
<td>Hemicellulase</td>
<td>Baking</td>
</tr>
<tr>
<td>Lipase</td>
<td>Fats, oils</td>
</tr>
<tr>
<td>Microbial rennet</td>
<td>Dairy</td>
</tr>
<tr>
<td>Xylanase</td>
<td>Baking, starch</td>
</tr>
</tbody>
</table>

#### 5.3 Milk Clotting Enzymes

Most of the today’s hard cheese products are made with a biotech enzyme called chymosin. This is produced by genetically engineered bacteria, which is considered more pure and is available in plentiful amounts than its naturally occurring counterpart, rennet, which is derived from calf stomach tissue. Biotechnology now makes it possible to replace rennet from calves’ stomachs in cheese production. Cheese enzymes produced by microorganisms provide an alternative to animal rennet and a more reliable supply for cheese makers. Traditionally, cheese has been made using rennet, which is obtained from the stomachs of very young calves. Because of public health concerns regarding the use of rennet pastes, some consumers asked for non-animal substitutes. Chymosin replaces animal rennet as the coagulant ingredient in making cheese.

This also eliminates possible public concerns with animal derived material. Moreover, these enzymes guarantee a constant supply. This is important in times when animal farm production have decreased the number of calves available for slaughter, and thus the supply of rennet. Also to reduce the cost of rennin, gene of calf rennin enzyme is being cloned in bacteria and yeasts. Genetically engineered fungi bearing gene of calf cells for rennin enzyme are now used in the UK to obtain rennet enzyme preparation. Enzymes offer an animal friendly alternative to animal rennet. While providing constant quality, they are also less expensive. Our group at NDRI, Karnal has achieved the expression of buffalo prochymosin in E. coli at 10 mg/litre level and is currently engaged in hyper expression of
the same in a Pichia host for large scale production. The recombinant buffalo chymosin could be an effective substitute to bovine calf rennet and particularly suitable to process buffalo milk to cheese.

5.4 Galactosidase

Galactosidase is produced by variety of microorganisms. Lactose is hydrolyzed to glucose and galactose. Commercially available enzymes are derived from *Kluyveromyces lactis*, *Aspergillus niger* or filamentous fungi. Yeast enzymes are used for hydrolysis of lactose in milk while fungal enzymes are used for hydrolysis of lactose in whey. The major applications of galactosidase are found in :

→ Liquid milk and milk powder, to improve the product for lactose – intolerant individuals, or to increase the sweetness of milk based drinks.
→ Concentrated milk products, to prevent crystallization of sugars.
→ Fermented milk products, to increase fermentation rate.

5.5 Lipase

Lipases of triacylglycerol hydrolases catalyze hydrolysis of the ester bonds of triacylglycerols. These enzymes have been found in many species of animals, plants and microorganisms. In particular, lipases from fungi are important in industrial applications, and have been widely used for biotechnological applications in dairy industry, oil processing, and production of surfactants and preparation of pure pharmaceuticals. Lipase, another enzyme produced by microorganisms, can replace the lipase today extracted from animal glands.

The exponential increase in the application of lipases in various fields in the last few decades’ demands extension in both qualitative improvement and quantitative enhancement. Quantitative enhancement requires strain improvement and medium optimization for the overproduction of the enzyme, as the quantities produced by wild strains are usually too low. The spectacular success examples of strain improvement in industry are mostly attributed to the extensive application of mutation and selecton. Improvement of microbial strains for the over production of industrial products has been the hallmark of all commercial fermentation processes. Such improved strains can reduce the cost of the process with increased productivity and may also possess some specialized desirable characteristics. Effectiveness of UV radiation (Physical mutagen) and NTG (N-methyl-N’-nitro-N-nitroso guanidine, a chemical mutagen) in strain improvement for enhanced lipase productivity has been done.

Lipases are also used in certain Italian cheeses to given them their characteristic flavour. These enzymes are used to create natural flavour and not for development of flavour concentrate. An example is the use of Picantase, a lipase produced by the fungus *Mucor michiei*.

5.6 Lactoferrin

Lactoferrin is an iron-binding glycoprotein present in most mammalian body fluids. It is a component of the host defense against microbial infection. Antimicrobial peptides have
been derived from the whey protein lactoferrin. Digestion with pepsin releases peptides from bovine and human lactoferrin, which have 10 to 1000 times' greater bactericidal activity than intact lactoferrin. However, the antimicrobial mechanism of Lactoferrin is more complex than simple binding of iron. Lactoferricin (bovine Lactoferrin f17-41) is a pepsin-derived peptide from bovine lactoferrin, which has antimicrobial activity against Gram-negative bacteria and Candida albicans. The peptide is a more potent antimicrobial agent than undigested lactoferrin because it causes disruption of the bacterial cell membranes. Our group is involved in cloning and expression of lactoferrin from different mammalian species in E. coli and family shuffling of these genes to further enhance its functional properties. We are also expressing human lactoferrin in Pichia pastoris for large scale production for its application in infant formulae and other incorporation in other dairy products.

5.7 Lysozyme

Lysozymes are the enzymes that cleave the –β-1,4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid of peptidoglycan found in the cell wall of microorganisms. Thus causes lysis of the cell. Lysozyme is yet another potential target for production by recombinant DNA technology for different application in the food sector. Our group has cloned and sequenced buffalo lysozyme in E. coli.

5.8 Bioactive Peptides

Milk proteins are currently the main source of a range of biologically active peptides, even though other animal and plant proteins contain potential bioactive sequences. These peptides, which are encrypted within the sequence of the parent proteins, can be released by enzymatic proteolysis, for example, during gastrointestinal digestion or during food processing.

Proteolysis by naturally occurring enzymes in milk, coagulants and by microbial enzymes, especially from lactic acid bacteria (LAB) used as starters, generate bioactive peptides (BPs) during milk fermentation and cheese maturation, thereby enriching the dairy products. Once produced, BP may act in the body as regulatory compounds which a hormone-like activity. Concentrates of these peptides are potential health-enhancing nutraceuticals for food and pharmaceutical applications. The bioactivities in milk include modulators of digestive an gastrointestinal functions, hemodynamic modulators with potential effects on the gastrointestinal tract, hormones and growth factors. The proteolytic activity for releasing the BPs varies with differentiation or functional state, or intramammary infection of the mammary gland.

The type of starter used is one of the main factors that influence the synthesis of antihypertensive or opiate BPs in fermented milks; for example, proteolysis by Lactobacillus helveticus is related to the production of antihypertensive peptides. LAB, used as starter microorganisms in cheese making or constituting the endogenous lactic acid micro flora of milk, release many different oligopeptides from cell wall-envelope proteinases (CEP), and peptidases hydrolyse such oligopeptides into shorter fragments and amino acids that directly, or as precursors, contribute to the flavor. Some of these peptides are bioactive.
Functions of bioactive peptides are:

- Opioid peptides (a and \(-\)-casomorphins and lactorphins act as opioid agonists, while casoxins behave as opioid antagonists).

- Peptides inhibitory to Angiotensin-I Converting Enzyme (ACE) (Peptides from a S1- and \(-\)-CN and human \(-\)- and k-CN).

- Immuno0stimulating peptides (Immuneptides obtained from a-S1- and \(-\)-CN and a-lactalbumin).

- Antithrombotic peptides (casoplatehins)

- Peptides inhibitory to HIV-1 proteinase

- Antimicrobial properties (Lactoferricin B, Isracidin

- Mineral binding (Phosphopeptides)

The protein fraction of milk contains many valuable components and biologically active substances. Moreover, milk proteins are precursors of many different biologically active peptides, which are inactive within the sequence of the precursors protein but can be released by enzymatic proteolysis. Many milk protein-derived peptides, such as caseinophosphopeptides, reveal multifunctional bioactivities. Caseinophosphopeptides can form soluble organophosphate salts and may function as carriers for different minerals, especially calcium. Furthermore, they have been shown to exert cytomodulatory effects. Cytomodulatory peptides inhibit cancer cell growth or they stimulate the activity of immuno competent cells and neonatal intestinal cells, respectively.

Several bioactive peptides derived from milk proteins are potential modulators of various regulatory processes in the body and thus may exert beneficial physiological effects. Caseinophosphopeptides are already produced on an industrial-scale and as a consequence these peptides have been considered for application as ingredients in both ‘functional foods’ and pharmaceutical preparations. Although the physiological significance as exogenous regulatory substances is not yet fully understood, both mineral binding and cytomodulatory peptides derived from bovine milk proteins are claimed to be health enhancing components that can be used to reduce the risk of disease or to enhance a certain physiological function. Casomorphins have also been produced by genetic engineering techniques followed by enzymatic or chemical cleavage of the microbial fusion protein to liberate the required peptide.

Food researchers are presently considering different bioactive peptides as health enhancing ingredients for use in functional foods and nutraceuticals. Several naturally occurring bioactive peptides may also be used as highly active drugs having a well-defined pharmacological effect, for example in the treatment of dental diseases as well as mineral malabsorption (casein phosphopeptides) and immunodeficiency (Immuneptides). The production of desirable bioactive peptides during food processing, e.g. by use of specific enzymes or genetically transformed microorganism, is an area of interest for research.

5.9 Single Cell Protein (SCP)

Microbial proteins produced by yeasts, bacteria, algae, fungi for animal and human food purpose are referred to as SCP. To overcome global food shortage, protein, proteinmalnutrition diseases like Kwashiorkor, Marasmus, cheaper and abundant food supplies that can prove as better alternatives to today’s animal and human food, are required. SCP
productions using oil-based chemicals or methanol consumption or paper industry wastes or potato wastes as food are all the gifts of fermentation technology. However, progress is fast with the biotechnology concepts which look to the disposal of wastes, reduction environmental problems and to produce low-cost feed for animals as simultaneous aims. Also the much-required characteristics are introduced in the SCP organism with genetic engineering techniques. Waste from fruit pulp, confectionery, and pineapple canning, etc. have a good amount of fermentable sugar and are suitable for *Candida utilis*. For industrialized countries, DCP production is an antipollution measure, since wastes are converted into products and high BOD/COD problem in effluent solved.

5.10 Nutraceuticals (Vitamins and Mineral Synthesis)

A nutraceutical is any substance that is a food or a part of a food that provides medical or health benefits, including prevention and treatment of disease. Micronutrient malnutrition is still a problem of unacceptable proportions in developing countries. This is underdeveloped countries is also being combated with biotechnology. Vitamin A deficiency is a common problem in poor countries. Vitamin A deficiency impairs growth, development, vision, and immune systems, and in severe cases can lead to blindness and death. Almost one-third of children in developing countries area affected to some degree by vitamin A deficiency and many more are iron deficient. Micronutrient deficiencies can be addressed by distributing vitamin and mineral supplements, by fortifying foods, or through food-based strategies, which attempt to modify people’s diets. Fermentation improves the bioavailability of minerals such as iron and zinc as a result of phytic acid hydrolysis. It also has other nutritional advantages such as increasing the content of riboflavin and vitamin B12.

5.11 Fibre for Human Consumption

Genetically engineered yeast has been used to obtain a range of fibers for human consumption. It is called fibercell and is glucan of yeast cell wall and is related to glucans that form fiber components of oat etc. It has desirable properties such as controllable bulking, controllable degradation by bacteria in colon and cholesterol reducing properties. It is tasteless, odorless and nongelling. The product contains 90% fiber as compared to 18-20% in the oat bran. Very soon the product will be commercialized as food additive.

5.12 Concerns About Biotechnology

As biotechnology has become widely used, questions and concerns have also been raised. One of the main areas of concern is the safety of genetically engineered food. IN assessing the benefits and risks involved in the use of modern biotechnology, there area a series of issues to be addressed so that informed decisions can be made. In making value judgments about risks and benefits in the use of biotechnology, it is important to distinguish between technology-inherent risks and technology-transcending risks. The former includes assessing any risks associated with food safety and the behavior of biotechnology-based product in the environment. The latter involve the political and social context in which the technology is used, including how these uses may benefit or harm the interests of different groups in society. The health effects of foods grown from genetically engineered crop depend on the composition of the food itself. Any new product may have either beneficial or occasional harmful effects on human health. These risks area systematically evaluated by FDA and identified prior to commercialization.
Most of the industry leaders interviewed are quite enthusiastic about the benefits of biotechnology – especially in terms of increased food availability, enhanced nutrition, and environmental protection. Most feel that biotechnology has already provided benefits to consumers. Almost all recognize that foods developed through biotechnology have already been part of consumers’ everyday diet. They clearly do not agree with most of the opponents’ claims and tend to have almost no trust in such groups. Their main concerns involve lack of consumer acceptance – not the safety of the foods. They express high levels of confidence in the science and the regulatory process. In fact, almost none feel that biotechnology should not be used because of uncertain, potential risks. Most food industry leaders do not feel it is necessary to have special labels on biotech-derived foods. They express concerns that consumers would perceive such labels as a warning. They also worried that the need to segregate commodities would post financial and logistical burdens on everyone in the system – including consumers. Food industry leaders recognize a major need to educate the public about biotechnology. They look to third parties, such as university and government scientists to provide such leadership.

Research shows that consumers will accept biotech foods if they see a benefit to themselves or society and if the price is right. Their responses to foods developed through biotechnology are basically the same as for any other food – taste, nutrition, price, safety and convenience are the major factors that influence our decisions about which foods to eat.

6.0 Conclusion

The applications of biotechnology are so broad, and the advantages so compelling, that virtually every industry is using this technology. Biotechnology is enabling the industries to make new or better products, often with greater speed, efficiency and flexibility. Biotechnology holds significant promise to the future but certain amount of risk is associated with any area. Biotechnology must continue to be carefully regulated so that the maximum benefits are received with the least risk. The future of the world food supply depends upon how well scientists, government, and the food industry is able to communicate with consumers about the benefits and safety of the technology.
1.0 Introduction

Understanding consumer needs and preferences are critical to successful marketing and enhancing marketing value of a product. Value added food products are gaining more attention in the marketplace, and an increasing number of markets are capitalizing on this interest. This stems from the fact that consumers are more sophisticated and aware. Present day consumers prefer foods that promote good health and prevent disease. Furthermore, these foods must fit into current lifestyles providing convenience of use, good flavor, and an acceptable price-value ratio. Such foods constitute current and future waves in the evolution of the food development cycle.

With increased competitive pressures, low prices and large volumes may not suffice as strategic advantage in the long-term. One way of gaining competitive advantages requires finding new ways of creating value addition based on technological development. It represents a technological upgrading process that leads to the production of value added products. The functional food market is a rapidly expanding market. It has characteristics of a changing market where flexible strategies are reflected in production and consumption of particular products.

In today’s arena, food safety is the primary topic in any discussion of food stability. Food safety encompasses over all the issues surrounding food preparation, processing, packaging and storage. Outbreaks of pathogens such as Listeria monocytogenes have prompted the food industry, the public and the governments to question the adequacy of current methods of food preservation. Food preservation is a vital parameter for ensuring the food safety. Besides, the consumption of more food that has been formulated with chemical preservatives has increased consumer concern and created a demand for more natural and minimally processed foods. In the recent years there has been a great interest in naturally produced antimicrobial agents. One promising choice appears to be the use of antimicrobial peptides or proteins produced by bacteria generally known as bacteriocins. The lactic acid bacteria (LAB) are the potential candidates for bacteriocin production and, in fact, have been exploited as reservoirs for antimicrobial peptides with food application.
2.0 Bacteriocins

Bacteriocins, as originally defined by Tagg et al. (1976), are ribosomally synthesized extra-cellular proteinaceous compounds that kill or inhibit closely related bacteria. This definition has been broadened somewhat in recent years, in that it has become evident that some bacteriocins may have inhibitory spectra, which include unrelated genera. Generally, bacteriocins are low molecular weight, cationic, amphiphilic, peptides which tend to aggregate and are benign to the producing organism. In cases where the mode of action is known, the cell membrane is usually the site of action. These agents are generally heat-stable, yet are apparently hypoallergenic and are readily degraded by proteolytic enzymes in the human gastro intestinal tract. They can be produced spontaneously or induced by certain chemicals such as mitomycin C. They are biologically one of the important substances, and have been found to be useful in membrane studies and also in typing pathogenic microorganisms causing serious nosocomial infections. The investigations show significant economic potential of bacteriocins in food and biomedical fields. Some of these have value to the system by virtue of their natural presence, but others have enough activity to warrant their use as part of a formulated preservative system and may further add value to the finished product.

3.0 Classification of Bacteriocins of LAB

Bacteriocins are a heterogeneous group of particles with different morphological and biochemical characteristics. They range from a simple protein to a high molecular weight complex: the active moiety of each molecule in all cases seems to be protein in nature. Bacteriocins from LAB have been classified, initially by Klaenhammer (1993), in four classes on the basis of common, mainly structural, characteristics. Later reviews restricted the LAB-produced bacteriocins to three classes because the existence of a fourth class as proposed by Klaenhammer (1993), i.e. complex compounds needing a carbohydrate or lipid moiety for activity, had been based on unpurified, and therefore ill-defined, compounds. Class I bacteriocins are small, heat-stable peptides containing thioether amino acids, like lanthionine, and are for this reason named lantibiotics. Class II are small, hydrophobic, heat-stable, nonmodified bacteriocins consisting of either a single peptide with antilisterial activity (class IIa) or two polypeptide chains (class IIb), and also include other peptide bacteriocins (class IIc). Class III consists of large, hydrophilic, heat-labile proteins. Even if chemical, enzymatic or physical characteristics of the food, food processing, or the physiological state of the bacteriocin producing micro-organism can limit the bacteriocin activity in situ, it has been claimed that micro-organisms producing bacteriocins possess a competitive advantage over other organisms living in the same natural environment (Vaughan et al., 2001).

4.0 Bacteriocins in Biopreservation of Foods

In response to various consumer concerns, efforts have been made to introduce minimal processing technologies and to find alternative food grade preservatives. Dairy products are nutrient-dense foods that are important for good health. At the same time, they are highly perishable commodities and attention is required for their preservation. The principle of ‘biopreservation’ involves the use of antagonistic microorganisms, or their metabolites, to inhibit or destroy undesired microorganisms in food. Bacteriocins are examples of metabolites that have considerable potential in the realm of bio-preservation. As broad spectrum bacteriocins inhibit a large number of food pathogenic organisms it is particularly suitable to use them as bio-preservatives in foods. One such bacteriocin, nisin,
has been granted GRAS (Generally Regarded as Safe) status by Food and Drug Administration and is widely used as biopreservative in more than 48 countries in a number of food products, e.g., semi-hard & hard cheeses (prevents late blowing), processed cheese, cheese spread (to inhibit C. butyricum, C. tyrobutyricum), sterilized milk (to inhibit thermophilic heat resistant spore formers), milk powder, canned foods, low acid foods and meat products. In other nations, nisin is used on a much wider scale as a food antimicrobial. Nisin and pediocin (a bacteriocin produced by Pediococcus acidilactici) have been demonstrated to be active against Listeria monocytogenes and other Gram-positive bacterial pathogens in fluid milk. Use of Pediocin PA-1 produced by Pediococcus acidilactici in cottage cheese, half and half cream and cheese sauce systems has been found to be effective in controlling the growth of Listeria monocytogenes. Pediocin 34, a broad-spectrum bacteriocin produced by Pediococcus pentocaseus 34 has been found to be quite effective in increasing the shelf life of some of the indigenous dairy products. Enterocins, the bacteriocins from enterococci have shown a potential for dairy applications as biopreservatives as these are insensitive to rennet and heat and have stability over a wide range of pH. Recently some strains of Streptococcus thermophilus have been known to produce bacteriocins, known as thermophilins. In general, these thermophilins are stable and active over a wide range of pH and temperature and can be used as biopreservatives.

5.0 Hurdle Technology

Bacteriocins have applications in hurdle technology, which utilizes synergies of combined treatments to more effectively preserve food. Some of the strains of LAB may naturally produce more than one bacteriocin and heterologous expression of bacteriocins has been demonstrated in constructed strains. Protein engineering has led to the development of nisin derivatives with altered anti microbial activities or greater solubility at pH 6.0 than the wild type nisin. Most effective combinations of hurdles can be made through an understanding of the mechanism of action of each hurdle for e. g. the incorporation of an emulsifier (Tween 80) along with pediocin AcH exhibits higher listericidal activity in slurries of skim milk and butter milk. Nisin in combination with a chelating agent and a surfactant has been found to be inhibitory to Gram positive and negative organisms in cheese. In milk and whey, combination of hydrostatic pressure and bacteriocin (Lactacin 3147) has proved effective in increased killing of S. aureus and L. monocytogenes. The combination of hurdles from vacuum packaging, refrigerated storage and bacteriocin inhibition would provide a product with enhanced microbial stability.

6.0 Strengthening Probiotics

Probiotics are live microbial food supplements, which benefit the health of consumers by maintaining, or improving their intestinal microbial balance. Probiotics have been shown to play beneficial roles in gut health, including reducing the duration or severity of a variety of diarrheal diseases and helping to protect against infectious diseases, by decreasing Helicobacter pylori infections. Additional possible health benefits attributed to probiotics include a decrease in colon cancer risk, enhancement of the immune system, reduction in symptoms of inflammatory disorders such as inflammatory bowel diseases, alleviation of some symptoms of irritable bowel syndrome, and prevention of allergic symptoms. Bacteriocin production is considered as one of the performances or a functional characteristic of probiotic strains as it helps to establish the probiotic organism in competitive environment of gut. Due to their perceived health benefits probiotic bacteria have been increasingly included in yoghurts and fermented milks during the past two
decades. Most commonly these bacteria have been lactobacilli such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. Probiotics, prebiotics and associated ingredients might add an attractive dimension to cultured dairy foods for augmenting current demand for functional foods. Furthermore, milk constituents of known or conjectured physiological effects add yet another dimension to the healthy profile of the food. Today, plenty of evidence exists on the positive effects of probiotics on human health. However, this has usually been demonstrated in diseased human populations only. Thus there is an urgent need for evidence for probiotic health benefits in average (generally healthy) populations. Probiotic strains with good bacteriocin production may serve the dual purpose of food preservation and possessing the health benefits as well.

7.0 Bacteriocin Producers as Protective Cultures/ Starter Cultures

Since lactic acid bacteria are commonly used as starter cultures in food fermentations, investigators have explored the use of bacteriocin producers as protective cultures. Till now the bacteriocin producers have been efficiently used as bioprotective/adjunct cultures, in various foods along with starter culture. While acid production is an important technological feature for a starter culture and antimicrobial activity being only of secondary concern, the contrary is true for a bacteriocinogenic strain to be used as a protective culture or as a co-culture. Recently, the focus is shifting towards the selection of starter cultures with the ability to provide additional attributes like exo-polysaccharides production, probiotic ability and/or bacteriocins production. The use of such functional starter cultures in fermented dairy products is preferred for both product quality improvement and preservation as compared to conventional starter cultures. Bioprotective cultures may act as starter cultures in the food fermentation process or they may protect foods without any detrimental organoleptic changes. Natural bacteriocin producers, such as *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Enterococcus faecalis* and *Enterococcus faecium* have been used as protective cultures in various products e.g. the outgrowth of clostridia spores in cheese milk was completely prevented when a nisin A producing strain was mixed at 10% rate with the starter culture. *Bacillus cereus*, a food poisoning bacterium, had been found to be inhibited by the bacteriocinogenic strain of *Enterococcus faecalis* AS 48-32 in milk and in a nonfat hard cow’s cheese. The use of bacteriocin producing *E. faecalis* strain for the manufacture of cheese has been reported to inhibit the growth of *L. monocytogenes* by 6 log cycles. Bacteriocin producing starter cultures have been used to prevent histamine formation by amine forming lactobacilli in cheese.

8.0 Antimicrobial Active Packaging

“Active packaging” is a new term and a newer approach for introducing hurdles to both oxidation and microbial growth. Incorporating preservatives directly into packaging materials creates an additional line of defense. BHA and BHT have worked well in the past as a constituent of packaging material, providing an antioxidant barrier at the food/packaging interface.

Active packaging (AP) performs some desired role other than providing an inert barrier between the product and external conditions and combines advances in food technology, biotechnology, packaging and material science, in an effort to comply with consumer demands for ‘fresh like’ products. The incorporation of anti-microbial agents directly into polymeric packaging is an exciting development, which allows industry to combine the preservative functions of anti-microbial with the protective functions of preexisting packaging concepts. One extensively discussed option is the incorporation of
silver-containing zeolite to the contact surface of polymer films. There are other synthetic and naturally occurring compounds that may be exploited by the packaging industry. These include organic acids, bacteriocins, spice extracts, chelating agents, antibiotics and enzymes, etc. The use of bacteriocins and other biologically derived antimicrobials in packaging materials is attracting increasing interest in recent times, particularly for their antilisterial activity, and patents have been filed in this area. Bacteriocins are adsorbed to the packaging materials, which would ideally be in direct contact with a food surface. The stability of the ensuing materials has been ascertained using activity retention tests and their functionality in food systems has also been determined.

Additionally, it has been documented that immobilizing antimicrobials by incorporating into edible alginate gels or spray application of nisin to the surface of foods followed by vacuum packaging enhances their antimicrobial decay. Adsorption of bacteriocins to plastic surfaces and to siliconized surfaces with retention of activity has been accomplished. Combining the bacteriocin directly into a plastic material could provide several advantages as a bacteriocin delivery mechanism. First, only the necessary amount of bacteriocin would be used. Secondly, the agent would not be a direct additive to the food product. Thirdly, if the plastic material were made from an edible and/or biodegradable plastic, environmental advantage would be realized.

9.0 Conclusions

Research during the past two decades has revealed that bacteriocins have properties that make it one of the most commercially important metabolite of lactic acid bacteria with massive use in biopreservation and hurdle technology. Furthermore, the exploitation of bacteriocins in active packaging technology and biotherapeutics reveal its advantages in the current scenario. The implementation of carefully selected bacteriocin producing strains as starter cultures or co-cultures in fermentation processes can help to achieve in situ expression of the desired property, maintaining a perfectly natural and healthy product without affecting the organoleptical quality. Thus the judicious and effective use of bacteriocin in dairy industry may enhance the value of final product in terms of quality, shelf life and consumers’ preference.

10.0 Suggested Readings


1.0 Introduction

Numerous dairy products include a fermentation step during manufacturing, the most common being natural and fresh cheese, sour cream and yogurt. To achieve that step, food-grade, lactose-fermenting bacterial starter cultures are added to milk prior to processing. Traditionally, culture usage has been limited to a specific rotation of strains for each fermented dairy food, with minimal understanding of the biochemical pathways involved. Starter cultures for fermented foods are today developed mainly by design rather than by screening. The design principles are based on knowledge of bacterial metabolism and physiology as well as on the interaction with the food product. In the genomics era, we have improved the understanding of aspects of gene regulation, gene expression and gene stability in lactococci: all of which are very important when developing commercial starter cultures. The design tools available are food grade tools for genetic, metabolic and protein engineering and an increased use of laboratory automation and high throughput screening methods. The large body of new data will influence the future patterns of regulation. It is currently difficult to predict in what direction the future regulatory requirements will influence innovation in the food industry. It can either become a promoting force for the practical use of biotechnology to make better and safer products, or it can be limiting the use of starter cultures to a few strains with official approval. A scientific basis for dramatic innovations that could transform the culture industry is currently being established. Successful cultures based on modern technology are being developed in areas as given below.

- Probiotics
- Bacteriocins and bioprotection
- General improvement of yield
- Improvement of performance for the existing culture market
- Introduction of cultures for fermenting other food products
- Improvement in flavour
- Thicker body and improved mouthfeel
- Phage resistance
2.0 Novel Approaches

Microorganisms associated with milk are extremely important in the bioprocessing, safety and spoilage of dairy foods. Historically, however, the selection of culture strains for use in dairy foods has been based on trial and error in standard growth studies. Thanks to advances in biotechnology during the past decade, researchers are now able to use genetic and microbial techniques to gain a better understanding of how these microorganisms function at the molecular level. Today's researchers first use genetics to definitively identify and characterize these microorganisms, which in turn allows a better understanding of their biochemical pathways. Once a culture’s biochemical pathways are understood, genes that express or control certain traits can be identified and isolated.

These gene segments can then be transferred between organisms to modify or adapt traditional starter cultures and non-traditional dairy cultures, enhancing their functionality in dairy foods. The most common approaches are either intergeneric, which refers to genetic transfers between organisms within the same family but of different genera, or intragenic, which refers to transfers within the same family and same genera. Intragenic transfers occur naturally through evolutionary processes and can be directed in research laboratories. The least common approach is transgenic, or the transferring of genes between different families. The dairy industry today uses a microbial-produced recombinant chymosin, which is the result of expressing a calf gene in bacteria. The enzyme chymosin, which is identical to the calf rennet, is then purified from the bacteria and used to coagulate milk in dairy fermentations. Newer possibilities are being explored based on genetically modified cultures for enhanced functionality.

2.1 Optimization of Performance of Cheese Starter Cultures

The primary reason starter cultures are used in cheese manufacturing is to ferment lactose into lactic acid. This is important for proper texture and flavor development in each cheese variety. Thus, starters influence the efficiency of the cheese make process (based upon rate of acid production), as well as the quality of the finished product. The ideal acid-production rate varies, depending on the desired moisture, pH and calcium levels needed to provide the characteristics of the cheese variety being produced. Until recently, the biochemical pathways controlling acid production were not well understood. However, biotech advancements have enabled researchers to understand how starter cultures function, and hence, to select specific cultures to achieve a desired acid-production rate.

Improved Milk Coagulation—Researchers at the Minnesota South Dakota Dairy Foods Research Center have identified a slow milk-coagulating mutant strain of the starter *Lactococcus lactis* ssp. *lactis* C2. They determined that the strain lacked pyruvate carboxylase activity, which is required for the proteolysis that contributes to milk coagulation. By cloning, sequencing and characterizing the pyruvate carboxylase gene from the parental strain, researchers were able to identify the gene deficiency in the mutant strain. Armed with this improved physiological understanding of lactococci in milk fermentations, researchers are currently applying their knowledge to identify optimal starter culture strains for the manufacture of Cheddar cheese.
Reduction in bitterness of Cheese- Bitter peptides produced early in the cheesemaking process by chymosin and starter bacteria can be degraded by intracellular peptidases from starter and adjunct cultures. To better understand the process, scientists at the Western Dairy Center in Logan, UT, and the Wisconsin Center for Dairy Research in Madison, are using genetics to identify and characterize the microbial enzymes responsible for both the production and degradation of bitter peptides in Cheddar cheese. Results will help identify or develop starter systems that eliminate or control the bitter flavor defect in Cheddar cheese. Work is also going on to construct Lactococcus lactis derivatives with enhanced activity of peptidases. These strains could be used in industry to reduce the incidence of bitterness in bacterial ripened cheeses. For example, the proteinase specificity of the starter culture is a major determinant in whether bitterness will develop.

Controlled and Accelerated Cheese Ripening-Also at the Western Dairy Center, researchers are developing a process-regulated expression system for dairy starter cultures that could be used to closely regulate enzyme production in cheese starter bacteria. This will enable the cultures to produce high levels of a desired enzyme during a specific stage of cheese manufacture. To accomplish this, researchers have completed a nucleotide sequence analysis of the Lactobacillus helveticus LH212 groESL operon and have confirmed that the groESL promoter is tightly regulated at the transcription level by heat shock. Further experiments will enable researchers to determine whether a natural metabolic switch can be employed to avoid problems presently associated with the addition of proteolytic enzymes to cheese. Results from this work will provide basic and applied information needed by the industry to control and accelerate enzyme functionality in cheese.

Researchers at the Wisconsin Dairy Research Center at the University of Wisconsin-Madison and the Western Dairy Center at Utah State University in Logan, have completed a draft genome sequence of Lactobacillus helveticus CNRZ32, a starter culture that can be used to produce ripened cheeses with reduced bitter off-flavors. Using the sequence, researchers can better understand the role of endopeptidases in L. helveticus CNRZ32. These enzymes reduce the accumulation of bitter peptides and hence the development of bitterness in ripening cheeses. The genome sequence will also be used to construct or isolate cultures that result in intensely nutty or sweet cheeses, which can be used as either table cheese or as ingredients.

Improvement in flavour-Additional genomic work at the Wisconsin and Western centers is exploring the construction of strains of Lactobacillus casei that over express a bacterial lipase known to enhance cheese flavor. Newly constructed strains could be used to produce fruity cheese. A second objective of this project is to manufacture processed cheese from Cheddar cheese having significantly elevated levels of free fatty acids or furanones and pyrazines. The resulting cheeses could be used as ingredients in cheese sauces and in baking applications.

2.2 Exploitation of Non-Starters

Non-starter lactic acid bacteria (NSLAB), which are non-pathogenic, are inherently present in milk and survive pasteurization, and/or are added indirectly to cheese milk during manufacturing. Researchers are using biotechnology to identify NSLAB in order to control their contribution to flavor during cheese manufacture.
At the Minnesota-South Dakota Dairy Foods Research Center, researchers have isolated various NSLAB from commercial cheese samples. Using DNA fingerprinting, they classified these organisms into 13 distinct strains, with each strain characterized for protease, peptidase and diacetyl production using classical biochemical assays. Significant differences were noted among strains Cheesemaking experiments in which select adjuncts were reintroduced to the cheese milk showed growth suppression of unwanted bacteria, thus reducing overall cheese variability and subsequently improving cheese flavor and quality.

2.2.1 Use of lacticin 3147 to control NSLAB in cheese

Lacticin 3147 has been investigated for use in the dairy industry to control NSLAB during the fermentation of dairy products. The control of NSLAB in cheese ripening would allow for a more predictable end product. The most efficient method of introducing lacticin 3147 to a fermented dairy product is to use a lacticin-producing culture as the starter or as a starter adjunct in the fermentation process. DPC3147, the natural lacticin 3147 producer, is unsuitable as a starter culture as the strain is associated with an off-flavour. However, this problem can be eliminated by taking advantage of the conjugative nature of pMRC01. This approach, which is a frequently used method for genetic improvement allows the directed transfer of the plasmid responsible for producing the bacteriocin, pMRC01, to a commercially used lactococcal starter. Over 30 lacticin 3147 transconjugants have been created to date. An important consideration when creating and using transconjugants as starters is to ensure that their important industrial traits have been retained and that they remain suitable for cheese manufacture.

Nisin-producing starters, for example, have often been associated with slow acid production, reduced proteolysis and poor heat resistance when compared with a commercial starter. It has been shown that a lacticin 3147-producing transconjugant starter (DPC4275) produced acid at comparable rates with the parental commercial starter. It was also shown that the level of bacteriocin produced was constant throughout the ripening process and at the end of cheese production the NSLAB population had been reduced by at least 100-fold. A reduction in the NSLAB population was also seen when DPC4275 was used to make low fat cheese at increased ripening temperatures. This offers the cheese-maker far greater control over the developing adventitious flora without the need to resort to expensive alternatives such as cold ripening. However, as mentioned earlier NSLAB may contribute positively to the flavour and quality of the cheese and therefore it may be desirable that certain NSLAB develop to high numbers during the ripening process. This objective was achieved when a lacticin 3147-resistant variant of Lactobacillus paracasei subsp. paracasei DPC5336 was isolated after repeated exposure to low levels lacticin 3147. This strain, which still remains sensitive to high levels of lacticin, was then used in conjunction with a lacticin 3147-producing starter in Cheddar cheese manufacture. While other NSLAB were inhibited during ripening, the resistant mutant could tolerate the levels of lacticin 3147 and became the dominant microflora in the cheese. Developments like this give the cheese-maker greater control of the microbial biota. Another advantage of using lacticin 3147 transconjugants as dairy starters is that the plasmid pMRC01 encodes bacteriophage resistance, specifically an abortive infection mechanism. The susceptibility of lactococcal starter cultures to phage attack is a serious problem in the dairy industry and can result in production and economic losses. Thus, the introduction of the lacticin-3147 genetic determinants to a starter culture has the added benefit of conferring protection from
lactococcal phage attack as a result of the linked abortive infection mechanism. This is in contrast to nisin-producing starters, which are associated with phage susceptibility.

2.3 Use of Adjuncts

Adjuncts are defined as cultures that contribute to flavor development without participating significantly in acid fermentation. Researchers at California Polytechnic State University in San Luis Obispo have shown that an adjunct strain of *Lactobacillus helveticus* produces a high-quality, full-flavored mild or aged Cheddar cheese with slightly sweet notes and no bitterness. They also have identified bacterial cells attenuated by heat treatment that preserve desired enzyme activities but have undesired enzymes inactivated by the heat treatment. The attenuated cultures provide a more controlled and consistent proteolysis, resulting in flavor and texture improvement in lower-fat cheese.

At the University of Minnesota, researchers identified an integrated culturing system that produces a high-quality, mild cheese flavor in lower-fat Cheddar after only two months of ripening. The system consists of three strains of lactic acid bacteria, one starter (*L. lactis* ssp. *cremoris* SK11) and two adjuncts (*L. lactis* ssp. lactis biovar. diacetylactis JVI and *L. casei* 7A). Researchers here have also an integrated culturing system that produces a high-quality, mild cheese flavor in lower-fat Cheddar after only two months of ripening. The system consists of three strains of lactic acid bacteria, one starter (*L. lactis* ssp. *cremoris* SK11) and two adjuncts (*L. lactis* ssp. lactis biovar. diacetylactis JVI and *L. casei* 7A).

Researchers at Utah State University have identified that *Brevibacterium linens*, a traditional smear culture for Brick and Limburger cheese, converts the amino acid tryptophan into flavorless compounds while non-starter and adjunct lactobacilli often convert it into off-flavor compounds. In addition, brevibacteria can neutralize off-flavor compounds produced by lactobacilli. Brevibacteria also are better at converting the amino acid methionine into the volatile sulfur compound methanethiol, which contributes to a desirable Cheddar cheese aroma and flavor. Researchers recently isolated and characterized the primary enzyme responsible for generating methanethiol, and plan to clone the enzyme onto typical lactobacilli cheese cultures. When used with a complementary starter culture, brevibacteria can improve the flavor of Cheddar cheese. It also accelerates flavor development in aged Cheddar cheese.

2.4 Interaction Among Starter, Adjunct and NSLAB

Starter, adjunct and non-starter lactic acid bacteria (NSLAB) all affect the development of flavor in Cheddar cheese; however, the cause and effect relationship between these bacteria has not been well understood. The ability to address population dynamics among these cultures will enable researchers to monitor changes over time, and then to select those culture systems that best prevent or reduce off-flavor development. Researchers at several universities are focused on enhancing cheese flavor. For example, research at the Southeast Dairy Foods Research Center at North Carolina State University is currently determining if there are any regional differences in aged Cheddar cheese flavor. It is well known that there are ‘perceived’ regional differences in Cheddar cheese flavor. A good example of this perception is the labeling of certain Cheddars as ‘New York’ or ‘Vermont-style.’ They are currently investigating flavor and texture differences of aged Cheddar cheese produced in different U.S. states and regions (California, the Northwest, the Midwest and the Northeast). Knowledge of the actual regional differences in Cheddar
cheese and the nature and source of these differences would enhance the ability to identify methods to alter or accelerate cheese flavor development where needed or desired.

Western Dairy Center scientists have studied the role of metabolic cross feeding between starter, adjunct and NSLAB. They found that each of these bacteria catabolize aromatic amino acids in defined media under conditions typically found in Cheddar cheese. The pathways involved in these reactions can facilitate the production of off-flavor compounds. They are now investigating the specific roles for selected enzymes in the production of these compounds. Through enzyme assays, gene probes and recombinant DNA technology, researchers will be able to provide new strategies to identify or develop starter systems that avoid or reduce off-flavor development.

2.5 Use of Bacteriocins in the Acceleration of Cheese Ripening

Cheddar cheese usually has a maturation time of at least six months, during which gradual autolysis of the starter cultures occurs. Lysis results in the release of intracellular enzymes such as lactate dehydrogenase (LDH) and postproline dipeptidyl minopeptidase (PepX) which break down the casein in the cheese to small peptides and amino acids. The amino acids released are the precursor compounds responsible for flavour development in cheese. As cell lysis is a slow process and often a limiting step in cheese maturation, controlled early lysis is known to be advantageous for improved flavour development.

Bacteriocins such as lacticin 3147 and nisin, which inhibit a broad range of food spoilage and food pathogenic bacteria, have obvious applications in the food industry. Class II bacteriocins, such as lactococcin ABM, only inhibit other lactococci and therefore their associated applications are limited. However, it has been shown that lactococcin ABM can have both a bactericidal and bacteriolytic mode of action on target cells. In fact, sensitive strains undergo complete lysis following exposure to an ABM producer, resulting in the release of intracellular enzymes. Cheese making trials using L. lactis subsp. lactis DPC3286 (an overproducer of lactococcin ABM) as a starter adjunct with the cheese-making strain L. lactis subsp. cremoris HP demonstrated increased starter cell lysis, elevated enzyme release and an overall reduction in bitterness when compared with the control cheeses.

The potential of using lactococcin ABM to induce starter cell lysis has been investigated. Addition of these bacteriocins to growing sensitive cells is associated with a gradual decrease in optical density (O.D.) in laboratory media and a concomitant increase in the release of intracellular enzymes. However, a problem that may be encountered when using bacteriocins to lyse starters is that the rate of acidification may be compromised if the target lactococcal strain is also the primary acidifier. To overcome this problem with lactococcin ABM, a three-strain system was developed). This included a lactococcin-producing adjunct (DPC3286), a bacteriocin-sensitive starter HP as a target and Streptococcus thermophilus, a bacteriocin-resistant species. Streptococcus thermophilus is included as it is not inhibited by lactococcin ABM, is insensitive to lactococcal phage and is an efficient acid producer. As this strain continued to grow throughout ripening overall acidification was not affected and elevated enzyme release was achieved by lysis of the starter. This indicates a potential use for lactococcin ABM as a tool in cheese manufacture to increase enzyme release, thereby accelerating the rate of ripening while not negatively affecting the quality of the cheese.
Lacticin 3147, produced by L. lactis IFPL105 can also have a bacteriolytic effect on sensitive cells. Lactococcus lactis IFPL105 and the lacticin 3147-producing transconjugant L. lactis IFPL3593 have been successfully used in the acceleration of cheese ripening. When the lacticin 3147 transconjugant, IFPL3593 was used as a starter culture in cheese manufacture, increased lysis of starter adjuncts allowing for the release of elevated levels of PepX resulted. Furthermore, as the starter strain had the lacticin 3147 immunity genes, it was not inhibited throughout ripening and therefore complete acidification was allowed and the resultant cheese was not adversely affected. It has been noted, however, that lysis with lacticin 3147, nisin and lactococcin ABM requires the presence of the autolysin gene, acmA, in the sensitive strain. Another interesting and novel phenomenon has been observed with lacticin 481 was that the O.D. of exposed cells did not decrease; in fact it increased even while intracellular enzymes were released. This suggests that lacticin 481 could be used to induce early cell lysis but also the target cell would continue to grow leaving its acid producing abilities unaffected.

To conclude, given that Cheddar cheese ripening can be a long and costly process, it would be economically advantageous to accelerate the process. Results suggest that the use of bacteriocins as tools to induce early cell lysis would appear to be a possible option. Also, as the use of bacteriocin-producing cultures involves the natural alteration of starter cultures, there would not be any additional technology costs or genetic modification events associated with the process.

2.5.1 Improving bacteriocin production in cultures

Some lactic acid bacteria can naturally preserve dairy foods through the production of proteinaceous bacteriocins, like nisin and pediocin, which exert an antagonistic effect against certain pathogenic and spoilage bacteria. Researchers at the Minnesota-South Dakota Dairy Foods Research Center are using biotechnology to increase nisin production by *Lactococcus* dairy starter cultures. They are accomplishing this by increasing the number of copies of the genetic determinants for nisin. Specifically, they are attempting to subclone the entire genetic unit from its chromosomal location to a multi-copy lactococcal plasmid. This nisin-producing plasmid could also be introduced into other lactic acid bacteria, extending the range of bacteria that can produce nisin.

2.6 Development of Cultures for Mozzarella Cheese

Typical thermophilic starter cultures in mozzarella manufacture are either *Lactobacillus delbruekii* ssp. *bulgaricus* or *L. helveticus*, both rods, and *Streptococcus thermophilus*, a coccus. Researchers are using advanced technologies to better understand how these cultures function. Armed with an understanding of what biochemical pathways take place during mozzarella manufacture, researchers also are exploring the potential use of different bacterial strains for improved fermentations.

Scientists at the Western Dairy Center, in collaboration with the University of Nebraska-Lincoln, have identified another lactic acid coccus, *Pediococcus*, to be used as a starter for mozzarella cheese. Pediococci typically metabolize galactose, but they are unable to rapidly ferment lactose. To overcome this limitation, researchers have used modern genetic recombination techniques involving a naturally occurring lactococcal lactose plasmid to transform strains of *Pediococcus acidilactici* and *Pediococcus*
pentosaceus to rapid lactose fermenters. These cocci can be used with a rod to manufacture reduced-browning mozzarella cheese.

The researchers have also identified *Lactobacillus casei* ssp. *casei* as a potential adjunct culture for lower-fat mozzarella cheese. *L. casei* produces strong proteolytic and peptidolytic activities, which improves the body and melt properties of lower-fat mozzarella. During manufacture, *L. casei* can either partially or totally replace *L. helveticus* to show these improvements.

### 2.7 New Uses for Exopolysaccharide Cultures

Exopolysaccharide (EPS)-producing cultures sometimes referred to as ropey or capsule producing cultures, have generally not been used in the manufacture of cheese. However, researchers are finding new uses for these organisms.

At the Western Dairy Center, researchers have been able to improve the melt and stretch characteristics of lower-fat mozzarella cheese by using specific starter culture strains that produce an exopolysaccharide capsule around the bacterial cells. When an EPS-producing culture such as *Streptococcus thermophilus* MR-1C is used to make cheese, a 1-3% increase in cheese moisture can be obtained. Ongoing experiments are characterizing the structure of the MR-1C EPS and the genes that encode its biosynthesis. Results will provide basic information needed to understand the influence of EPS on cheese quality and functionality.

EPS-producing cultures, especially those that release the EPS into the milk, also have application in yogurt and various dairy-based beverages. These cultures provide a desirable thickness and smooth mouthfeel without the addition of other ingredients. This contributes to a consumer-friendly ingredient statement. Researchers from the Western Dairy Center at Oregon State University have identified an EPS culture that improves the flavor of sour cream when it is used as the starter culture.

### 2.8 Identifying Phage-resistant Starter Cultures

Researchers are beginning to better understand how to prevent viruses from attacking starter bacteria during cheese making. These viruses, called phages, are found throughout the cheese manufacturing system and can attack at any time. They are harmless to humans, but cause the starter to fail in the cheese vat, which results in production inefficiencies or lower quality cheese. At North Carolina State University in Raleigh, NC, researchers found that a select few industrial *L. lactis* starter culture strains could be used for longer periods of time without a phage attack. They used genetics to identify natural defenses that these cultures possess, and were able to transfer these genes to other dairy starter cultures, thus making them more resistant to bacteriophage attack. This technology has resulted in the commercialization of a starter culture rotation system wherein genetic “traps” are built into bacterial strains that have the same cell surface properties, but house different internal defense systems. The strains all invite attachment of the phage onto the cell surface. Once inside, the phage is destroyed by the cell’s internal defense. By rotating culture strains with different internal defenses each day, any phage that eludes one genetic trap is invited into and caught by the next one. The system is
designed to stop virus evolution by killing bacteriophage before they learn to resist existing defenses.

Also at North Carolina, current studies in this area involve targeting transcription regulators as a general strategy for phage defense of Lactococcus species. Thus far, researchers have cloned and characterized genes from lactococcal bacteriophages that encode transcriptional regulators. This will enable the design of genetically engineered phage defense mechanisms.

Oregon State University researchers have clarified the mechanism of the early step in lactococcal phage infection, which is another strategy of phage defense. By identifying and eliminating the receptors on the surface of lactococcal cells, bacterial strains can be developed that are resistant to phage infection. Without a receptor, the phage is unable to identify the new strain as a host, and no infection occurs. A protein that acts as a phage infection protein (PIP) was identified for one type of lactococcal phage, and although it is not the phage type currently most problematic in the U.S. cheese industry, it provides an additional option for the design of phage protection for cheese starter cultures.

The Western Dairy Center is introducing a species barrier to phage. During mozzarella cheese manufacture, two unrelated bacteria, Streptococcus species and Lactobacillus species, are used. Of these two, the streptococci are more prone to bacteriophage attack. Since bacteriophage is known to be unique to individual species, use of a different species would provide bacteriophage protection. Use of a culture from a different species would be even better. Pediococci, which are cultures used for meat and vegetable fermentations, lack the ability to ferment lactose. Without this ability, they cannot produce sufficient lactic acid in milk to be used for making cheese. Using biotechnology, researchers enabled some Pediococcus species to ferment lactose. Being from a different genus, when the pediococci are used in conjunction with streptococci, these newly bioengineered cultures provide mozzarella cheese makers with a genetic barrier that forms an almost impenetrable shield against bacteriophage attack.

### 2.9 Improving Culture Distribution

At North Carolina State University, researchers are using preconditioning treatments like sub-lethal stress to elevate the tolerance of Lactobacillus cultures to freezing and storage. Such conditions are used to store and transport live, active cultures, and often result in cells dying or losing activity. Using genetic indicators, researchers are evaluating stimuli that turn on tolerance and stress response genes. By preconditioning the cells for this activity, they can then be transported in harsher environments with greater odds for survival.

### 2.10 Identifying Probiotic Cultures

Increased food industry interest in the area of probiotics is driving researchers to further investigate these beneficial bacteria and their application in dairy foods. Technologies such as DNA sequencing and fingerprinting are enabling researchers to properly identify probiotics. This will help ensure sufficient levels of specific functional strains in commercial food products.
Researchers at California Polytechnic State University (Cal Poly), in collaboration with North Carolina State University, are involved in a large-scale effort to sequence the *Lactobacillus acidophilus* chromosome. Once the complete chromosomal sequence is identified, researchers hope to identify which genes are important to the functionality of probiotic bacteria. Results should also provide a directed means to improve the expression, regulation and attributes of important probiotic characteristics. Going one step further, North Carolina researchers are currently looking for specialized genetic signals that are turned on by exposing *L. acidophilus* to milk. They are attempting to correlate the expression of critical probiotic functions to delivery via milk.

Cal Poly researchers are seeking a better understanding of the clinical effects of eating probiotic-containing dairy foods. To do this, they are using a DNA-based rapid identification method to quantitatively describe changes in microbial populations in human fecal samples obtained from subjects eating probiotic-containing yogurt. At the University of Minnesota, scientists are using DNA-based techniques to rapidly identify and characterize probiotic species. With this technology, researchers are able to genetically characterize strains of bifidobacteria present in commercial cultures. They are attempting to determine survival through the gastrointestinal tract by identifying the cultures before consumption and in the feces. This technology provides researchers with the tools to gather clinical data to support the claims that have been theorized for so many years.

### 2.11 Improved Lactase Activity

Lactose intolerance is perceived by consumers to be a significant and widespread problem. Efforts to alleviate symptoms associated with lactose intolerance have had marginal success. At the Southeast Dairy Foods Research Center in Raleigh, NC, scientists are investigating overexpression of lactase (β-galactosidase) in various lactic acid bacteria. Because these organisms are associated with both dairy foods and the human gastrointestinal tract, they are ideal candidates for overexpression and delivery of β-galactosidase. Efforts continue in attempt to create a food-grade, high copy replicon for lactase overexpression in probiotic lactobacilli.

### 3.0 Conclusion

Continued advancements in biotechnology are enabling researchers to aggressively study the genetic make-up and the biochemical pathways of cultures that either currently have or potentially have application in dairy foods. Such technologies will improve culture functionality and, ultimately, improve manufacturing efficiencies and finished product quality. The dairy industry has already begun to reap some benefits from researchers' increased understanding of cultures. This includes a reduction in phage and improved Cheddar flavor development in cheese. Long-term, manufacturers should be able to reduce ripening times for cheese, but still attain great flavor. There is also potential that dairy foods can be viewed by consumers as the ideal carrier for functional, beneficial probiotic cultures.
4.0  Suggested Reading


CALCIUM FORTIFIED MILK – THE INNOVATIVE SOLUTION FOR EXTRA CALCIUM

Dr. Sumit Arora
Scientist (Senior Scale)
Dairy Chemistry Division
NDRI, Karnal

1.0 Introduction

Food fortification is one of the relevant modes of action to address the issue of micro nutrient malnutrition in developing countries (FAO 1995). There are several steps in the implementation of a food fortification programme in a developing country like India. Having identified the need for nutritional intervention on behalf of a population and the required level of fortification, then a suitable carrier must be identified, appropriate fortificants selected, the technologies used in the fortification process determined and some mechanism put in place to determine whether the nutritional objectives of the programme are being met. Foods that have been successfully fortified in developing countries include: milk and milk products, sugar, wheat flour, corn flour, salt, fats and oils. The levels at which nutrients are added to milk depends on a number of factors including level of milk consumption and nutritional requirement of the target population, effect of added nutrients on functional or sensory characteristics of milk and stability of the nutrients during processing and storage of milk (Food fortification, 1993).

Indians are more prone to osteoporosis, a debilitating bone disorder resulting from porous bones, with an estimated five crore people suffering from life threatening disease. Major factors responsible for high prevalence of osteoporosis in India are inadequate intake of calcium and Vitamin D. As a part of preventive measure, the Indian society for bone and mineral research (ISBMR) suggested to the authorities and industry to go for calcium fortification of food products (Hindustan Times, 2002). Calcium requirements vary throughout an individual’s life and for different population groups. Thus it is imperative that dietary intake of calcium be increased in population groups that have sub – optimal calcium nutritional status. Poor dietary habits are also seen to be responsible for this situation, especially when consumption of fast food is gaining acceptance. If a calcium supplement is given to a population which already has an adequate supply, this does not affect growth in terms of height, but increases bone density (Danone, 1999).

Cow and buffalo milk contains 125 to 150 mg/100 g and 180 to 200-mg/100 g, calcium, respectively. ICMR (1989) recommends RDA for calcium as 400 to 600 mg (1000 mg in pregnancy/lactation) whereas NIH (1994) recommends 1200 to 1500 mg of calcium per day. High intake of calcium will only be possible through formulating calcium enriched dairy products.
2.0 Fortification with Calcium

Calcium is one of the most essential nutrients and must be provided regularly in the diet. This mineral alone constitutes about 2 per cent of the total body weight and most of this is distributed in the bones, therefore, an adequate daily intake of calcium is vital for maintaining structural integrity of the body skeleton and strength of the teeth. Besides its structural role, calcium is equally important in regulating various metabolic processes in the human body. It also plays a role in the transmission of impulse in nerve cell and in case of injury forms an important component of the blood clotting. More recently, central role of calcium in the prevention and treatment of a number of diseases i.e. osteoporosis, hypertension, colon cancer, kidney stones and lead absorption has also been acknowledged (National Dairy Council, 1992).

The average rate of calcium accumulation in the skeleton between birth and maturity is of the order of 60 mg per day but the most rapid deposition occurs during adolescence when the positive balance required rises to about 300 mg or more daily. There is general agreement that extra dietary calcium is required for optimum skeletal growth at this time, with recommended intakes at puberty around 1200 mg or more (Vital news, 2000).

2.1 Selecting the Calcium Fortificant

The ideal calcium source for fortification of foods should be highly absorbable and available to enhance bone mass, inexpensive and safe for human consumption. The ultimate nutritional benefit of the calcium source must aim at increasing peak bone mass during growth and to reduce bone mass later in life (Weaver, 1998). Several commercial calcium salts are available to the food manufacturers for use in food and dietary supplements e.g. inorganic salts like calcium carbonate, calcium chloride, calcium phosphate and organic salts like calcium citrate, calcium lactate, calcium gluconate. The salt best suited for fortification should possess a high nutritional value and low interference with the absorption of other nutrients besides its cost effectiveness and minimal effects on consistency, mouthfeel and taste of the product. In general, organic salts of calcium are more bioavailable than the inorganic salts (Goldscher and Edelstein, 1996). The inorganic salts (Calcium carbonate 40 %, phosphate 31% and chloride 36%) contain more calcium than organic salts (lactate 13 %, citrate 21% and gluconate 9%) but their application in food and beverages remain limited due to their poor solubility and the way in which they influence taste in final applications.

According to Weaver (1998) there is no significant difference (Table1) in the bioavailability of most of the calcium compounds available for fortification of food and the manufacture of nutritional supplements, so for most applications there is no need to use an expensive calcium source. Using milk calcium, as a fortificant is also gaining momentum because it has beneficial sensoric properties such as neutral taste and bland odour. It also offers marketing benefits in terms of labeling and claims as it is derived from milk (Witte, 2000). Evidence suggests that salts such as calcium oxalate and calcium carbonate with molecular weights under 100 Kd can be absorbed intact by passive diffusion rather than by vitamin D dependent active mechanism which necessitates calcium dissociation from any complex (Benway, 1993).
Table 1. Solubility and Absorbability of Calcium Salts

<table>
<thead>
<tr>
<th>Source</th>
<th>Solubility M/L</th>
<th>Fractional Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Oxalate</td>
<td>0.04</td>
<td>0.102±0.040</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.14</td>
<td>0.296±0.054</td>
</tr>
<tr>
<td>Tri calcium phosphate</td>
<td>0.97</td>
<td>0.252±0.130</td>
</tr>
<tr>
<td>Calcium citrate</td>
<td>7.3</td>
<td>0.242±0.049</td>
</tr>
<tr>
<td>Calcium citrate Malate</td>
<td>80</td>
<td>0.363±0.076</td>
</tr>
<tr>
<td>Bisglycin calcium</td>
<td>1500</td>
<td>0.440±0.104</td>
</tr>
</tbody>
</table>

Soymilk has also been fortified (Valente Alvarez, 1996) using a mixture of calcium gluconate and calcium-D-saccharate as the calcium source and protecting the stability of the soymilk product by using sodium hexametaphosphate as a calcium sequestering agent. Lactaid® low fat, which is a lactose free milk marketed by McNeil consumer healthcare, Ft.Washington, PA., fortified with tribasic calcium phosphate, has 500 mg of calcium per serving, 200 mg more than standard milk products (Klahorst, 2001). In addition to traditional dairy products such as milk and Yoghurt drinks, new concept and ideas are being developed for calcium and other micronutrient fortified beverages to help consumers meet the recommended daily intake.

2.2 Calcium Sources and Bioavailability

Calcium source is only present in significant amounts in a limited range of foods. Some vegetable, e.g., green leafy one, such as broccoli provide quite large amounts of calcium, others include cabbage, beans, watercess. Milk and milk products are a major source of calcium. Calcium levels are not reduced when milk is skimmed. The adsorption of calcium is affected by its solubility and chemical form and by the presence of inhibitors in the food or total diet (Allen, 1982).

The bioavailability of calcium has been shown to be reduced by a variety of dietary components capable of forming insoluble complexes with calcium in the intestine. These include phytate, oxalate and uronic acid and other components of non-starch polysaccharide (NSP) (dietary fibre). Calcium in spinach is less well utilized than calcium from milk or from other green vegetables such as broccoli. The high oxalate content of spinach has been assumed to reduce the absorption of calcium by forming insoluble calcium complexes in the intestine (Heaney, 1988). Calcium in milk is easily absorbed by the intestine and is considered to have higher bioavailability than calcium in vegetables or cereals. Two factors in milk – lactose and casein, have been suggested to be responsible for promoting calcium absorption and leading to apparent higher bioavailability (Miller, 1989).
Lactose delays the precipitation of calcium in vitro by forming calcium-lactose complexes (Charley and Saltman, 1963) and it may also do so in the intestinal lumen. According to Kansal(1998) Lactose stimulates passive component of calcium absorption in the ilium and the colon in rats. Lactose enhances calcium absorption in Vitamin D deficient as well as Vitamin D adequate animals. Casein may also act by increasing the proportion of soluble calcium in the intestinal lumen (Lee et al., 1979). It is possible that the higher bioavailability of milk calcium may merely be due to the absence of factors, which normally inhibit the absorption of calcium such as phytate and oxalate. The type of calcium source did not affect calcium absorption, but milk components increased the apparent calcium absorption.

Milk and other dairy products are the most important sources of calcium in readily available form. There is an increasing trend in producing calcium and vitamin D enriched milk and other drinks. A part of calcium in milk is associated with the casein fraction, where it associated with phosphorylated serine. The caseinophosphopeptides (CPP) released in the gastrointestinal tract during normal digestion can improve passive transport of calcium in the lower portion of small intestine. These CPP forms soluble complexes with calcium phosphate salts and thus improve calcium absorption (Kansal, 1998). Sodium intake raises the urinary excretion of calcium thus increasing the amount of calcium that has to be absorbed from diet to maintain calcium balance; otherwise calcium would be resorbed from the skeleton to compensate the reduced bioavailability (Goulding and McIntosh, 1986).

In infants, the protein and calcium requirements are generally met from milk. In fact, the micellar form of calcium found in milk is best absorbed and has high (almost 2 times) bioavailability compared to that from nuts and vegetables (like sesame seeds, almonds, whole wheat bread, spinach etc). The reason for the poorer bioavailability of calcium through vegetarian source is the presence of chelators like phytate and oxalate in vegetables, which makes calcium insoluble and unavailable for absorption. However, regular intake of milk will help in meeting the daily protein and calcium requirements (Neelam and Hemalatha, 2001).

2.3 Interaction Between Calcium and Other Minerals

It has been suggested that a high intake of calcium may impair the absorption of a number of other minerals including zinc, copper, magnesium and iron. However, the results of studies in human indicate that under normal dietary conditions, the absorption of zinc, copper and magnesium is not impaired to any great extent (Sandstrom and Lonnerdal, 1989; Lonnerdal, 1989). Jackson and Lee (1992) concluded from a review of the effect of dairy products on iron absorption that their nutritional benefits outweigh any small inhibitory effect they might have on iron availability.

2.4 Dietary Factors Affecting Calcium Bio Availability

Optimal bone mineralization obviously requires calcium, but phosphorous must be present for the formation of hydroxyapatite. The dissociation of calcium intake from that of phosphorous may restrict calcium and phosphorus retention. A calcium source with a ratio Ca/P > 1 is a plus. Lactose is reported to stimulate calcium passive absorption (Vital news, 2000).
Because it is bound to peptides and proteins, milk calcium is more likely to remain in solution when the pH is unfavourable. Milk calcium may be absorbed in distant intestine in the absence of vitamin D under the influence of lactose. Dairy products do not contain anything likely to inhibit the intestinal absorption of calcium like phytates, oxalates, uronic acids or the polyphenols of certain foods. The hyper calciuric effect of sulfates from the sulphur containing amino acids of milk is offset by the hypocalciuric effect of phosphorous (Vital news, 2000). The uptake of calcium is increased when it is ingested during a meal and combined with protein and lactose as in case of dairy products (Danone World News Letter, 1999).

The choice of calcium fortification compound may be made more on technological grounds, since there is little variation in the bioavailability of the calcium from different compounds (Couzy, 1996). Only a fraction (25 to 35%) of the calcium in the diet is absorbed, most of the dietary calcium stays in the intestinal lumen and contributes to the daily loss of calcium.

### 2.5 Mechanism Involved in Fortification

Calcium fortification of milk and milk based beverages has been carried out. Calcium fortificant preparations including stabiliser and emulsifiers have been used for this purpose to maintain calcium in suspension so as to improve mouthfeel and appearance of products (FAO, 1995). An important relationship exists between calcium addition and protein concentration, that is one of the determining factors for successful heat stability (Valente Alvarez, 1996). The ratio of calcium-to-protein may not exceed 40 mg calcium per gram of protein in order not to swamp the protein with calcium. It is the overload of calcium on the protein that is the first determining factor. Another important processing step involves the careful control of final pH followed the calcium salt addition. Finally, the use of a calcium-sequestering agent must be a controlled addition, dependant upon the actual calcium load of the system. Upon addition of calcium salt, there is a marked drop in the pH, which will render the system unstable unless it is corrected to near neutrality by the addition of a food grade base.

### 3.0 Conclusion

Fortification of foods can be a useful tool in combating micronutrient deficiencies. The successful application of food fortification technology is based largely on consideration of the compatibility of vehicle, fortificant and process. Calcium enrichment of food and dairy products has gained interest with increased awareness about importance of higher calcium intake. Calcium plays many important roles in the body. Dairy products are a very good source of dietary calcium, they are further enriched with calcium in order to achieve higher calcium intakes per serving.
4.0 Suggested Readings


Couzy.F (1996) Foods naturally rich in calcium or fortified with calcium. Abst no 073 published in 1st World Congress on calcium and vitamin D in human life. 8 – 12 Oct, Rome, Italy.


Food Fortification (1993). Encyclopedia on food science, food technology and nutrition. 3, 190.


Hindustan Times (2002). Indians more prone to osteoporosis: PTI. Hyderabad, oct 21st.


1.0 Introduction

Since time immemorial, ghee has been used in Indian diet as the most important source of fat. Ghee, the Indian name for clarified butterfat is obtained by heat clarification and desiccation of sour cream, cream or butter. It is the largest indigenous milk product having an important place in Indian dietary, because of its characteristics flavor and pleasant aroma, besides being a source of fat-soluble vitamins.

In recent past, there has been great deal of questioning about the role of milk fat in the metabolism of cholesterol and other body functions. Ghee being a saturated fat and contains some cholesterol, is suspected to render the individuals prone to coronary heart disease (CHD). However, critical analysis of scientific literature shows no evidence of any association of milk fat with increased risk of CHD. On the other hand, in Ayurvedic system of medicine, ghee is considered to induce several beneficial effects to human health and is used extensively for therapeutic purposes, such as in the preparation of a number of formulations for treating skin allergy and respiratory diseases, and is considered capable of increasing mental powers and physical appearance, and curative of ulcers and eye-diseases. These practices suggest that ghee is a valuable form of dietary fat, but scientific validation of these claims is obscured. However, literature shows that milk fat contains several components (conjugated linoleic acid, sphingomyelins, butyric acid and β-carotene), which have therapeutic potential against carcinogenesis (Parodi, 1996). CLA, besides being a powerful anticarcinogen, has antiatherogenic, immunomodulating and lean body mass enhancing properties (Pariza, 1997).

Contrary to milk fat, vegetable oils despite of containing considerable amount of linoleic acid (known to promote carcinogenesis) have got the label of ‘health friendly oils’ because of literature showing hypocholesterolemic effect of polyunsaturated fatty acids (PUFA) and extrapolating it to decreased risk of CHD. Today, these vegetable oils have almost replaced dairy ghee from Indian kitchen. Diet dictocrats are promoting vegetable oils as if they are the complete solution for modern age deadly diseases, such as cancer and CHD. But actual situation is just the reverse. In spite of tremendous alterations in dietary fat patterns, mortality and morbidity due to these diseases is continuously increasing. In fact, inclusion of vegetable oil with a purpose to reduce serum cholesterol level has resulted in increased number of non-cardiovascular death especially cancer (Williams et al., 1981; McMichael et al., 1984; Delahaye et al., 1992; Tamakoshi et al., 1994). Further, excess consumption of oils rich in PUFA have been shown to contribute to a large number of diseases including heart disease, immune system dysfunction, damage to liver,
reproductive organs and lungs, digestive disorders, depressed learning ability, impaired growth and weight gain (http://www.westonaprice.org/know_your_fats/know_your_fats.html).

Epidemiological studies supporting the vegetable oils and discrediting milk fat are subjected to potential biases due to several factors. Most importantly, total energy intake, which is a stronger predictor in the pathogenesis of cancer, is not generally taken into account. Even when total energy intake is controlled, it may be impossible to completely separate the effects of dairy intake from that of other dietary factors that alters cancer risk. Person with a high consumption of dairy product may also be likely to consume large amounts of meat or other fat foods that could also contribute to an increased risk of cancer. Further, other factors such as lifestyle, physical activity cannot be controlled in epidemiological studies. Besides this, various methods used in epidemiological studies, including food frequency questionnaires and diet records or food diaries, may not be reliable and some misclassification of intake is unavoidable.

Unlike epidemiological studies, almost every animal study conducted so far has witnessed protective role of milk fat against vegetable oil in carcinogenesis. Certainly, animal studies done under controlled conditions and taking particular item as variable keeping other items constant are the better alternatives to generate reliable information. Keeping in view of this we undertook studies to investigate the effects of dietary intervention of dairy ghee (cow and buffalo) vis-à-vis vegetable oil (soybean oil) on gastrointestinal and mammary carcinogenesis, arteriosclerosis and immunomodulation in rats (Bhatia, 2005).

2.0 Gastrointestinal Carcinogenesis

Intervention of dairy ghee (cow or buffalo) and soybean oil on gastrointestinal (GI) carcinogenesis was studied in 21 d old male albino rats fed for 33 wk. Carcinogenesis was induced by dimethylhydrazine dihydrochloride injected (IP) weekly for 20 wk starting fourth wk past start of the experiment.

→ During post-injection period, the rats on cow ghee grew faster, and at the end of experiment weighed more than the rats on soybean oil or buffalo ghee.

→ The incidence of tumors in GI tract was considerably higher in animals on soybean oil (73.30%) than on cow ghee (55%) or buffalo ghee (40%).

→ Tumor multiplicity (tumor / tumor bearing rat) and tumor volume were less on ghee diets than on soybean oil (3.64; 677 mm$^3$), and cow ghee was more effective (1.73; 59 mm$^3$) than buffalo ghee (2.88; 472 mm$^3$) in restricting these measures.

→ The levels of thiobarbituric acid reactive substances (TARS), a measure of tissue lipid peroxidation, in liver and colorectal tissue on soybean oil were significantly greater than on ghee diets.

→ Compared with soybean oil diet, CLA accumulation on ghee diets in colorectal tissue and liver was 5 and 7.5 fold, respectively.

3.0 Mammary Carcinogenesis

Intervention of dairy ghee on mammary carcinogenesis has been compared with that of soybean oil in 21 d old female albino rats fed for 42 wk. Tumors were induced by 7,12-dimethylbenz (a) anthracene (DMBA) administered (6 mg/animal) through oral intubations at 46 d of age.
A large number of animals died in all dietary groups within 10 days due to acute DMBA toxicity. The mortality incidence was greater on soybean oil than in ghee groups. Thereafter, animals in ghee groups recovered and became healthy and survived till conclusions of experiment, but in soybean oil group, the condition of rats did not improve and mortality continued till termination of experiment due to neoplastic / non-neoplastic diseases.

Animals on cow ghee, during post-induction period grew faster and weighed more than those on buffalo ghee or soybean oil. Cow ghee, therefore, attenuated growth inhibitory effect of DMBA.

Tumor incidence did not vary among three dietary groups; however, tumor multiplicity was fewer on ghee. Tumor weight and tumor volume on cow ghee were not as much as on soybean oil or buffalo ghee. Thus, cow ghee opposed to buffalo ghee or soybean oil favorably intervened in promotional stage of carcinogenesis.

Dairy ghee opposed to soybean oil diminished DMBA induced mortality and other neoplastic / non-neoplastic disorders.

Accumulation of TARS in liver and mammary tissue of rats was significantly greater on soybean oil than on ghee diets. The difference was more conspicuous in target tissue (mammary tissue) than in liver.

Compared to soybean oil, CLA accumulation in mammary tissue on cow and buffalo ghee was 12 and 9 fold, respectively.

Significantly lower activity of superoxide dismutase (SOD) in liver was observed on soybean oil than on ghee diets.

4.0 Lipid Profile, Immunomodulation and Antioxidative Status

Intervention of dairy ghee (cow or buffalo) and soybean oil on lipid profile, and antioxidative and immune status was studied in male albino rats (110 d old) fed hypercholesterolemic diet for 110 days.

Mean body weights of animals on ghee diets (cow and buffalo) were significantly lower than on soybean oil, despite average feed intake was similar in three dietary groups.

5.0 Lipid profile

Plasma total cholesterol level increased in all dietary groups (0-90 d period), however, mean cholesterol level during the entire study was significantly less on cow (71.3 mg/dl) and buffalo ghee (75.7 mg/dl) than on soybean oil (86.8 mg/dl).

HDL-cholesterol also increased in all dietary groups (0-90 d). The rise in HDL-cholesterol level was highest on cow ghee (125%) and lowest on soybean oil (49%). Buffalo ghee registered 96 percent rise in HDL-cholesterol level.

The increase in VLDL + LDL-cholesterol was inversely related to rise in HDL-cholesterol. The increase in VLDL + LDL-cholesterol on soybean oil was significantly greater than on ghee diets. Thus, the rise in plasma cholesterol on soybean oil was due, largely, to increase in VLDL + LDL-cholesterol (62%), whereas on ghee diets, HDL-cholesterol contributed to major part of rise (63-74%) in plasma cholesterol.
Atherogenic index (VLDL + LDL-cholesterol / HDL-cholesterol) decreased significantly in ghee groups, but increased on soybean oil.

Mean triglycerides level was significantly lower on cow ghee than on soybean oil and buffalo ghee.

Deposition of cholesterol in liver was significantly less on ghee diets than on soybean oil. Cow ghee more efficaciously reduced the deposition of cholesterol and triglycerides in aorta compared with buffalo ghee and soybean oil.

6.0 Antioxidative Status

Superoxide dismutase activity in RBC increased on all the three diets; the magnitude of increase was significantly greater on ghee diets than on soybean oil.

Superoxide dismutase activity in liver and colorectal tissue was significantly higher in ghee groups opposed to soybean oil.

There was no difference in the activity of glutathione-S-transferase in liver and colorectum between soybean oil and ghee groups.

7.0 Immunomodulation

Activities of β-galactosidase and β-glucuronidase secreted by peritoneal macrophages were higher in ghee groups than in soybean oil group, and cow ghee oppose to buffalo ghee was more effective in augmenting these activities.

Percent phagocytosis (number of macrophages phagocytising foreign particle/100 macrophages) and phagocytic index (measure of foreign particles engulfed) were greater in ghee groups than on soybean oil.

8.0 Conclusions

Soybean oil containing polyunsaturated fatty acids promotes carcinogenesis, while dairy ghee, cow ghee in particular, attenuates the effect of carcinogen.

Compared with soybean oil, dairy ghee attenuates dietary hypercholesterolemia and decreases atherogenic index by way of increasing high-density lipoproteins. Cow ghee opposed to buffalo ghee is more effectual in improving lipid profile and that decreases deposition of cholesterol and triglycerides in aorta.

Dairy ghee opposed to soybean oil, improves immune system and antioxidative status, and cow ghee is more effectual than buffalo ghee. In addition, increased accumulation of CLA and decreased lipid peroxidation in tissues also correlate with its protective effects of dairy ghee.

9.0 Suggested Readings


1.0 Introduction

Increasing awareness among consumers to know which specific molecules present in their food possess disease preventive or curative properties has led to the concept of “Functional Foods”. Now the attention of scientific investigations has moved towards exploring the role of biologically active components on human health. Basic temptation in human being towards nature and the products that are natural, for every little disturbances related to health resulted in flourishing of market with products containing various therapeutic ingredients. Functional foods, pharma foods, designer foods and nutraceuticals are synonymous for foods that can prevent and treat diseases. Functional foods have been defined as foods that, by virtue of the presence of physiologically active components, provide a health benefit beyond basic nutrition. Epidemiological studies and randomized clinical trials carried out in different parts of the world have been demonstrated or at least suggested numerous health effects related to functional food consumption, such as reduction of cancer risk, improvement of heart health, enhancement of immune functions, lowering of menopause symptoms, improvement of gastrointestinal health, anti-inflammatory effects, reduction of blood pressure, antibacterial & antiviral activities, reduction of osteoporosis etc. Nutritional significance of plant molecules is well documented and increasing cases of cancers, coronary heart diseases, diabetes and many other chronic diseases, have been attributed to under consumption of fruits and vegetables in our diet. But beyond these known nutrients i.e. vitamins, fibers, plants have clearly more to offer and scientists are scurrying to discover exactly which plant components might fend off specific diseases. An ever-expanding array of previously unknown plant molecules with hard to pronounce names is being uncovered. But there exact metabolic role and how these can be utilized in designer food, need to be clarified.

All over world there has been growing demand for functional foods. Currently Japan leads the world in the production, with more than 100 production and consumption of such products.

2.0 Defining Functional foods and Phytochemicals

The term functional foods was first introduced in Japan in the mid-1980s and refers to processed foods containing ingredients that aid specifically bodily functions in addition to being nutritious. To date, Japan is the only country that has formulated a specific regulatory approval for functional foods, known as foods for specific health use (FOSHU). Currently
100 products are licensed as FOSHU foods in Japan. In US, the Functional foods category is not recognized legally. However, The Institute of Medicine’s Food and Nutrition Board (IOM/FNB, 1994), defined functional foods as “any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains”. To qualify as functional food, it should meet following three highlighted conditions:

→ It is a food (not capsule, tablet or powder) derived from naturally occurring ingredients.
→ It can and should be consumed as part of daily diet.
→ It has particular benefits when ingested, serving to regulate a particular body process. Such as:
  → Improvement of biological defence mechanisms
  → Prevention of specific disease.
  → Recovery from specific disease
  → Control of mental and physical conditions
  → Retarding the ageing process

“Phytochemicals”- phyto from Greek word for plants, denoting their plant origins. Most likely phytochemicals developed as a part of the plants own defense mechanism against environmental insult and only fortuitously provide benefits to man. The number of identified physiologically has increased dramatically in the last decades and overwhelming evidence from epidemiological, in vivo, in vitro and clinical trial indicate that plant rich diet can reduce the risk of certain chronic diseases (Hasler, 2000) Health professionals are gradually recognizing the role of phytochemicals in health improvement.

3.0 Potential Phyto-nutrients

Some phytochemicals with proven health benefits can be grouped under following categories.

3.1 Phytoestrogens

Phytoestrogens are a broad group of plant-derived compounds that are structural mimics of endogenous 17 beta-estradiol. Two major phytoestrogens, which are of great importance from a nutritional and health perspectives, include lignans (Flaxseed) and isoflavones (soy bean).

Isoflavones are flavonoid compounds and major flavonoids that have been identified in soyabeans are genistein, daidzein and glycitin. These compounds exist naturally in Soya in several glycoside forms, but it is the aglycone form of isoflavones that is biologically active.

These compounds either compete with or antagonize estradiol action. Exact biochemical mechanism involving CYP3A monooxygenase activity in presence of phase I enzyme inducers such as dixamethane, on carcinogens was explained by Ronis et al. In another study dietary soy products, showed antitumer activity, by directly affecting the tumor cell proliferation and reduction in tumor angiogenesis (microvessele density). This activity may prevent prostrate cancer. Research has shown that diets rich in soya help to reduce blood levels of LDL (bad) cholesterol by an estimate of 12-15%. The isoflavones in
soy foods are converted in the gut to phytoestrogens that may reduce LDL blood cholesterol. The health effect of these compounds depend upon the exposure level of phytoestrogens, the binding constant relative to estradiol and the selectivity of different tissue receptors.

### 3.2 Organosulfer Compounds

Termed as “promise of garlic” and most widely quoted herb in the literature. Besides its popularity as a recipe seasoning, garlic has long been promoted as a medicinal agent. Garlic and other allium vegetables – onions, chieves, leeks and scallions contain allylic sulphides.

Garlic has acquired a reputation in many cultures as a formidable prophylactic and therapeutic medical agents. Plant extracts containing organosulfur compounds have been shown to exhibit antimicrobial, hypolipidemic, hypoglycemic, antithrombotic, antioxidant and anticarcinogenic (Shenoy and choughuley, 1992). The cardiovascular properties of garlic are less understood and it was suggested that antihypertensive properties of compounds present in garlic play a major role. Animal studies indicate that garlic supplementation in diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, Glucose-6-phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) reductase.

Active components identified is a family of thioallyll compounds and the intact garlic bulb contain an odorless amino acid called alliin, which is converted enzymatically by allinase into allicin, when the garlic cloves are crushed. Formation of allicin is responsible for characteristic garlic flavour. Allicin then immediately decomposes to form numerous sulphur-containing compounds. It has role in prevention of CVD possibly through antihypertensive properties. Animal feeding studies demonstrated the regulation of fatty acid and cholersterol synthesis via modulating. The role of certain enzymes, by garlic components. However, it is still unclear which component of garlic is responsible for cholesterol lowering effect.

Recent investigations also reveals the lowering of plasma concentration of cholesterol is mainly due to an inhibition of hepatic cholesterol synthesis. The human and animal studies to identify the active sulfur compound (s) of garlic responsible for hypocholsterolemic effect was carried out by Yeh and Liu (2001). On the basis of their studies with aged garlic extract (AGE) containing a wide range of organosulfur compounds, they concluded that cholesterol lowering properties of garlic/organosulfur compounds may be attributed in part from inhibition of hepatic cholesterol synthesis. They also observed the major role of water-soluble organosulfur compounds specially SAC as the active principle.

Epidemiologic studies in the past 15 years have established an inverse relationship between garlic consumption and the incidence of certain types of cancer include gastrointestinal, laryngeal, breast and colon cancer (Milner, 2001). The proposed mechanisms include, inhibition of N-Nitroso compounds formation, suppression of metabolic activation of carcinogen, enhanced DNA repair, reduced cell proliferations and /or induction of apoptosis. It is believed that many of these events occur simultaneously at cellular level and are responsible for chemo protection role of garlic compounds. Milner (2001) has reviewed the role of garlic compounds in cancer prevention. Garlic used in various forms as food ingredient and processing involve may also affects the level of organosulfur compounds and efficacy of there. Heating has a negative influence on beneficial effects of garlic.
Song and Milner (2001) demonstrated that heating of garlic for 30 Sec in microwave oven resulted in only 10% allinase activity of original. However, 60 Sec microwave heating not only completely inactivated allinase but also eliminated garlic’s ability to suppress DMDA induced DNA-adduct formation.

3.3 Glucosinolates

About 100 glucosinolates have been identified in more than 450 plant species including 16 higher plant families, marine sponges and red algae. Cruciferous vegetables comprising cauliflower, broccoli, radish, horseradish cabbage, Brussels sprout contain relatively high content of glucosinolates, a potent anticarcinogen. Glucosinolates are a group of glycosides stored within cell vacuoles of all cruciferous vegetables. The functions of glucosinolates in plants are not very clear but their potent odour and taste suggests a role in herbivore feeding and microbial defense (Finley, 2005). Glucosinolates consist of a beta-D-thioglucose group; a sulfonated oxime group and a side chain derived from methionine, phenylalanine, tryptophan or branched chain amino acids. The sulfate group of a GS molecule is strongly acidic and plants accumulate. GS by sequestering them as potassium salts in plant vacuoles. Glucosinolates not bioactive in animals that consumes them until they have been enzymatically hydrolyzed to an associated isothiocynates.

Myrosinase, an enzyme found in plant cell, catalyze these GS to a variety of hydrolytic products, including isothiocynates in doles. Diindolylmethane (DIM), indole-3-carbinol (I3C), phenethly isothiocynate (PEITC) and sulphoraphane seems to be promising chemo preventive molecules, in brassica plants. In addition to the induction of phase I and II xenobiotic metabolizing enzymes resulting in the inhibition of the oxidative activation of carcinogens. I3C may reduce cancer risk by modulating estrogen metabolism. The C-16 and C-2 hydroxylations of estrogens involve competing cytochrome P-450-dependent pathways, each sharing a common estrogen substrate pool. Studies indicate that the increased formation of 2-hydroxylated estrogen metabolites relative to 16-hydroxylated forms, may protect against cancer. Another isothiocynate isolated from broccoli and termed as sulforphane has been shown to be the principal inducers of a particular type of phase-II enzyme, quinone reductage.

3.4 Carotenoids and Flavonoids

Consumption of B-carotene rich fruits and vegetables, has since long been known to prevent certain diseases like cataract. More recent studies have promoted their role in caner, and CVD prevention primarily owing to their anti-oxidative properties.

Carotenoids, C-40 polyisoprenoid structure with an extensive conjugated double bond system. Of the 600 or so carotenoids that have been identified, about 50 serve as precursors for vitamin A. The most researched among them are B-carotene & lycopene. These carotenoids inhibit the oxidation of LDL to its aterogenic form. Lycopene’s ability to act as an anti-oxidant and scavenger of free radicals that are often associated with carcinogenesis is potentially a key for mechanism for its beneficial effects on human health. (Khachik et al., 1995). Lycopene may prevent carcinogenesis and athrogenesis by interfering passively with oxidative damage to DNA & lipoproteins. Lycopene is the most effective quencher of singlet oxygen in biological system.

Flavonoids are polyphenolic compounds and originally regarded as nutritionally inert, these is new increasing interest in the apparent anticarcinogenic properties of certain flavonoids, although exact biochemical mechanisms is not clear. A great deal of attention
has been directed to the polyphenolic constituents of tea, particularly green tea. Catechins are the predominant and most significant of all tea polyphenols. The four major green tea catechins are epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate and epicatechin.

However, epidemiological studies are inconclusive, on the other side research findings in laboratory animals clearly support a cancer chemopreventive effect of tea components. Other flavonoids, with promising health stimulating effects include quercetin, kaempferol, myricetin, apigenin and luteolin. Source of these compounds are chocolate, coca, fruit juices, nuts, red wine, raspberry, blackberry and citrus fruits.

3.5 Phytosterols

Phytosterols are another important terpene subclass. Two sterol molecules that are synthesized by plants are β-sitosterol and its glycoside. In animals, these two molecules exhibit anti-inflammatory, anti-neoplastic, anti-pyretic and immune-modulating activity. A proprietary mixture of β-sitosterol and its glycoside were tested in vitro, in animals and in human clinical trials. Phytosterols were reported to block inflammatory enzymes, for example by modifying the prostaglandin pathways in a way that protected platelets. Recently, the cytostatic activity of steroidal saponins from *Ruscus aculeatus* against leukemia HL60 cells has been reported.

In the body, phytosterols can compete with cholesterol in the intestine for uptake, and aid in the elimination of cholesterol from the body. Saturated phytosterols appear to be more effective than unsaturated ones in decreasing cholesterol concentrations in the body. These actions reduce serum or plasma total cholesterol and low-density lipoprotein (LDL) cholesterol. In mammals, concentrations of plasma phytosterol are low because of their poor absorption from the intestine and their faster excretion from liver, and metabolism to bile acids, compared to cholesterol.

3.6 Other Phytochemicals

There are certain other compounds present in plant foods, with significant health promoting effect include plant fatty acids, tocotrienols, phenolic derivatives and dietary fibers etc. Docosahexaenoic acid (DHA), which is one of the most important structural component of brain and ratina, and denovosynthesis of this compound is very rare. The decline in DHA intake could have serious implications for public health, since low plasma, DHA concentrations have been correlated with increased incidence of number of important chronic diseases such as depression, attention deficit disorders and Alzheimer’s dementia. Crypthecodinium cohmii strain of marine algae is used for the commercial production of DHA rich oil. Spirulina, termed as wonder alga is one of riches source of omeg-3-fatty acids, quality protein and many other therapeutic molecule.

Plant polyphenols are secondary metabolites widely distributed in higher plants. Their distinguishing characteristics are: water solubility, molecular weights that range from 500 to 3000-4000 D, 12-16 phenolic groups and 5 – 7 aromatics rings per 1000 relative molecular mass, intermolecular complexation, and classification as condensed proanthocyanidins, galloyl and hexahydroxydiphenoyl esters and derivaties, or phlorotannins. Polyphenols historically have been considered as anti-nutrients by nutritionists, because some, eg. tannins have such adverse effects as decreasing the activites of digestive enzymes, energy, protein and amino acid availabilities, mineral uptake
and having other toxic effects. Recognition of the antioxidant activities of many polyphenols has realigned thinking toward the health benefits provided by many of these compounds.

4.0 Promising Functional Foods with Phytochemicals

Nutrients dense foods that provide benefits beyond basic nutrition have been already developed and commercially available. Such foods can be defined as “An accepted and tolerable food that has natural, naturally concentrated photochemical derived from fruits and vegetables or related food ingredients that a) indicated to be epidemiologically important in disease prevention, b) have shown to be useful in preventing cancer or other chronic diseases in animal bioassays, c) are unique in structure, class, pattern and metabolism. The concept of fortification is not new and some new formulations based on phytochemicals are discussed here.

4.1 Functional Drinks

The beverage market is on the cutting edge in functional food development. Energy drinks, isotonic (spare) beverages, herbal and green teas, fortified waters, smart drugs, caffeinated drinks and fringe. Beverages are relatively cheap to blend, fortified, bottle and distribute. These beverages are making inroads in the conventional drink market, currently dominated by aerated synthetic beverages. Soluble fiber containing beverages are already available. Tea and coffee, constituents can be incorporated into new formulations with other phytochemical to develop herbal drinks. Recently the anti-oxidants or free radial scavenging ability of ascorbic acid, B-carotene lycopene, trocalnenols has been promoted. Free radicals are formed by autoxidation, photosensitization, and enzymatic reactions and due to pollutants.

Some of the fruits and vegetables more important ones from beverage formulation standpoint include licorice, ginger, tea, citrus, carrots, tomatoes berries, mint and other herbs and spices. These have been much interest in developing beverages, which contain these types of compounds.

4.2 Dairy Products

Probiotic cultures in fermented dairy products, have multifunctional role. In recent years, yogurts with oligosaccharides have been developed. These cultured dairy products may also incorporate, isoflavones of soy protein, B-glucan of oat, carotenoids and flavorioids of certain fruits in their formulation. Blackberry and raspberry as fruits or their extracts added in ice creams, dairy drinks, frozen desserts, not only as flavouring component, but also as source of anthocyanins and other phenotic derivatives. Similarly imitation dairy products may also include omega-3-fatty acids from flax seed oil and DHA from algae. Some infant formula have already available in market containing DHA. Dairy analogue, primarily based on soymilk, may be modified to increase the level of phytoestrogens, particularly isoflavones.

4.3 Confectionary Items

Bars either based on fruits or on cereals may serve as vehicle of phytonutrients. Commercially wide variety of nutritional bars has been developed for specific purpose.
These bars can be enriched with fibers, anti-oxidants, phenolic substances, ant proteins, glucosinolates, in them. Oat bar, based on soluble oat fibers. Also contain omega-3-fatty acids to further strengthen its heart health image. Fruit preserves, fruit spreads, have been developed, using traditional herbs and products were further enriched by adding other phyttonutrients. Encapsulation technology offers the product developer a new tool in protecting sensitive and expensive nutrients. A coated candy confectionary was patented in USA. The product has a soft candy center with 5-40% (w/w) water. Two coating was applied to the product, first one as moisture barrier and second one was used as a carrier for a number of additional ingredients, including vitamins, enzymes, phytochemicals and vegetable extracts.

Chocolate considered as a culprit for CHD, obesity and dental problems, also moves into healthy arena. The chocolate has neutral effect on cholesterol levels, low level of caffeine, as compared to beverages, anti-oxidant activity of polyphenols, potential benefit of procyanidins and epicatechins on CVD. Replacement of a part of cocoa butter with PUFA; may further improve nutritional quality of chocolates.

4.4 Breakfast Cereals

Raspberries and blackberries, reservoirs of phyttonutrients are considered as good option available for cereal product formulations. Initial studies conducted with DHA in bread are encouraging. Likewise, breads with garlic compounds and isothiocynates of cruciferous vegetables are prospective options. Soy flour is already important ingredient in bread, not only because of its nutritional significance, but excellent functional properties it imparts.

4.5 Meat Products

Soy based meat analogues, had clicked the market in early 70’s. Texturizing properties of soy proteins, has offered the opportunity to develop a number of meat analogues. Addition of oat gum to meat patties resulted in decreased cooking loss. A comminuted beef product was developed that would have nutraceutical properties, exhibit resistance to rancidity and posses improved water holding capacity and cooking yield.

5.0 Safety Issues

The optimal levels of the majority of the biologically active components currently under investigation have yet to be determined. In addition, a number of animal studies show that some of the same phytochemicals (e.g. allyl isothiocynate) highlighted in this review for their cancer-preventing properties have been shown to be carcinogenic at high concentrations.

The benefits and risks to individuals and populations as a whole must be weighed carefully when considering the widespread use of physiologically-active functional foods. For example, what are the risks of recommending the increased intake of compounds (e.g. isoflavones) that may modulate estrogen metabolism? Soy phytoestrogens may represent a “double-edged sword” because of reports that genistein may actually promote certain types of tumors in animals. Knowledge of toxicity of functional food components is crucial to decrease the risk:benefit ratio.
6.0 Conclusion

Mounting evidence supports the observation that functional foods containing physiologically active components, either from plant or animal sources, may enhance health. It should be stressed, however, that functional foods are not a magic bullet or universal panacea for poor health habits. There are no “good” or “bad” foods, but there are good or bad diets. Moreover, diet is only one component of an overall life-style that can have an impact on health; other components include smoking, physical activity, and stress.

Health-conscious consumers are increasingly seeking functional foods in an effort to control their own health and well-being. The field of functional food, however, is in its infancy. Claims about health benefits of functional foods must be based on sound scientific criteria.

7.0 Suggested Readings


Hasler, C.M. 1998. Functional Foods: Their role in disease prevention and health promotion. Food Technology 52(11), 63-70 pp


1.0 Introduction

The compositional features of human milk ensure provision to newborn infant the balanced nutrition from postnatal period until weaning into the solid foods. In addition, certain unique biochemical and immunological factors provide protection to the new born against infective agents in his/her new environment. All these features ensure intake of energy and nutrients needed to support optimum growth of infant. However, under situations where infants cannot be nursed by mothers, it becomes imperative to have infant formulas which closely imitate human milk so as to provide comparable nutritional and health benefits.

The most appropriate alternatives to breast milk will continue to be infant formulae based on bovine milk. However, infant’s underdeveloped intermediary metabolism in the critical period of first three months of ‘extra-uterine’ growth is expected to have certain physiological repercussions when fed on formulas that are compositionally different from human milk. Thus industrially prepared formulae should ideally simulate the composition and biological properties of human milk. They should contain adequate levels of biologically significant substances, which may offset losses during manufacture of infant foods and have abilities to alter gastrointestinal microflora of infants, implanting beneficial microorganisms and removing pathogens.

Considerable R&D work has been undertaken with regard to simulation studies of bovine milk to that of human milk in the area of gross and intricate composition of various constituents viz. fat, protein, carbohydrates, minerals and vitamins. This has led to the development of processes for several different types of infant formulae for the dietary requirements of normal and pre-term infants and infants suffering from specific physiological disorder. However, very little work has been done as yet for incorporating bio-immune factors that assume special significance in the bottle-feeding practice for providing protection against enteropathogenic bacteria among infants.

2.0 Formulation Aspects of Infant Formule

Infant formulae tend to vary in composition, but within fairly narrow and quite precise limits. In general, as a complete substitute for human milk, formula should provide protein (of appropriate biological quality) at 7 to 16 per cent of calories, fat at 50 to 54 percent of calories, linoleic acid at 2 to 3 per cent of calories, and the remaining calories from carbohydrate sources.
Fat plays an important role in infant nutrition. For best fat absorption, it is desirable to have available a portion of monoglycerides with palmitic acid in the 2 position. Both cow’s and goat’s milk fat represent sources of naturally occurring triglycerides with this fatty acid configuration. Vegetable fats have little or no such chemical entities. Nonetheless, a reasonable likeness of human milk fat is simulated through the judicious blending of various vegetable and milk fats. Possibly corn oil is somewhat more acceptable because of its relatively high level of oleic acid. A complete replacement of milk fat is possible, or blends of milk fat and vegetable fat possibly serve even better. In addition, evidence exists to suggest a beneficial effect in fat absorption from presence of the amino acid taurine and the betaine carnitine. Human milk contains 26.6 µmoles/100 ml of the former and 59 nmol/ml of the latter. Bovine milk formulations are assumed to be equivalent or higher in content of carnitine, but soy formulas, lacking supplementation, could be considered deficient. In human milk, the ratio of fat to protein is 2 (or more):1.

Protein in milk-based formulas is best divided between whey proteins and casein in a 60:40 ratio. Of the major whey proteins is cow’s milk, α-lactalbumin more nearly mirrors the whey proteins of mother’s milk. Absence of the other major whey protein of cow’s milk (i.e., β-lactoglobulin) eliminates a possibly significant allergen. Given 0.9 percent α-lactalbumin and 0.6 per cent casein, a reasonable likeness of the protein profile of mother’s milk is produced. The ideal protein composition of formula would be 1.0 to 1.2 g protein/100 ml, and made consistent with human milk in the levels of taurine and cystine. Major sources of protein for infant formulas include milk, whey, and soy. In the latter case, highly refined soy protein (soy isolate) is used in most instances. Nonetheless, various methods of purifying soy protein create significant differences in the composition of associated ingredients, particularly mineral components. All soy protein supplies should be carefully monitored, and the protein obtained from different suppliers should not be assumed to be identical. Chemical analysis will generally serve most monitoring and/or quality control functions. New protein sources (nonmilk, nonsoy) should undergo extensive metabolic and clinical studies prior to use.

Calorific density of infant formulas of 670 kcal/liter appears nearly optimal for normal full-term infants. This is obtained by adjusting the ratios of Fat:protein:carbohydrate content of the formulation. The formulation should provide optimal calcium-phosphorus ratio of 1.5:1 at least through most of the first year of life. Calcium should be of a chemical form that is biologically available and should be present to a minimum of 50 mg/100 kcal and phosphorus 25 mg/100 kcal. Minimum and maximum amounts of sodium, potassium and chloride must also be observed. These levels are met within the ranges 6 to 17, 14 to 34 and 11 to 29 milliequivalents (mEq), respectively, in a formula providing 670 kcal/liter. One milliequivalent is equal to the atomic weight (in milligrams) of the element divided by valence. Osmolarity – in moles of solute/liter – should not exceed 400 mOsm.

Carbohydrate sources include lactose (or milk and whey products that contain lactose), sucrose, corn syrup solids (a source of glucose), and starch. Actually, modified starch is used not so much as source of carbohydrate as a stabilizer. Although there is no reason to believe that starch poses a digestive problem to infants, neither have studies been carried out to assess its relative digestibility. However, generally more formulas are prepared without than with it.

A large number of mineral compounds are approved and made mandatory for incorporation in formulated infant foods (PFA, 1999). Suitability of any given mineral
additive depends on composition and moisture level of the food product. Certain mineral compounds are needed in fairly large amounts in infant formula. Calcium and phosphorus are two examples. Other mineral elements are needed only in very small (trace) amounts. At these minute levels, these minerals are nonetheless essential. Thus, trace minerals in ingredients of infant formula must be considered.

In formulating mineral composition of dairy-based infant formula involves certain unique technological challenges. The content of calcium in cow’s milk varies somewhat on a seasonal and regional basis. The level of certain trace minerals – among them zinc, iodine, copper, sodium, manganese, and cobalt – vary to more or less an extent by the amount present in feed. The iron content of cow’s milk seems uninfluenced by the intake of feed. Some amount of iron, copper, molybdenum, manganese, and magnesium is associated with the fat globule membrane and will be lost upon separation and exclusion of milk fat as an ingredient in formula. Perhaps a third of both calcium and phosphorus is associated with casein and remains with casein upon rennet coagulation. Most magnesium (about 75%) and essentially all zinc are complexed with casein. Off course certain amount of mineral depletion is necessary to provide a formula of appropriate osmolar strength. In the process, however, some amount of some trace minerals will be lost.

When trace minerals are added to formula, sulphate salts are commonly used. Because of the potential to cause methemoglobinemia, nitrate salts are usually not added to formula. Some small (safe) amount may occur in formula made up of vegetable products. Nitrates also occur, and occasionally at high levels, in some water supplies. Copper is another potentially toxic component of water. Soy products not adequately processed may contain goitrogens, necessitating the presence in formula of added iodine as a defense against goiter. Minerals commonly added to formulas include calcium, phosphorus, magnesium, iron, copper, iodine, zinc, potassium, sodium, manganese, and chlorine (as chloride).

→ Additives - Codex standards for infant food (1981) has approved various kinds and amounts of food additives such as thickening agents, emulsifiers, antioxidants, and compounds for adjusting pH. Most of these agents have GRAS status in the United States. Similarly, these standards also recommend the vitamin additives for use in infant formula. In some cases, several different chemical forms of the vitamin or provitamin have approval for use. Processing requirements, availability, and/or stability in the specific food system will dictate which form(s) will serve best.

### 3.0 Bioprotective Attributes

During early infancy, risk for mortality and morbidity from common pathogens like coliform, salmonella, and shigella are very high. A number of innate factors in human milk influence, the intestinal microflora of the neonate that ultimately inhibit these enteropathogenic bacteria. These humoral protective factors are either derived from blood serum or synthesized in mother’s memory glands. Little attention has been paid for incorporating these bioprotective factors that assume special significance in bottle-feeding practice for providing humoral protection among infants. The following text describes possible actions and usage of these humoral protective factors for infant formulae.

#### 3.1 Lactoferrin

Under physiological conditions, lactoferrin is capable of chelating iron, thus depriving bacteria of the iron required for metabolism, resulting in their inhibition. Digestion
of lactoferrin with certain proteolytic enzymes does not significantly alter its iron binding property, suggesting that protein was quite capable of exerting antibacterial activity against the *E. coli* and other enteropathogens in the digestive tract of the infant. Lactoferrin also plays a useful role in the absorption of iron from the feed. Anti-carcinogenic properties of lactoferrins, especially as a possible cure for leukemia have also been demonstrated. Lactoferreroxin, an opioid peptide with six amino acid residues originating from hydrolysis of lactoferrin has been shown to be physiologically significant, although its usefulness in human health needs to be further elucidated.

### 3.2 Lactoperoxidase

The lactoperoxidase system (LP) consists of three components – lactoperoxidase enzyme, hydrogen peroxide and thiocyanate, an oxidizable substance. In the newborn infant saliva is quite rich in lactoperoxidase activity and the enzyme is known to be resistant to low pH and proteolysis. The saliva that enters the stomach and rich in lactoperoxidase is expected to reach the intestinal tract in an active form for activation of LP system. The lactic acid bacteria metabolically generate hydrogen peroxide. Thiocyanate, the third factor of LP system occurs naturally in animal tissues and secretions. Thus, the system oxidizes the chemically inert SCN into OSCN. Exposure of bacterial cell to this oxidative product results in leakage of potassium and amino acids from the cell and inhibition of carbohydrate uptake and synthesis of DNA and RNA leading to cell lysis specially of the gram negative organisms. Bovine milk that contains 30 µg/ml lactoperoxidase and 2 to 8 ppm thiocyanate could be an important source for the supply of SCN required for activation of LP system. Controlled application of the system in infant formula may be very useful for protecting neonatal health.

### 3.3 Lysozyme

In view of the antibacterial effect and possibly general immune system of neonates, there has been a considerable interest in developing food applications of lysozyme. The incorporation of lysozyme in infant formula could be useful in view of its influence under the ecological system of the intestinal tract. The concentration of lysozyme in human milk is 300 times greater than bovine milk. The biological significance of lysozyme lies with its possible protective effect against gram-negative potential pathogens. Although lysozyme is isolated from egg bovine lysozyme has greater lytic activity. It is stable at low pH and resists gastric juices under *in vivo* situations. Lysozyme containing formulations tend to enhance bifidobacterium population in the intestinal tract.

### 3.4 Immunoglobulins

It is well established that feeding of colostrum and milk increases the resistance of both human and neonates against enteric infections. The secretory Ig-A is a major antibody in human milk. Ig-G and Ig-M are known to involve to a greater extent in providing both the bacteriostatic and bactericidal effects. These immunoglobulins if incorporated in an active form the infant formula, will complement the bacteriostatic components present in the formula. Membrane driven processes have been standardised in our lab for the preparation of immunoglobulins on pilot scale suitable for incorporation in the infant formulas.

In this manner, it may be possible to augment the future infant formulations with the unique properties of human milk with respect to containment of humoral protective factors.
apart from the nutritional properties to fulfil the requirements of an human infant in the absence of availability of sufficient quantities of mothers milk or in case where mothers are unable to feed their infants due to socioeconomic reasons or fear of transmittable diseases.

4.0 Biological Aspects

4.1 Pro-and/or Prebiotics in Infant Feeding

The predominance of several of the probiotic organisms as natural inhabitants of the gastrointestinal tract of breast-fed infants has led to detailed studies on their role on the health and well being of infants, their association with the components of breast milk and their probable incorporation in infant feeds. The industry is investigating means to make the gut ecology of formula-fed infants similar to those of breast-fed infants. The bifidus activity of milk of Indian women were studied by Pahwa and Mathur (1982) who found that the activity was approximately 30 and 50 times as much as those of buffalo and cow milk respectively. Cell concentrate of *B. bifidum* was added to an infant formula mix (BCF) and fed to 24 infants. The physiological response of the BCF-fed infants was very close to the breast-fed children. Both the groups had bifidobacteria implanted in their intestines. Animal bioassays involving the two formulae revealed that the calcium and iron absorption was greater in the BCF-fed rats.

Langhendries *et al.* (1995) reported that the addition of lactobacilli to infant formula resulted in a gut ecology similar to those of breast-fed infants. It has been demonstrated that supplementation of infant formula with *B. bifidum* and *S. thermophilus* can reduce the incidence of acute diarrhoea and rotavirus shedding in infants admitted to hospital (Saavedra *et al.*, 1994; Phuapradit *et al.*, 1999). The use of antibiotics, particularly those with broad-spectrum action, destroys the intestinal flora, causing diarrhoea. Several studies mention the possibilities of employing probiotics as alternatives to antibiotics to help the gastrointestinal tract to resist such aggression. Vanderhoof *et al.* conducted a trial in children receiving antibiotics, wherein they were given *Lactobacillus casei* subsp. *rhamnosus* (*Lactobacillus GG*) or placebo. The percentages of children with loose stools and diarrhoea were significantly lower in the group receiving the probiotic organisms.

Certain glucosamines (of nonprotein nitrogen) may serve to stimulate growth of *B. bifidum*. For this purpose, N-substituted D-glucosamines have been suggested, and also the co-enzyme A precursor, pantetheine phosphate. In addition, lactulose (4-0-β-d-galactopyranosyl-d-fructose), a ketose derivative of lactose, has been shown to enhance growth of the organism. This is true even though the compound is not found to occur in raw (unheated) milk of either humans or cows. The formula in which the bifidus-stimulating phenomenon was originally noted consisted of 1.2 g lactulose/70 kcal, with lactose content of the diet held at 2.5 times the protein level. Oligosaccharides and other prebiotics indicate that incorporation in humanised infant formulae may boost the survival of probiotic organisms in the gut of formula-fed infants. The proportions, as well as the absolute numbers of bifidobacteria in the intestinal contents of infants increase when they are fed formula supplemented with prebiotics (Boehm *et al.*, 2001; Knol *et al.* 2001). The Scientific Committees on Food (SCF) of the European Commission has confirmed the safety of oligofructose for use as an ingredient in baby food (Franck, 2002) after examining experimental evidences. This prebiotic along with galacto-oligofructose can be used in follow-up foods in a concentration of up to 0.8 g/dl in the product ready for consumption and can be given to infants aged between 0 and 6 months. Thus, consumption of cereal supplemented with fructo-oligosaccharides is associated with fewer infectious periods and
less severe diarrhoeal disease. However, more clinical studies among paediatric subjects are needed to elucidate the mechanisms involving prebiotics and clinical benefits.

5.0 Conclusion

There is still a place for artificial milks, although the emphasis of evidence suggests that breast-feeding is the ideal to be sought wherever possible. There is no doubt, nevertheless, that there are circumstances which demand bottle-feeding. The need of the hour appears to develop advanced infant foods with antimicrobial and protective components of milk such as lysozymes, lactoperoxidases, lactoferrin, vitamin binding proteins Infant formulae with added prebiotics and with or without probiotics are also gaining recognition. Such formulae help to improve the gut microflora as well as nutrient absorption.

6.0 Suggested Reading


# Table 1: Major constituents of Human and Bovine Milk

<table>
<thead>
<tr>
<th>Component</th>
<th>Human</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>700</td>
<td>680</td>
</tr>
<tr>
<td>Fat (g/l)</td>
<td>40</td>
<td>38</td>
</tr>
</tbody>
</table>

**Fatty acid composition (mole %):**

<table>
<thead>
<tr>
<th>Component</th>
<th>Human</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>–</td>
<td>3.3</td>
</tr>
<tr>
<td>C6:0</td>
<td>Trace</td>
<td>1.6</td>
</tr>
<tr>
<td>C8:0</td>
<td>Trace</td>
<td>1.3</td>
</tr>
<tr>
<td>C10:0</td>
<td>1.3</td>
<td>3.0</td>
</tr>
<tr>
<td>C12:0</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>C14:0</td>
<td>5.1</td>
<td>14.2</td>
</tr>
<tr>
<td>C16:0</td>
<td>20.2</td>
<td>42.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.8</td>
<td>5.7</td>
</tr>
<tr>
<td>C18:1</td>
<td>46.4</td>
<td>16.7</td>
</tr>
<tr>
<td>C18:2</td>
<td>13.0</td>
<td>1.6</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.4</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**Comparative triglyceride structure of fats (mole %):**

<table>
<thead>
<tr>
<th>Component</th>
<th>Human</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSS</td>
<td>9.0</td>
<td>35.0</td>
</tr>
<tr>
<td>SSU</td>
<td>40.0</td>
<td>36.0</td>
</tr>
<tr>
<td>SUU</td>
<td>43.0</td>
<td>29.0</td>
</tr>
<tr>
<td>UUU</td>
<td>8.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Protein (g/l):**

<table>
<thead>
<tr>
<th>Protein make-up</th>
<th>Human</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (g/l)</td>
<td>3.1</td>
<td>26.0</td>
</tr>
<tr>
<td>Alpha lactalbumin (g/l)</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Beta lactoglobulin (g/l)</td>
<td>Absent</td>
<td>3.0</td>
</tr>
<tr>
<td>Factors</td>
<td>Human milk</td>
<td>Bovine milk</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Complement (C1-C9)</td>
<td>Present</td>
<td>Doubtful</td>
</tr>
<tr>
<td>Secretary Ig A</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Present</td>
<td>Trace</td>
</tr>
<tr>
<td>Bifidus stimulating factors</td>
<td>Present</td>
<td>Trace</td>
</tr>
<tr>
<td>Vitamin binding proteins</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Amino acids</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Copper</td>
<td>Moderate</td>
<td>Low</td>
</tr>
</tbody>
</table>
Table 3: Vitamin Content of Human and Bovine Milk (Per l)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Human</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit. A (IU)</td>
<td>1900</td>
<td>1030</td>
</tr>
<tr>
<td>Vit. C (IU)</td>
<td>44</td>
<td>17</td>
</tr>
<tr>
<td>Vit. D (IU)</td>
<td>22</td>
<td>34</td>
</tr>
<tr>
<td>Vit. E (IU)</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Vit. K (IU)</td>
<td>15</td>
<td>68</td>
</tr>
<tr>
<td>Vit. B1 (mg)</td>
<td>150</td>
<td>370</td>
</tr>
<tr>
<td>Vit. B2 (mg)</td>
<td>380</td>
<td>1800</td>
</tr>
<tr>
<td>Vit. B6 (mg)</td>
<td>130</td>
<td>460</td>
</tr>
<tr>
<td>Vit. B12 (mg)</td>
<td>0.7</td>
<td>4</td>
</tr>
<tr>
<td>Biotin (µg)</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>Folic acid (µg)</td>
<td>43</td>
<td>55</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>1700</td>
<td>900</td>
</tr>
</tbody>
</table>

Table 4: Minor Constituents of Infant Formulas (Per 100 kcal)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (IU)</td>
<td>250.0</td>
<td>239.7</td>
</tr>
<tr>
<td>D (IU)</td>
<td>80.0</td>
<td>79.9</td>
</tr>
<tr>
<td>K (µg)</td>
<td>4.0</td>
<td>4.1</td>
</tr>
<tr>
<td>E (IU)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>C (ascorbic acid) (mg)</td>
<td>8.0</td>
<td>7.99</td>
</tr>
<tr>
<td>B1 (thiamine) (µg)</td>
<td>40.0</td>
<td>42.2</td>
</tr>
<tr>
<td>B₂ (riboflavin) (µg)</td>
<td>62.0</td>
<td>62.8</td>
</tr>
<tr>
<td>Vitamin/Mineral</td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>B&lt;sub&gt;6&lt;/sub&gt; (pyridoxine) (µg)</td>
<td>36.0</td>
<td>36.5</td>
</tr>
<tr>
<td>B&lt;sub&gt;12&lt;/sub&gt; (µg)</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Nicotinamide (µg)</td>
<td>260.0</td>
<td>264.0</td>
</tr>
<tr>
<td>Folic acid (µg)</td>
<td>5.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Pantothenic acid (µg)</td>
<td>300.0</td>
<td>320.0</td>
</tr>
<tr>
<td>Biotin (µg)</td>
<td>1.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**Minerals**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg)</td>
<td>50.0</td>
<td>52.5</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>25.0</td>
<td>26.2</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Iodine (µg)</td>
<td>5.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.55</td>
<td>0.57</td>
</tr>
<tr>
<td>Copper (µg)</td>
<td>62.0</td>
<td>63.9</td>
</tr>
<tr>
<td>Manganese (µg)</td>
<td>5.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>20.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>80.0</td>
<td>84.4</td>
</tr>
<tr>
<td>Chloride (mg)</td>
<td>55.0</td>
<td>57.1</td>
</tr>
<tr>
<td><strong>Choline (mg)</strong></td>
<td>7.0</td>
<td>7.3</td>
</tr>
</tbody>
</table>

PFA (1999)
SAFETY ASPECTS OF FOOD COLOURS

Dr Sudhir Singh
Sr. Scientist
Indian Institute of Vegetable Research
Varanasi (UP)

1.0 Introduction

Colours are used in food for centuries to increase consumer acceptability (Walford, 1980). The colouration of food is technological necessity in processed foods especially canned and pulped fruits and vegetables, which often lose their natural, shade during the heat treatment. However, because of cheapness, easy availability, several low cost food colours have tempted unscrupulous traders to use non-permitted food colours in various food products. The degradation of food colours in intestinal tract causes breakdown of degrading components with liberation of toxic amines that are harmful to the human beings.

The consumption of food colours regularly in large quantities leads to the formation of toxic amines after degradation. Besides the degradation of food colours through gut flora, the ingested food colours react with proteins and carbohydrates thus altering the digestion processes. Certain food colours such as amaranth, tartrazine and erythrosine react with copper, iron and zinc in foods, thus affecting their absorption in small intestine. The food colours are extracted by selective extraction process based on differential solvent solubility method. Food colours should not contain more than 1-3 ppm arsenic, 20 ppm chromium, 10 ppm each of copper and lead. The maximum permissible limit should be strictly adhered to for the benefit of consumers.

2.0 Incidence of Non-Permitted Food Colours in Traditional Dairy Products

During the survey of 1,154 coloured samples, of khoa and channa based sweets of rural and urban markets of Lucknow, 498 samples contained permitted and 656 samples contained non-permitted colours during 1965-67 (Khanna et al., 1973). The permitted green colour, fast Green FCF, is at present not synthesized in India. It is an imported colour; therefore, unscrupulous traders; use either hazardous non-permitted green colour, I malachite green, or mix cheap quality non-permitted yellow or blue colour to make green shade in khoa-based sweets. A statistically designed survey of khoa-based sweets in rural markets in UP also confirmed the mixing of brilliant Blue FCF with tartrazine or indigo carmine with tartrazine or blue VRS and metanil yellow (Khanna et al., 1985).

The comparative usage pattern of synthetic dyes in khoa-based sweets among rural and urban markets of UP showed 25% adulteration with non-permitted food colours in
urban-sold khoa-based sweets whereas 78% adulteration was noted in khoa-based sweets of rural markets. The non-permitted colour in majority of the cases was detected as metanil yellow, orange II and blend of metanil yellow and orange II in khoa-based sweets of rural and urban markets of UP (Khanna et al., 1986).

Another survey of samples of sweets and confectionery products during festival season in 1993-94 in twin cities of Hyderabad and Secunderabad by National Institute of Nutrition, Hyderabad, confirmed wide usage of non-permitted colours. The major adulterants were metanil yellow, orange II and rhodamine B in sweets and confectionery products. As per the estimate of adulteration of non-permitted colours, it is observed that 5% of the samples contained orange II, 41% rhodamine B, 30% auramine and 24% samples had combination of rhodamine B and auramine (Anon, 1996).

3.0 Health Hazards of Non-Permitted Food Colours

Non-permitted food colours are used alone or in combination or mixed with permitted food colours in various food products such as khoa and channa-based sweets, ice creams, namkins, turmeric powder, chilli powder etc. It is evident from the survey work at NIN; Hyderabad that Metanil yellow and Orange II are most commonly used non-permitted food colours in various food products. Our studies with adult wistar male albino rats after daily intake of 3.0% metanil yellow in synthetic diet (20% casein) for 45 days showed significant decrease in food intake and body weight than the control group of rats with normal control diet (20% casein). Similarly there has been significant decrease in the weight of body organs such as kidney, heart, lung, stomach, caecum and testes than the control group of rats.

Our experiments further revealed that feeding of 3% metanil yellow in control synthetic diet showed substantial increase in reductive and hydrolytic activity in the caccal micro flora of rats than the control group of rats. This clearly shows that people consuming foods containing hazardous food colours are capable of producing enzymes responsible for cleavage of food colour at a rapid rate, thus causing accumulative toxicity (Singh, 1993). The type of diet and frequency of feeding affects, the type of micro flora in the intestine.. Western diet rich in animal protein and fat contained more bacteroids and facultative anaerobes and lactobacilli were observed to be lower in the faeces of the Western population (Aries et al, 1969; Drasar et al., 1973).

The reduction of azo compounds in the gut has a number of important toxicological effects. The amines produced by azo reduction may themselves be toxic or be converted into toxic metabolites in the tissue after absorption. The introduction of L-amino-2-naphthol and 2-amino-1-naphthol as the breakdown product of Orange II, induced local tumor formation into the lumen of the bladder of mouse (Daniel, 1962; Clayson & Garner, 1976; Watabe et al., 1980).

The interspecies differences also lead to different toxicity response to food colours. This suggests that the toxicity of an azo dye depends to a large extent on the rate at which bacterial systems reduce azo linkage. Besides azo dyes, the other poorly absorbed colours are metabolised by gut microflora. Therefore, the intact sulfonated triphenylmethane compounds are largely excreted in the faeces unchanged, which accounts for low toxicity. Unlike many polar azo dyes and its metabolites, the triphenylmethane compounds are not responsible for any alteration in gut morphology (Brown et al, 1980; Phillips et al., 1980).
Besides the degradation of food colours through gut flora, the ingested food colours along with food cause adverse changes in the body. As per the recent evidence, some food colours such as erythrosine, orange II and rhodamine B at the permissible levels react with proteins and carbohydrates thus harming the carbohydrate digestion. Amaranth, tartrazine and erythrosine react with mineral ions such as copper, iron, and zinc in foods, thus affecting their absorption in small intestine. This may lead to deficiency-linked disorders in the human body. Other food colours such as tartrazine and metanil yellow reduce the level of vitamin A and E in the body during continuous intake of colours in the diet (Anon, 1996).

4.0 Status of Synthetic Food Colours

Out of the 8 colours permitted for food use under Prevention of Food Adulteration Act, there are 3 red, 2 yellow, 2 blue and 1 green synthetic colours. However, 9 colours are listed as non-permitted colours on the basis of toxicological studies. India produces about 135 tonnes of synthetic food colours, which is just about 1.5% of the world production of food colours. Among the permitted food colours, sunset yellow FCF and tartrazine accounts for 70% net production.

Apart from fast Green FCF and brilliant blue FCF that are still imported, the other permitted food colours cater to the domestic requirements only. The Indian population consume 220 mg of food colours per year which is 50 times less than the per capita intake of food colours in USA and 35 times less than the UK consumption (Walford, 1980).

5.0 Identification of Food Colours

Synthetic food colours are characterised by their high water solubility, ability to produce different shades of colour and their relative stability in foods with temperature and acids. Food colours exhibit good fastness properties with light, alkali, fruit acids, benzoic acid, sulfur dioxide and stabilities at higher temperature such as 105-205°C. Synthetic food colours develop characteristic colour with concentrated acids and base such as hydrochloric acid, sulfuric acid and sodium hydroxide which enables their qualitative identification.

6.0 Extraction and Resolution of Metabolites

Selective extraction method based on differential solvent solubility is generally carried out to extract the food colours (Dixit et al., 1995). The sample is soaked in petroleum ether with occasional shaking followed by filtration. After filtration, the residue is dried at room temperature and shaken with ethanol with occasional heating in a water bath, after which the solvent is decanted. The combined filtrate is evaporated to remove ethanol and the residue is taken in distilled water. The colours in water phase are then selectively extracted in 2-3 ml of acidic n-butanol. The small volumes of acidified n-butanol serve the purpose of qualitative detection. However, repeated extraction with n-butanol is followed for quantitative determination. The combined n-butanol extracts are dried under vacuum. The butanolic coloured layer was applied on to a whatman No.1 chromatography paper strip, together with standard colour sample, and run for 8-10 hr in either of the following 2
solvent systems (Crossby, 1981). 1 Solvent A: Trisodium citrate: ammonia: water (2g: 15ml : 85 ml) for pink-red, orange and yellow synthetic colours.

Solvent B: Isopropanol: ammonia: water (7: 2 : 1 v Iv) for blue, green and yellow synthetic colours.

Rf values of separated colours are compared and matched with Rf values of reference dyes. The separated spots of various aromatic amines were visualised either by viewing under ultraviolet viewer or after spraying the chromatogram with Bratton-Marshall spray reagent.

7.0 Impurities in Food Colours

Food colours contain various metal contaminations such as lead, cadmium, manganese, nickel, zinc, copper, chromium and arsenic which may arise from raw materials during processing. Joint FAO/WHO Expert Committee on Food Additives recommended that increased attention should be given to reduce the trace element impurities in food colours, therefore particular attention should be paid to ensure that free amine content of colours is kept below 0.02%. The use of sophisticated analytical techniques such as high-pressure liquid chromatography has resulted in the identification of impurities like \(\alpha\) and \(\beta\) naphthylamine in certain azo colours. These contaminations arise mostly from non-sulfonated amine moieties, which may occur as impurities in raw materials.

Food colours should contain not more than 1-3 ppm arsenic, 20 ppm chromium, 10 ppm each of copper and lead (Khera, 1979). Food containing lead chromate causes vomiting and diarrhoea in several persons and many human populations developed severe allergic reactions after consuming foods with carmine, a natural dye extracted from a group of insects (Anon, 1996). The maximum permissible limit should be strictly adhered to for the benefit of consumers. Strict adherence to maximum metal limit would encourage the industry to select good quality raw materials, processing equipments and containers and ultimately eliminate the inherent additional health hazards to a vast section of consumers.

8.0 Conclusion

The use of food colours has become a prominent feature of the present-day food processing industry. The restrictive supply avenues of permitted colours together with their high cost are detrimental factors in promoting their use. On the contrary, the easy availability in small quantities and in cheap packing make non-permitted colours much more easily accessible. This coupled with ignorance plays a significant role in the continued popularity of non-permitted colours. Easy retail availability of permitted food colours in tamper proof small strip packs along with suitable rapid and reliable methodology will considerably help in overcoming the large scale indiscriminate use of non-permitted food colours in various food products.
9.0  Suggested Readings


1.0 Introduction

Direct relationship between the type of diet consumed and health status of an individual has led to the concept of functional foods. Functional foods and nutraceuticals provide a means to reduce the increasing cost on the health care system by a continuous preventive mechanism. The interest in functional foods has started in early 1990s, becoming one of the fast growing sector of global food industry.

Gastrointestinal organ system in human body is an important link between the food and resultant health benefit. The delicate balance between the intestinal microflora and the host organism is very critical and any disturbance may lead to acute gastro enteritis and more chronic disorders like inflammatory bowl syndrome, colonic cancer etc. Many factors influencing the gut microflora include medication, age, stress, living condition and above all diet. Hence, dietary management strategies that help in sustaining or even improving the normal gastrointestinal microflora need to be addressed. Probiotics are the well-known means to target the GI microbes with proven disease preventing/curing attributes. Complex carbohydrates including dietary fibers, resistant starch and oligosaccharides not only contribute significantly towards nutrient metabolism, but also perform certain physiological functions that affect GI microflora positively. Oligosaccharides, a common constituent of plant and animal cellular constituents have been recognized with number of health attributes and termed as “New age fiber”.

2.0 Oligosaccharides

Oligosaccharides (O.S) are found as major components of many natural products either in free or in combined form. These are hydrolyzed polymers of monosaccharides that contain 3-10 linked molecules of simple sugars. Certain other compounds like lactulose and galactobiose also exhibit similar functional characteristics and are widely regarded as oligosaccharides. Oligosaccharides can be synthesized by chemical reactions or by controlled enzymatic hydrolysis of complex polysaccharides or enzyme assisted transglycolation reactions. Food grade OS are not pure compounds but they are mixture of oligosaccharides with different degree of polymerization, monomer sugars, or parent polysaccharides. O.S are crystalline compounds, which are soluble in water and relatively less sweet than sucrose. They also exhibit wide range of physico-chemical properties like water activity lowering ability, modification of viscosity, freezing point depression, emulsifying, gel forming and water binding ability. They also possess higher stability over wide range of pH and temperature. OS are resistant to human salivary amylases, porcine
3.0 Novel Physiological Functions of Oligosaccharides

One of the most critical aspects of OSs is that they must remain viable after ingestion when it is exposed to gastric acid or certain small intestine secretions. Human intestine lacks enzyme able to hydrolyze β-glycosidic bonds with the exception of lactose, which make OSs with β-linkages nondigestable. After entering into the large intestine, the OSs are selectively utilized as sole source of carbon by beneficial bacteria of the *Bifidobacterium* species and other harmful bacteria like *E.coli, Clostridia* harbouring the large intestine can not utilize it. The proliferation of *Bifidobacteria* by this selective utilization is named as “bifidogenesity” and is also recognized as key property for physiological functions in host organisms. Such ingredients could be called "prebiotics" and also be called as "colonic foods". The intestinal microbes transform these OSs into short-chain fatty acids (SCFAs) like acetate, propionate and butyrate, which are absorbed and metabolized into carbon dioxide and hydrogen. Briefly SCFAs are thought to be efficiently absorbed and utilized by human colonic epithelial cells, to stimulate salt and water absorption. The secondary physiological function OSs perform include improved mineral absorption particularly calcium and magnesium and the tertiary function attributed to them include allergy prevention and cancer prevention.

3.1 Oligosaccharides as Bifidogenic Factor

A limited range of microorganisms, which include the members of *Bifidobacterium* species ferment some OS Due to the selectivity of the growth substrates and health promoting properties of *Bifidobacteria* considerable attention is now being focused on the use of these OSs as bifidogenic factor. *Bifidobacteria* is an intestinal microbe that exhibits number of putative functions in host organisms. *Bifidobacteria* has been thoroughly investigated as therapeutic and prophylactic agents for human and animal health. Cultured dairy products containing *bifidobacteria* have been shown to demonstrate a wide variety of functional attributes including inhibition of pathogens, maintenance and restoration of normal intestinal flora, stimulation of immune system and removal of toxicants such as blood ammonia, phenol and urinary indican in the Gastrointestinal tract). Other potential health benefits ascribed to *bifidobacteria* include reduction of serum cholesterol levels and colon cancer risks, improvement of lactose intolerance, calcium absorption and vitamin synthesis (Gibson and Roberfroid, 1995).

Human studies have shown an increase in *Bifidobacterium* resulting from OSs ingestion and a reduction in detrimental bacteria such as *Cl. perfringens*. They found that ingestion of 210mg /day for several weeks effectively increased bifidobacterial population in intestine (an average of 7.5 times) and decreased *Cl. perfringens* (an average of 81%). *Lactobacilli* also increase from 2 to 3 times on ingestion of OSs. A number of in-vitro experiments indicate that wide range of Lactic acid bacteria including *Bifidobacteria* and *Lactobacilli* possess the ability to ferment various groups of OSs, while harmful bacteria like *Clostridia, Bacteroides* cannot ferment them. Recently it has been experimentally demonstrated in infants that a mixture of galactooligosaccharides (GOSs) and fructans is very effective in stimulating a *bifidobacteriae* and changing the stool characteristics to become closer to those found in breast-fed infants. The bifidogenic ability of OSs makes them an ideal ingredient in probiotic food products, infant formulae as well as many other functional foods.
3.2 Oligosaccharides as Immunomodulatory Agent

Oligosaccharides either prevent or reduce the adhesion of infectious agents to epithelial surface and it is an important mechanism in increasing the defense against intestinal infections. The production of SCFAs and subsequent lowering of pH inhibit the growth of many food borne pathogens. Human experiment with lactulose—a milk oligosaccharide, indicated that it shows promise for the treatment of Shigella carrier but appears ineffective in treatment of acute shigellosis. Some of the plant oligosaccharides have also been effective against certain pathogenic organisms. The role of OSs in development of balanced intestinal microflora seems to be one of the main mechanisms for the host defense activity. Immnuomodulation property of OSs is mainly effective in preventing certain types of allergy. Human clinical trial indicated that certain OSs might prevent atopic dermatitis. However this particular function need more clinical trials and validation.

3.3 Oligosaccharides and Mineral Absorption

Many OSs especially inulin and fructooligosaccharides (FOS), have been linked to an increase of mineral absorption in the large intestine. The higher production of SCFAs in the presence of OSs lower down the pH effectively and help in solubilization of minerals particularly calcium and magnesium. The anti femur fracture properties are increased by administration of Bifidobacterium and lactulose. They promote Ca absorption, which in turn increases bone strength. A significant increase in calcium absorption was observed in adolescent girls who were given a drink fortified with inulin and FOS (4g/day) and a daily supplement of calcium (1.5g/day). Thus for effective absorption of calcium the diet should not be deficient in calcium. Galactooligosaccharides recovered the absorption of magnesium and thus suppressed the calcification of heart specially when the concentration of calcium and phosphorus was high. A preterm infant formula containing 90% GOS and 10 inulin resulted in increased renal calcium excretion as compared to that found in a non-supplemented formula, indicating the higher calcium absorption. The higher calcium and mineral absorption is beneficial in maintaining the bone health and combating the conditions of osteoporosis. FOS intake also improved iron absorption and might relieve anemia caused by iron malabsorption. However, the various investigations did not show a consistent trend regarding mineral metabolism and OSs intake. There is need to consider experimental design, type of OSs and mineral intake.

3.4 Oligosaccharides in Cancer Prevention

In humans, colorectal cancer is thought to have a bacterial origin, with around 10 different carcinogens identified that have been as a result of microbial activity. Several bacterial enzymes like β-galactosidase, β-glucosidase and nitroreductase may play a role in colon carcinogenesis by converting procarcinogens to proximal carcinogens. Dietary manipulation that helps in reduction of accumulation of such compounds is an attractive approach to deal with the problem of colon cancer. Oligosaccharides have been shown to reduce the activity of microbial enzymes involved. It is mainly due to activation of the immune system by Bifidobacterium cells, cell wall components and extracellular components. Lactulose can directly protect against DNA damage in animal models challenged with colonically active carcinogens.

In vivo, however, 4 g FOS (neosugar)/d decreased β-glucuronidase and glycocholic acid hydroxylase activities in 12 subjects but did not affect nitroreductase activity. Similarly,
in the study of Bouhnik et al, 12.5 g FOS/d had no effect on the activities of nitroreductase, azoreductase, or β-glucuronidase in feces. Likewise, Kleessen et al were unable to show changes in β-glucuronidase and β-glucosidase activities with inulin consumption in constipated elderly subjects. Studies of fecal enzyme activities are notoriously difficult to interpret and the in vitro system may well be a better model of what is going on in the more proximal gut. Furthermore, whether changes in enzyme activity translate into increased product formation depends on substrate availability, pH and a host of other factors.

3.5 Oligosaccharides and Coronary Heart Diseases (CHDs)

Oligosaccharides have been suggested to modify serum triglyceride levels and cholesterol metabolism. The human lipid metabolism is a complex process and comprehensive investigations are difficult to undertake. They often result in yielding conflicting outcomes. Data from FOS show either no effect or slight decrease in circulating triglycerols and plasma cholesterol concentrations, whereas higher molecular weight inulin have shown more success in lowering triglyceride levels. The mechanism for lowering serum triglycerides and cholesterol content can be attributed to similar functionality that is usually observed with dietary fibers. The hypotriglyceridemic and hypocholesterolemic effects of OSs may be attributable to the reduction of hepatic synthesis/or absorption of these compounds.

3.6 Oligosaccharides in Improving the Intestinal Diseases

Inflammatory Bowl Disease (IBD) encompassing both Cohn’s and Ulcerative colitis is a chronic inflammatory intestinal disorder of unknown etiology. OS act as fecal bulking agents due in part to their osmotic effect. Small amounts are recommended throughout the day due to this effect. Studies have shown that greater than 10 grams per day can cause increased diarrhea and cramping. Eternal tubefeeding products on the market contain approximately 4 to 8 grams per 1,000 calories. Studies have used any where between 3 and 20 grams per day. OSs are currently used to treat encephalopathy; animal studies suggest a decreased risk of colon cancer. Butyrate can maintain remission in IBD patience by promoting mucosal proliferation and accelerating the healing process in animal models and human subjects. Thus, the use of these compounds enhances the butyrate production in the colon of UC patience.

4.0 Common Oligosaccharides

During the last two decades, many types of oligosaccharides, such as fructo-, galacto-and xyl- oligosaccharides were actively developed. In Japan, the leader in functional food category has allowed seven types of oligosaccharides with ‘Food For Specific Health Use’ (FOSHU) status. Some of the promising oligosaccharides with proven health benefits have been discussed hereunder.

4.1 Fructo-oligosaccharides

Fructo-oligosaccharides (FOS) are naturally occurring carbohydrates in wheat, rye, triticale, asparagus, onion, and Jerusalem artichoke and a number of other plants. FOS and higher molecular weight fructans can comprise 60-70% of the dry matter in Jerusalem artichokes. FOSs have been commercially prepared by the action of a fructofuranosyl furanosidase from Aspergillus niger on sucrose. They are about 30% as sweet as sucrose,
have a taste profile similar to sucrose, are stable at pH values above 3 and at temperatures up to 140°C. Since fructo-oligosaccharides are non-reducing oligosaccharides, they do not undergo the Maillard browning reaction.

These are short and medium chain of β-D fructose in which fructosyl units are bound by a β (2-1) glycosidic linkage. Their synthesis in plant cells starts by the transfer of a fructosyl moiety between two sucrose molecules. Some of these molecules have a glucose unit as the initial moiety. The β (2-1) glycosidic bond of FOS is not hydrolyzed by mammalian digestive enzymes but is susceptible to attack by certain bacteria. FOS have been defined as a combination of three sugars 1-ketose (GF$_2$), nystose (GF$_3$) and 1$^\beta$-fructofuranosynystose (GF$_4$). Depending upon the chain length and degree of polymerization, these are named oligofructose (average degree of polymerization 4 to 80) or inulin (degree of polymerization=12). The term FOS will be used to encompass both oligofructose and inulin, which are commercially available as raffilose and rottiline respectively.

When *Bifidobacteria* grow on such substrates, they seemingly do so at the expense of bacteroids, *clostridia* or coliforms, which are maintained at low levels or may even, are reduced. This specificity of *Bifidobacteria* is due to production of β fructosidases. The accepted mechanism for the inhibition of growth of other bacteria by *Bifidobacteria* is thought to involve a decrease in pH as a consequence of the production of large quantities of carboxylic acids, mainly acetate and lactate. The administration of FOS affects the fecal bacteria. The reduction in levels might be due to secretion of bacteriocin type substance that is active against *Clostridia, E.coli* and many other pathogenic bacteria such as *Listeria, Shigella, Salmonella and Vibrio cholera*.

FOS are almost quantitatively utilized so as to give a mixture of short chain fatty acids, lactate and CO$_2$. It has been hypothesized that highly fermentable carbohydrates could, possibly via the production of lactate in the colon, improve the metabolic absorption of various ions including Ca, Mg and Fe.

The safety of the FOS is well documented in various studies. Results indicated that there is no evidence that FOS possess any genotoxicity. Subchronic and chronic toxicity as well as carcinogenicity studies in rats revealed no significant adverse effects at dose upto 2170 mg/kg/day. However at higher level (more than 5% of diet in rat) resulted in soft stools or diarrhoea.

### 4.2 Inulin

Inulin is found in more than 36,000 plants and present in significant quantities in asparagus, artichokes, onion, garlic, wheat, dahelia roots. Commercially inulin is manufactured from chicory roots using hot water extraction, purification followed by drying. Inulin is made up of linear chains of fructose molecules linked through β (2-1) linkage. Inulin is considered as soluble dietary fiber and it has proven effect on reduction of glycemic response, blood cholesterol, stool pH, constipation and elevation of stool weight, HDL/LDL ratio. Almost all chains, which have a length upto 60 units, terminate in one glucose unit. It has a prebiotic effect and is an attractive fat mimetic and bulking agent. Often mistakenly referred to as FOS, inulin is prized for its ability to hold water, replace fat and contribute minimal calories. It is also hailed for its bland flavor profile. Typically composed of approximately 10% oligosaccharides, the long chain polysaccharide structure of inulin gives the substance water-holding abilities, enabling its application in a wide variety of products where a bulking agent can add processing functionality and help consumers meet their daily need for fiber. Inulin has also been studied by the scientific community for its
fermentation profile, ability to stimulate the growth of probiotic gut microflora and potential health benefits. Its predominantly long chain structure ensures long fermentation times in the colon.

4.3 Lactulose

Lactulose (4-o-β-D-galactosylpyranosyl – D-fructose) is an isomer of lactose. Lactulose is formed during heating of milk in small amounts. Pasteurized milk does not contain any detectable amount of lactulose, highly pasteurized milk (< 50 mg/lt), UHT milk (100–400 mg/litre), and sterilized milk (600–1200 mg/litre). Determination of lactulose may give an indication regarding the severity of the thermal treatment of milk and milk products and milk products. International dairy federation (IDF) has set the upper limit of lactulose content in UHT milk as 600 gm/liter.

Lactulose is white crystalline odourless powder with melting point of 169°C. Lactulose is water-soluble and solubility increases with increases with temperature, exhibiting 86% solubility at 90°C. The relative water activity lowering ability of lactulose solution is significantly lower than equimolar solution of sucrose; hence it can be used as humectants in wide range of processed food products. The degree of sweetness ranged between 0.48 – 0.62 compared to 1.0 of sucrose, however with increase in concentration the difference in sweetness decreased, lactulose is 1.5 times more sweet than lactose.

4.3.1 Lactulose as bifidus factor

Lactulose plays a role in proliferation of Bifidobacterium spp. that has a very close relationship with human health. Lactulose is generally not metabolized in small intestine and passed directly in large intestine, where it is broken down by the microbes, most specifically by Bifidobacterium and acidophilus bacteria with lactic acid as end product. Bifidobacteria has been thoroughly investigated as therapeutic and prophylactic agents for human and animal health. Bifidobacterium is a predominant microorganism in the intestine of breast feeding infants because of lower protein and buffering capacity of human milk. Investigation regarding the effect of incorporation of lactulose in infant formula on the intestinal bifidobacterial flora in rats indicated that 0.5 – 1.0% lactulose content in formula had no adverse effect on the absorption and retention of nitrogen, calcium, phosphorus and iron from the formula by bifidobacterial flora. It also did not affect the growth, blood composition or histopathology of liver and shows no adverse effect on continuous consumption duering growth period. Incorporation of 0.5% lactulose in formulation was considered adequate to stimulate bifidobacterial flora and prevent laxative effect of it. Borculo- Domo ingredients, a major European dairy manufacturers, have developed a prebiotic ingredient named “Elixir”. The product is both in powder or syrup forms and contains 60% galactooligosaccharides, 20% lactose, 19% glucose and 1% galactose. As per manufacturer’s claim the product in pH and thermostable, has a bifidogenic effect and increase intestinal absorption of minerals.

Health benefit associated with lactulose among elderly is its ability to act as mild purgative, thus it help in reducing the growth of ammonia producing organism. This can be attributed to Lactobacilli stimulation ability of lactulose. This particular property of lactulose has successfully utilized by medical practitioners in the treatment of portal systemic encephalopathy and chronic constipation. Lactulose also has been gaining attention for being prospective prebiotic substance.
4.4 Galactooligosaccharides

Human milk contains various types of oligosaccharides and most predominant among them is galactooligosaccharides (GOS). The presence of GOS is breast milk is linked with higher bifidobacterial count in infants. They are produced commercially from lactose using the glycosyl transferase activity of enzyme β-galactosidase EC 3.2.1.23. GOSs consist of a number of β- (1→6) linked galactopyranosyl unit linked to as terminal glucopyranosyl unit via an α- (1→4) glycosidic bond. These galactooligosaccharides were earlier considered as unwanted products but now they are considered as prebiotics because they function as bifidobacteria growth promoting factors, reduce risk of colon cancer, prevent bone loss and lower serum cholesterol concentration.

4.4.1 Production of galacto-oligosaccharides

Commercially GOSs are produced from lactose solution using enzymatic preparations; hydrolysed solution is then demineralized, filtered, concentrated and then dried. However it is also available in concentrated syrup form. Enzyme lactase is utilized for hydrolysis of lactose. Lactase is the common name for the enzyme β - D - galactosidase or more formerly β - D – galactoside galactosehydrolase. In addition to the ability to catalyze the hydrolysis of lactose to its monosaccharides β-galactosidases are able to catalyze a series of transferase reactions involving both lactose and its hydrolysis products, particularly galactose to form a family of galactose - rich oligosaccharides termed as galactooligosaccharides. Sugar residue forming the glycone part of the substrate may be transferred to either water or to some other hydroxyl acceptor, such as another sugar or an alcohol. This reaction is called trans-galactosylation when the substrate is galactose. When the hydroxylic acceptor is another sugar, the oligosaccharidars are formed. If the sugar, which acts as acceptor is the monosaccharide, the product formed is a disaccharide, if the acceptor is a disaccharide, the product formed is trisaccharide and so on.

Type of oligosaccharides and their relative concentration is determined by number of factors. Source of enzyme ids the most important factor and the most investigated β-galactosidases are from the yeasts Kluyveromyces fragilis, K. lactis and the fungi Aspergillus oryzae, A. niger. Large numbers of β-galactosidases preparations have been studied for their ability to produce GOSs. Initial higher lactose concentration shifts the reaction towards oligosaccharide production. Lactose concentration in the range of 15-50% resulted in higher GOS production. The maximum concentration of OS was around 10% of the total sugar when a substrate containing 4.4% lactose was used. If the concentration of lactose was increased to 13.3%, as much as 26% of total sugar was OS. On comparing a free enzyme reaction with the immobilized enzyme, it was found that immobilized galactosidase produced more di-, tri- or tetra oligosaccharides In specific cases higher temperature,pH and salt concentration give rise to higher levels of oligosaccharides.

4.4.2 Physiological functions of galactooligosaccharides

In-vitro studies revealed that GOS are not hydrolyzed by human digestive secretion, but many beneficial intestinal microflora including bifidobacteria and lactic acid bacteria can effectively metabolize them. Literature also showed that GOSs could be used more readily and selectively by the bifidobacteria than other oligosaccharides. Clinical trials with GOS consumption with animal and human subjects indicated its positive role improving constipation, reducing blood ammonia level, cancer and osteoporosis prevention. The constipation may be improved by an increase in the osmosis of the higher concentration of SCFAs in the intestinal as result of GOSs consumption. GOS consumption along with
Bifidobacterium breve supplementation decreased the blood ammonia levels in patients with hyper ammonia associated liver cirrhosis. Bifidobacteria not only suppress growth of ammonia producing organisms like bacteroidaceae but utilize ammonia as sole source of nitrogen.

5.0 Application of Oligosaccharides in Processed and Functional Foods

People are choosing foods that offer health benefits, and a majority seeks out fortified and enhanced foods. Foods that communicate healthy messages, while delivering taste and convenience with a natural focus will continue to attract consumers. The oligosaccharides’s application in processed foods though in infancy stage, but it is one of the potential ingredients for healthy and functional foods. Another important trend in food products involves the huge youth segment. Use of novel ingredients to improve health, such as prebiotics, will abound. Functionally oligosaccharides possess certain unique properties like higher water absorption capacity, gel formation, emulsification, colour formation make them an ideal ingredient for improving the health attributes of certain processed foods. Some of the promising areas for its application in food products are discussed here.

5.1 Synbiotic Food Products

Synbiotic products- those that combine prebiotics with probiotics for enhanced health benefits—are the major new trend in the dairy sector. Fermented dairy products including cheese, yoghurt, cultured cream, dahi have been targeted to develop in symbiotic form. In such products addition of oligosaccharides perform two functions, stimulating the growth of probiotic organisms and improving the organoleptic quality of the product specially textural attributes. Addition of 0.5% FOS in normal as well as L. acidophilus and Bifidobacterium infantis supplemented yoghurt improve the acceptability of the product. The FOS containing yoghurt were less chalky, more creamier, sweeter and with less sour/fermented taste and aftertaste. Synbiotic yogurts are promising enhanced health benefits, such as improved immune function and increased calcium absorption in addition to overall health benefits. These products are at the forefront in providing consumers with a combination of health benefits in the tasty, convenient forms they enjoy and currently purchase.

5.2 Dietetic Foods

Oligosaccharides are though less sweet than sucrose may find their applications as sugar substitute in confectionary products. These are having low cariogenisity, lesser calorific value; hence prevent tooth decay as well as obesity in children and youths. A large production of oligosaccharides is used for the manufacture of fiber-enriched food products. Emerging products incorporating prebiotic fibers include kefir, yogurt and other dairy drinks, sports products, functional waters, nutrition bars, weight loss products, soymilk, green foods, probiotic supplements, mineral supplements, medical foods and pet foods. These products incorporate prebiotic fibers for various health benefits, including enhanced mineral and isoflavone absorption, fiber contribution, gut integrity, immune function and cholesterol control. The largest sector that utilizes oligosaccharides as ingredient is beverage industry.
5.3 As Fat Replacer

Oligosaccharides especially those obtained from plant sources have been investigated for their fat replacing ability and gaining popularity as “fat mimic”. They can form stable fat like cream with water and give a better balanced flavour, improved body and mouthfeel and stabilizes emulsions as well as dispersions. The oligosaccharide utilization also reduces calorific value in ice cream, cheeses, dairy beverages, spreads, cream and processed meat products.

6.0 Conclusion

Food ingredients and diet play an important role in the maintenance of the health of the host. Viable probiotic bacteria such as Lactobacilli and Bifidobacteria can survive to assist the colon to become metabolically active. Their effect has been confirmed in human tests and they have been applied to functional and nutritional foods. The large potential for the use of OS in the food industry provides an opportunity for the dairy industries to manufacture value added health foods. In the future, prebiotic fibers will have a strong position in the nutraceuticals industry. As people increasingly seek simple solutions to wellness issues, they will become even more scrupulous in their choices. The move toward products that provide a combination of benefits with ingredients that have sound, scientific support will explode. For the interests of consumers to be served, the food industry and the scientific community must provide accurate information in order to promote good choices.

7.0 Suggested Readings


Playne, M.J. & Crittenden, R. Commericially available oligosaccharides. IDF Bulletin No 313, 10-22 pp


1.0 Introduction

Colour of food is the most important characteristic by which quality of the food is judged. The colour also aids the recognition of food and has psychological appeal on its enjoyment. Thus, colour is one of the major factors influencing consumers' acceptance of the food. Therefore, overall objective for addition of colour in food is to make it recognizable, attractive and appealing. In addition, there are several other technical reasons for addition of colour in the food. In recent years increasing evidences for toxicological effects of synthetic colours have prompted regulatory agencies, world-over, to drastically prune the list of permitted synthetic food colours. Consequently, several synthetic food colours have been deleted and some more are likely to be banned in near future. Some countries (e.g. Norway) have removed all artificial food dyes from their permitted list. Today, consumers are also aware about safety and risk of synthetic ingredients in food. Therefore, manufacturers want to label their products as having "all natural ingredients". The pressure brought about by changes in legislation and consumers' preference has forced the food industry to focus its attention in exploiting the use of natural alternatives for the food colours. On the other hand growth of the food industry, with many new products being developed, has created a rapidly rising demand for safe and suitable colouring agents. Moreover, food manufacturers involved in exports, because of their greater international acceptance, prefer natural colours. Therefore, future prospect of natural food colours is quite promising.

Though the natural colours weigh more on safety front, they do suffer from several limitations. The major problems of natural colours of plant origin include seasonal production, variation in quality from source to source, availability of only limited colour shades and their instability during extraction, processing and storage. The Most natural colours have much lower tinctorial strength and consequently higher level of additions is required. The low yield, high unit cost of production and higher levels of addition in food make the natural colourants 6 to 8 times more expensive. Some natural colours also suffer from poor stability to heat, light, preservatives and changes in pH. The majority of natural colours are only oil-soluble.

Number of approaches has been considered to overcome some of the problems associated with natural food colours. Attempts are made to improve pigment yield by selection of particular cultivars, application of enzymes for the extraction, microorganism-aided extraction and use of super critical carbon dioxide as an extracting solvent. The use of citric acid, acetic acid and tocopherols, etc has been suggested to reduce pigment loss during extraction. The potential of membrane processing for concentration of pigments has also been investigated. Improved stability, high concentration, water solubility or dispersibility and diversified food applications have been achieved by physical modification of colour preparations such as emulsion,
suspension and powder forms. Addition of colours after heat processing and use of opaque packaging of products have been suggested to improve the stability. To overcome problem for availability of limited colour sheds, blends of natural colours in various combinations have been developed. The novel sources of natural colours for food has also been explored, some of the promising novel sources are monascus (mold), gardenia plant and algae. Attempts have also been made for chemical synthesis of natural food colour, known as nature identical food colours. This helps to eliminate variability in quality.

Natural colours are pigments obtained from naturally occurring materials such as plants, animals, microorganisms or minerals. The natural colouring materials are grouped into two broad categories, namely organic and inorganic. The organic group is further subdivided into six classes according to their chemical structure. These are carotenoids (bixin, capsanthin, β-carotene etc), anthocyanins (cyanidin and malvidin), melanoidins (caramels), porphyrins (chlorophylls) betalines (betanin) and quinoids (carminic acid). There are some natural colourants, not falling into any of the six major classes, which include turmeric, riboflavin, monascus, etc. The food colourant of inorganic group comprises gold, silver, aluminium, ultramarine, iron oxides and hydroxides, titanium dioxide and calcium carbonate.

According to PFA Rules some natural colouring principles whether isolated from natural sources or produced synthetically maybe used for food application. These include β-carotene, β-apo-8’-carotenal, methyl ester of β-apo-8’ carotenic acid, ethyl ester of β-apo-8’ acid and canthaxanthin, chlorophyll, riboflavin (lactoflavin), caramel, annatto, saffron and curcumin (turmeric).

2.0 β-Carotene

The most common source is carrot, but it also occurs in other vegetables and fruits like peach, apricot, broccoli, spinach, etc. It is also produced commercially by chemical synthesis. For application as a food colourant, β-carotene is available as liquid emulsion, suspension, water-dispersible powder and beadlets and also as dry crystals. It is susceptible to oxidation and sensitive to light, but the problems are minimized when it is incorporated into food. It has good stability in respect of pH and heat. It imparts yellow colour in food. The most important application is in colouring butter and margarine. It may also be used to colour a variety of fat-based products such as cheese, oils, fats and simulated dairy products. In a water-soluble form the pigment may be used to colour water based products like ice-cream, non-dairy creamers, dessert toppings, baked goods, confectionery, cake mixes, yoghurts, desserts, jellies, preserves, dressings and pasta. In its water dispersible form, it is used in soft drinks.

3.0 β-Apocarotenal

The most common source is skin and flesh of oranges. It is also produced commercially by chemical synthesis. Its solubility in oils and fats is higher than the β-carotene and may be further improved by gentle heating. To obtain higher solubility it is converted into β-apocarotenonic acid, which is then converted into methyl or ethyl derivative. It is the most susceptible of the synthetic carotenoids. β-apocarotenonic is used at 1-20 ppm levels to impart orange to red colour. It is often blended with β-carotene to produce a rich orange shade. Its major application is as an effective colourant for salad dressings. Other applications include natural and processed cheese, cheese sauces and spreads, oils and fats. In beadlets form it finds use as a colouring agent for soft drinks, snack foods, ice cream, pastry fillings, toppings and cake mixes.
4.0 Canthaxanthin

It is the principal pigment of pink edible mushroom, *Cantharellus annabarina*. It is also isolated from algae, hydra and the brine shrimp. It is produced commercially by chemical synthesis. Comparatively it is least susceptible to oxidation and less sensitive to light. It is available commercially as a dry powder and as water-dispersible beadlets. Canthaxanthin is used at 5 to 6 ppm level to impart red colour to food products. Its blend with β-carotene produces orange shades. It is used to enhance and standardize the colour of meat and fish products. In the water-soluble form it may be incorporated into confectionary products such as hard candy fruit juices, marzipan, sugarcoated tablets and fondant.

5.0 Annatto

Annatto is the orange-yellow colouring material derived from the pericarp of seeds of *Bixa orellana* L. Chemistry, properties, food applications and other details on annatto are presented in a separate topic.

6.0 Saffron

Saffron is a yellow extract and its oleoresin is obtained from stigma and styles of flowers of *Crocus sativus* L. Until the 1980s this was the only source of saffron, but now it is also extracted from dried fruits of Cap jasmine (*Gardina jasminoides*). This is a relatively less expensive source, but the colour extract does not have flavour of saffron. The carotenoid pigments, crocin and crocetin are the principal pigments of the yellow crocus. Saffron is readily soluble in water but insoluble in oils and fats. It is somewhat sensitive to pH changes and is unstable towards light and oxidative conditions. It exhibits moderately good resistance to heat. Sulphur dioxide and iron and copper ions have adverse effect on the pigment stability. As a spice saffron is used in a wide variety of foods. It imparts a very attractive golden yellow colour to foods and beverages and a spicy taste. Saffron is typically used at a level of 1 to 260 ppm in packed goods, rice dishes, soups, meat dishes and sugar confectionary. Its use as a natural colorant has been severely restricted due to very high price.

7.0 Chlorophylls

Commercially chlorophylls are obtained from Lucerne or grass for use as food colorants. Various derivatives of chlorophylls may be obtained by chemical modification of the pigment. These derivatives are better suited for colouring food products than the natural chlorophylls. The natural chlorophyll is sensitive to light, heat and acid. Its green colour is easily destroyed even under mild processing conditions. However, replacement of magnesium atom of natural chlorophyll with copper results in a brighter complex with an increased stability. The dosage rates used for food colouring vary between 0.05 to 0.1% for oil-soluble chlorophylls and 0.002 to 0.01% for water-soluble chlorophylls. The chlorophyll derivatives have wide applications in confectionery products including chewing gum, hard candies, gums, jellies, marzipan and fondant. They have also been successfully incorporated into cakes and biscuits in both the cream bases and cream fillings, dairy products and pet foods. In beverages at low pH, the acid resistant form are required for use.
8.0 Turmeric

Turmeric is the spice derived from the rhizomes of herb plant *Curcuma long* L. The yellow colouring pigment is curcumin. Turmeric is insoluble in water but soluble in alkalis, alcohol and glacial acetic acid. Its extract has strong characteristic odour and sharp taste, therefore, utilized for flavour and colour. Turmeric exhibits poor stability to light, oxidation, sulphur dioxide, alkaline conditions and metallic ions (Cu and Fe). Under alkaline condition, it turns brown. However, turmeric stands up well to heat processing. Turmeric is used to colour pickles, canned products, soups, mustard, wrapped confectionary, baking products and beverages. In its powdered form it may be used in dry mixes, cereals and baked goods.

9.0 Riboflavin

This is also known as lactoflavin or vitamin B$_2$. As a colouring agent it was originally derived from quercitron bark, but can now be manufactured synthetically. Chemically it is isoalloxanic with a ribitol side chain, which may form ester phosphate to yield riboflavin-5'-phosphate. Riboflavin is only slightly soluble in water; however, riboflavin phosphate is considerably more soluble. It is soluble in dilute alkali but decomposes in alkaline conditions. It is also sensitive to light but with regard to heat, riboflavin is relatively stable. Ascorbic acid can help to stabilize riboflavin. it may be used to colour confectionary like hard candies and fondant, but the products must be protected from light. It is also a useful colouring agent for ice cream, provided that the ice-cream does not receive an excessive exposure to display lighting in retail freezer cabinet. Similarly, its use in other dairy products such as milk and yoghurts requires careful choice of packaging to prevent the penetration of light. Riboflavin may be successfully used to achieve a pale yellow colour in salad dressing due to protective influence of emulsified oil droplets and acidic conditions. Its use to colour preserves like lemon curd, cereals and water ices is also recognized and use in beverage is also possible as long as the products are vacuum-packed or filled in inert atmosphere.

10.0 Caramel

Caramel colour is a melanoidin pigment. It is an amorphous dark brown colouring material resulting from the controlled heat treatment of various food-grade carbohydrates including dextrose, lactose, invert sugar, malt syrup; molasses, starch hydrolysates and sucrose. Caramel can be classified according to their method of manufacturing:

- Positively charged caramel formed by ammonia process;
- Negatively charged caramel formed by ammonium sulphate process (acid proof);
- Spirit caramel formed by sodium hydroxide (caustic caramel).

In solution, caramel forms a colloid. These colloids carry an electric charge, which is dependent upon the method of manufacture and pH of the product.

As an added food colourant, it was first used in the form of burnt sugar in alcoholic drinks and later for colouring of spirits, particularly rum. In the brewing industry, caramel is used to provide both colour and flavour. The caramel chosen must have positive charge. The major uses of caramel are in meat products, confectionary, bakery products and soft drinks. In bakery products and confectionary, it provides a uniformity dark rich shade. Caramel also finds wide use in vinegar pickles. Powdered form of caramel is utilized in dry mixes for bread and cakes. When applied to carbonated soft drinks, negatively charged acid-proof caramels are used.
11.0 Anthocyanins

The most important commercial source of anthocyanins is the extract from skin of black grapes. Other sources are red cabbage, blackberry blueberry and purple corn. Anthocyanins belong to class flavonoids and they are glycosides of anthocyanidins. Anthocyanins are soluble in water but insoluble in fats and oils. Owing to their amphoteric nature they are highly susceptible to pH changes. The pH shifts causes profound change in their colour hue and stability. In acid solutions (pH 1.0 to 3.5) anthocyanins exist as red flavylum salt but as pH increases the intensity of colour decreases and at pH 4.0 to 5.0 become colourless. However anthocyanins derived from purple corn retains the red to purple colour up to pH 6.0. HTST processing and storage at low temperature have been suggested to minimize the colour deterioration. Oxygen exerts a deleterious effect upon anthocyanins. Therefore, in canning and bottling of products use of nitrogen headspace, packaging under vacuum or complete filling of the containers is suggested. Hydrogen peroxide produced during oxidation of ascorbic acid causes pigment destruction. Anthocyanins being phenolic and glycosidic in nature, they are attacked by peroxidase, oxidase and glycosidase. Steam blanching retards the enzyme activity.

Anthocyanins are mainly used in acid type products and particularly well suited to use in soft drinks. Anthocyanin may also be used to colour confectionary products like fruit chews, chewing gum, gelatin starch jellies and fondant. Other successful applications of anthocyanins include yoghurts, fruit syrups for milk-based products, cake and biscuit fillings, preserves, water ices and table jelly tablets.

12.0 Betalaines

Betalaines are found only in members of plant family Cactaceae. However, the only commercially exploited source is red table beet (Beta vulgaris), where the pigment is concentrated in the underground root of the plant. Water-soluble pigments of beet red are collectively known as the betalaines, which includes two classes of pigment viz. betacyanins (red colour) and betaxanthins (yellow colour). The hue of the pigment extract is, therefore, determined by the ratio between red and yellow pigments. The betacyanins content is generally far greater than the betaxanthins. In beet root among betalyanins betanin is the principal pigment, accounting for 75 to 95% of the total betacyanins.

Betanin is fairly stable to pH changes within a range of 3.5 to 7.0; however, the maximum stability is between pH 4.0 to 5.0. Heat treatment turns the red colour to light brown. The problem of heat stability may be avoided by addition of the colour at the end of heat processing or after the heat treatment. Oxygen and light also exercise an adverse effect on stability of beetroot red. Some protection from oxidation may be offered by use of antioxidants such as citric and ascorbic acid. Water activity is also an important factor in the stability. The betanin stability increases with decreasing water activity. Metallic ions like Fe, Al, Cr, Cu and Sn have detrimental effect on beetroot red, but chelating agents can help to stabilize the colour. Tea extract and protein systems in food have protective effect on stability of the colour.

Betalain red is used to colour a variety of products. The use in confectionery includes marzipan, fruit-chews, hard gelatin gums and fondant. The pigment can be used to colour hard candies produced by traditional batch method where pigment is dissolved in glycerol or propylene glycol and added after heat processing. The application in continuous deposition technique is not suitable as the thermal conditions imposed are too severe. High water content of soft drinks restricts the use of beetroot red in these products but the pigment may be incorporated into dry mixes. Further applications for beetroot pigment include table jellies, biscuit jellies, biscuit creams,
pizza sources and gelatin desserts. Beetroot powder can serve as a replacement for nitrates in colouring sausages.

13.0 Paprika

Paprika oleoresin is the red/orange oil-soluble extract from the sweet red pepper, *Capsicum annum*. The oleoresin contains xanthophylls (capsanthin and capsorubin) in form of their diurate esters, β-carotene and characteristic natural flavouring components. Total carotenoids amounting to about 4% of the paricarp. Capsanthin, the major pigment represents 40 to 45% of the total carotenoids. Paprika oleoresin is oil-soluble but water-dispersible forms are available. The oleoresin is sensitive to light. Loss of colour in paprika may be prevented by addition of flavonoid marin at levels less then 500 ppm. The colour loss can also be prevented by addition of browning (caramalisation or Maillard reaction) products at levels of 0.005 to 0.5%.

The use of paprika oleoresin as a colour additive overlaps its use as a spice due to its characteristic flavour. Consequently, while using paprika oleoresin as a colouring agent, it has to be taken into account that whether the associated characteristic flavour is compatible or not. The paprika oleoresin is suitable for use in salad dressings, sauces and meat products. Other applications include meat seasonings, snacks, confectionary and baked goods. Paprika is often used in combination with annatto to provide a more red hue to processed cheese.

14.0 Suggested Readings


1.0 Introduction

Though there is no general consensus on definition and classification of food additives and nutraceuticals, the following definitions can be adopted to focus various aspects of their production using fermenters.

Food additives are ingredients that provide specific desirable attributes to the food. The partial list of these includes hydrocolloids, enzymes, acidulants, colours, essential oils, flavours and fragrances. Nutraceuticals may be defined as bioactive chemical compounds that have health promoting, disease preventing or medicinal properties. Direct fed microbials (probiotics), vitamins etc. fall in this category. Different aspects of fermenters will be covered in this note followed by discussion on production of lactic acid, xanthan gum and riboflavin as examples.

Food additives and nutraceuticals can be produced by either chemical synthesis or biosynthesis using fermenters. Recent developments and breakthroughs in biotechnology are making the biosynthesis route more attractive. Another criterion for the assessment of fermentative manufacturing processes that make use of genetically modified organisms is that of their environmental impact as compared with that of manufacturing methods based on chemical synthesis. Because it uses almost 90% renewable natural raw materials as starting substrates, the fermentative process uses less energy and causes less environmental pollution than does the traditional method. Less solvent and fewer auxiliary substances are required. For this reason the amount of air pollutants and waste materials is about two thirds less than with the traditional manufacturing process.

Before microorganisms that synthesize food additives or other fine chemicals can be used for industrial production, the biotechnological process concerned must be shown to be economically efficient. This is favored by the use of inexpensive starting substances such as whey or molasses formed as byproducts of food production processes. The yield of product can be further increased by optimizing temperature, nutrient composition, oxygen concentration, and pH level.
2.0 Considerations for Fermenter Construction

Fermentation is the term used to describe any process for the production of a product by means of the mass culture of a microorganism. The product can either be the cell itself called biomass production or its own metabolite: referred to as a product from a naturally or genetically improved strain. It could even be a microorganism’s foreign product: referred to as a product from recombinant DNA technology or genetically engineered strain, i.e. recombinant strain.

For pure culture fermentation, aseptic conditions must be maintained to prevent contamination from unwanted organisms. Fermenters must be capable of operating aseptically for several days, sometimes months. The material from which it is constructed must thus withstand repeated in situ steam sterilization and cleaning cycles. Most large-scale fermenters are made from corrosion-resistant stainless steel to withstand full vacuum to 3-atmosphere positive pressure at 150-180°C. The reactor vessel should be free from crevices and stagnant areas. Interior surfaces are polished to a mirror-like finish and all welds on the interior of the vessel are ground flush before polishing. The reactor vessel should have a minimum number of internal structures. Glass is used to construct fermenters up to 30-litre capacity. These fermenters are usually equipped with stainless steel head plates having entry ports for medium, inoculum, air and sensors. Valves and stirrer shaft seals are potential entry points for contaminants. Their construction must be suitable for aseptic operation. Pinch and diaphragm valves are recommended for aseptic operation of fermenters. Mechanical seals are commonly used for stirrer shaft entry to the large-scale reactor vessels. On small scale, magnetic drives can be used to couple the stirrer shaft with the motor. The high efficiency particulate air filters are integral part of air supply line for aeration of fermenters.

3.0 Fermenter Configurations

Various fermenter configurations have been developed to suit specific applications, which are described below:

3.1 Stirred Tanks

It consists of a cylindrical vessel with a stirrer to agitate the contents. The stirred tank with a top drive assembly is shown in Fig. 1. It is the most commonly used fermenter because of its ease of operation, neat design, reliability and robustness. The vessel, medium and probes are usually sterilized together, minimizing the number of aseptic operations required. The glass vessel can be protected by a removable stainless steel mesh or jacket. The stirred tanks are available in stainless steel also. They are more expensive than the glass vessels but they are more rugged and reliable.

3.2 Air-lift Fermenters

In all aerobic fermentations, air is an essential requirement. Air-lift fermenters have no mechanical agitation system but utilize the air circulating within the fermenter to bring about the mixing of the medium. This is a gentle kind of mixing and is ideal for plant and animal cell cultures as there is no shearing action because of absence of mechanical mixer or agitator. The air-lift fermentation is based on the difference in specific weight between the air-enriched volume and low-air volume. As the fermenter is aerated, the lower density broth (air enriched medium) creates an upward thrust, which results in the circulation of the
broth. The type of circulation depends upon the vessel configuration. The basic design for a laboratory scale air-lift fermenter is an outer glass hollow tube with an inner stainless steel tube. A tubular loop type fermenter is designed to increase the volume of the fermentation while maintaining the residence time.

3.3 Tower Fermenters

This type of fermenter is designed for continuous yeast fermentation process. Continuous brewing of beer or lager can be carried out successfully in a tower fermenter. The design of these fermenters is simple and they are less expensive than the conventional stirred tank fermenters. Also due to absence of any complex mechanical agitation system they are easier to construct.

3.4 Fixed-bed Fermenters

There are several systems developed with a tubular packed bed reactor for laboratory scale applications. The main problem associated with these reactors is getting the fixed bed fully aerated. If air is restricted then anaerobic microorganisms take over. The type of fermentation in fixed beds is a heterogeneous reaction, whereas it is homogeneous in stirred tank fermentation. The fixed bed reactors are widely used in wastewater treatment plants.

3.5 Fluidized-bed Fermenters

These fermenters are hollow chambers in which dense particles containing a microbial film or microbial mass are fluidized and recycled. One of the oldest fermentations known to man, vinegar fermentation utilizes this principle.

3.6 Rotating-disc Fermenter

They have circular discs, which rotate through the medium at slow speed. The microorganisms adhere to the discs and the microbial film is exposed alternately to both the nutrient solution and the air. These types of reactors are being used for wastewater treatment.

4.0 Production of Lactic Acid

Lactic acid is a versatile organic acid. In the food industry it is used as acidulant and/or as a microbial preservative in beverages and acid coagulated dairy products. It is also used in feed, chemical and pharmaceutical industries. The choice of an organism primarily depends on the carbohydrate to be fermented. *Lactobacillus delbrueckii* subspecies *delbreuckii* are able to ferment sucrose. *Lactobacillus delbrueckii* subspecies *bulgaricus* is able to use lactose. *Lactobacillus helveticus* is able to use both lactose and galactose. *Lactobacillus amylophylus* and *Lactobacillus amylovirus* are able to ferment starch. *Lactobacillus lactis* can ferment glucose, sucrose and galactose. *Lactobacillus pentosus* have been used to ferment sulfite waste liquor.
Lactic acid fermentation is known to be end product inhibited fermentation by an undissociated form of lactic acid. Several studies have been carried out to overcome this problem. It has found that using extractive lactic acid fermentation technique could give a lactic acid yield of 0.99g/l and lactic acid productivity of 1.67 g/l/h over a conventional batch reactor which gave a yield of 0.83 g/l and lactic acid productivity of 0.31 g/l/h (Srivastava et al. 1992).

Mainly the two reactor systems result in high yields and productivities of lactic acid: - a continuous cell recycle fermentation process and fed batch fermentation. A high volumetric productivity can be achieved using membrane cell recycle bioreactor. Continuous fermentations using whey permeates have been reported with high productivities. Lactic acid production has been studied with immobilized cell systems. Lactobacillus delbreuckii were immobilized in calcium alginate beads and used them in continuous flow column reactors.

The broth containing calcium lactate is filtered to remove cells, carbon treated, evaporated and acidified with sulphuric acid to get lactic acid and calcium sulphate. The insoluble calcium sulphate is removed by filtration; lactic acid is obtained by hydrolysis, esterification, distillation and hydrolysis.

5.0 Production of Xanthan Gum

Xanthan gum is a high molecular weight exocellular polysaccharide derived from the Xanthomonas species bacteria. It is a cream coloured powder that is soluble in hot or cold water with a high viscosity even at low concentration. This solubility behavior is related to the polyelectrolyte nature of the xanthan molecule. These properties are useful in the food industry where xanthan is used as a thickener, and to stabilize suspensions and emulsions. The thickening ability of xanthan solutions is related with viscosity; a high viscosity resists flow. Xanthan solutions are pseudoplastic, or shear thinning, and the viscosity decreases with increasing shear rate. The viscosity also depends on temperature (both dissolution and measurement temperatures), the biopolymer concentration, concentration of salts, and pH.

For the production of xanthan gum first, the selected microbial strain is preserved for possible long-term storage by proven methods to maintain the desired properties. A small amount of the preserved culture is expanded by growth on solid surfaces or in liquid media to obtain the inoculum for large bioreactors. The growth of the microorganism and xanthan production are influenced by factors such as the type of bioreactor used, the mode of operation (batch or continuous), the medium composition, and the culture conditions (temperature, pH, dissolved oxygen concentration).

Table 1 illustrates the type and scale of each step, and provides an indication of the associated analytical and developmental support necessary to achieve the optimum process performance.

At the end of the fermentation, the broth contains xanthan, bacterial cells, and many other chemicals. For recovering the xanthan, the cells are usually removed first, either by filtration or centrifugation. Further purification may include precipitation using water-miscible non-solvents (isopropanol, ethanol, and acetone), addition of certain salts, and pH adjustments. For food grade xanthan gum, the use of isopropanol for precipitation is prescribed. After precipitation, the product is mechanically dewatered and dried. The dried product is milled and packed into containers with a low permeability to water.
6.0 Production of Riboflavin

Many bacteria and fungi synthesize riboflavin using different substrates. Among these microorganisms, the gram-positive bacteria *Bacillus subtilis*, the yeast *Candida famate*, and the filamentous fungus *Eremothesium Ashbyii* and *Ashbya gossypii* are mainly used for large-scale production of riboflavin (Stahmann, 2000).

So far most of the study have been done in the submerged culture fermentation system. During cultivation, filamentous fungi demonstrate mycellial growth. At high concentration the fermentation broth becomes highly viscous. This results in a decrease in mass transfer (Ozbas and Kutsal 1992) for oxygen. Attempts to improve the rheology of filamentous fungal culture by increasing rates of aeration and agitation result in lower viability and productivity. Continuous cultivation is made difficult by growth of mycelia flocks on the walls of vessel, impellers, electrodes, baffles, and outlets. To overcome this problem, immobilized cell or enzyme rectors have been suggested (Kalingan and Krishnan 1995). There are several advantages of the immobilized viable cells over enzymes (Fukuda, 1995). The operations of enzyme extraction and purification are not required; application to a multistep enzyme reaction is possible, operational stability is higher. The advantages of immobilized cell system over the suspended culture are the improvements in biomass holdup, mass transfer, yield, and improved downstream processing (Webbs and Dervakos 1996).

The choice of reactor design depends on several factors. These include immobilization method, particle characteristics, e.g. shape, size, density, robustness, nature of substrate, inhibitory effects and hydrodynamics and economics considerations (Fonseca et. al 1986). Generally stirred tank reactors are not used for entrapped cell system as the impeller blade may break the bids with high impact, thus releasing the cells in the suspension. Therefore fluidized bed reactors and airlift fermenters are recommended for use with the immobilized filamentous fungi (vassilv and vassileva 1992).

7.0 Conclusion

Food additives can be biosynthesized from a wide range of renewable materials using fermenters. Selection, design and optimization of appropriate microorganism, fermenter configuration, media composition, operating conditions and downstream processing are vital for efficient production of high purity food additives. Importance of some of these crucial considerations was demonstrated in the discussion on production of lactic acid, xanthan gum and riboflavin.

8.0 Suggested Readings


Table 1: Steps in Xanthan Production

<table>
<thead>
<tr>
<th>Process Step</th>
<th>Scale and operation</th>
<th>Analytical Support requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture preservation of X. campestris</td>
<td>Long-term: lyophilized; frozen; short-term: solid media slants or plates</td>
<td>Strain improvement:  Test for culture viability</td>
</tr>
<tr>
<td>Inoculum build-up</td>
<td>Shake flasks: inoculum fermenters</td>
<td>Growth medium composition; Controlled operational conditions; Test for contaminants</td>
</tr>
<tr>
<td>Production stage</td>
<td>Bioreactor</td>
<td>Equipment design; Production medium composition; Fermentation conditions; Controlled operational conditions</td>
</tr>
<tr>
<td>Harvest</td>
<td>Thermal, chemical, or enzymatic; centrifugation or filtration</td>
<td>Process development of cell deactivation and removal</td>
</tr>
<tr>
<td>Isolation</td>
<td>Precipitation; filtration</td>
<td>Development of extraction and purification methods</td>
</tr>
</tbody>
</table>
The following figures show the configuration of two important types of fermenters, namely, stirred tank fermenter and air lift fermenter.
ANNATTO: THE NATURAL COLOUR OF CHOICE IN MODERN FOOD INDUSTRY

Dr. K. D. Aparnathi
Associate Professor
Dairy Chemistry Department
SMC College of Dairy Science
Anand Agricultural University
Anand – 388 110

1.0 Introduction

Annatto is a natural colourant derived from pericarp of annatto (Bixa orellana L.) seeds. Annatto is fast growing shrub, which produces cluster of pods containing 10 to 50 seeds. The seeds are covered with thin pulpy, bright orange resinous coating, which serves as a source of the colour. Annatto plant is native to tropical America, but is now widely distributed in most of the tropical regions of the world, both in the wild and on cultivated farm. India is one of the major producers and suppliers of annatto seeds in the international market.

2.0 Chemistry

The Colouring matter of annatto comprises various carotenoids of which cis-bixin is the major pigment, constituting about 82% in the total pigment. Since bixin is the principal Colouring matter, the chemistry and performance of annatto colour is essentially that of the bixin.

\[ \text{HOOC} \quad \text{COOCH}_3 \]

3.0 Structure of Cis-Bixin

Upon saponification, the terminal methyl group of bixin splits off and gives water-soluble salt of the pigment. It is the tinctorial agent present in water-soluble annatto colour, also known as annatto cheese colour. When bixin is heated in edible vegetable oil to an extraction temperature, it undergoes isomerization and degradation reactions. Therefore, the yellow pigments (thermal degradation product), trans–bixin and cis-bixin are the constituents in oil-soluble annatto colour, also known as annatto butter colour.
4.0 Methods of Manufacturing

Annatto colour is prepared by leaching the pericarp of the seeds in a suitable extractant. The selection of an extractant depends on intended end use of the extract.

4.1 Water-Soluble Annatto Colour

This is traditionally prepared by direct extraction of the seeds in aqueous & alkali and subsequent heating of the extract at 70°C temperature, followed by filtration. According to BIS, (IS: 2557,1963) the alkali (NaOH or Na₂CO₃) may be used at a level of 0.5 to 3.0%.

4.2 Oil-Soluble Annatto Colour

This is traditionally prepared by direct extraction of the seeds in edible oil under vacuum of about 20 mm Hg at a temperature not exceeding 130°C, followed by filtration to give clear extract. According to amendment in PFA Rules, now for manufacture of oil-soluble annatto colour only edible vegetables oils listed in Appendix B to the rules should be used.

4.3 Emulsion/Suspension of Annatto

An emulsion is prepared by using edible organic carrier liquid(s), stabilizers and emulsifiers and processing treatments. These forms of annatto have advantages such as high concentration, improved stability and expanded applications in a variety of food products.

4.4 Powder of Annatto

The dried form is prepared by adding appropriate edible carrier substance in the aqueous alkaline extract, followed by recovery of pigment-carrier complex by precipitation or drying. The carrier increases stability and solubility of the pigment.

4.5 Pigment Concentrate

The annatto pigment is extracted from the seeds in a suitable organic solvent, followed by removal of the solvent to give pigment concentrate. It is then used for manufacture of water-soluble, oil-soluble, emulsion and suspension preparations of annatto.

5.0 Safety Aspects

Annatto has been used since generations without problems. Scientific investigations about toxicity, carcinogenic effect, mutagenic action and metabolic aspects have also proved the safety of the colour. Annatto extract injections in high dosages may show some hypersensitivity reaction in individuals with pre-existing skin complaines. Finally, the permanent ADI use full form the first time for human being has been established by the Joint FAO/WHO Committee on Food Additives. The ADI is 0.065-mg/kg body weight/day. Allocation of permanent ADI indicates that the colour has been well tested; its safety has been proved and approved for use in foodstuffs.
6.0 Legal Status

Annatto colour is listed as a permitted natural colour for foods under the provision of PFA Act (1954). Similarly, under FD & C Act (1963) of USA annatto does not require certification for its use in food. Annatto is also on the list of natural food Colours suggested by FAO/WHO and EEC.

7.0 Importance As a Food Colour

Absence of any adverse action on regular and prolonged consumption is the main requirement in choice of a dye as food colour. It is also necessary that it should impart attractive and natural colour to the food. Annatto fulfills both these requirements. The use of annatto as a food colour has several technical advantages. Some of these include high heat stability, resistance to enzyme and microbial attack and stability towards some oxidizing and reducing agents.

8.0 Limitations in Use As a Food Colour

Some of the problems encountered with natural colouring matter, including annatto are variation from source to source, seasonal production and instability during extraction and storage. Annatto pigment has a tendency to precipitate at low pH and in presence of calcium ions. However, the problem of instability during storage or in food has been overcome with the advent of emulsion and powder forms.

9.0 Application in Food Products

Annatto extract is one of the oldest known dye and has been in use since antiquity for colouring of food. Originally, it was used in the United States and Europe for over 130 years as colour additive in butter and cheese. However, at present it has become a widely popular food colour for the entire food industry all over the world. Today annatto seeds and their extracts rank second in economic importance worldwide among all natural colours. Annatto colour is generally used at a level of 0.5 to 30 ppm in food products, resulting in the hue ranging from light yellow to dark orange. The type of colour preparation employed also dictate the end effect.

Oil-soluble annatto was formerly used in fat-based products like butter and margarine. However, now it is also used in creams, spreads, baked goods, soy-based products, desserts and salad oils. Water-soluble annatto was traditionally used for cheese. Today its applications have been extended to ice-cream cones, ketchup, sauce, cereals, cakes, snacks, drinks and salmon. Emulsions and suspension of annatto are used in processed cheese, ice-cream, yoghurts, cream desserts, drops, candy, glues, jellies, drinks with pulp, juices, beverages and liquors. The powder form of annatto provides added flexibility for use in dry mix. Therefore, its application may be extended to powder products like instant foods and drinks, dip mixes and health food powders.

In combination with other pigments, annatto creates a wealth of colour for various food applications. Annatto and curcumin are mixed when more yellow hue is required. A combination of annatto and paprika oleoresin is used to give orange to red colour. Annatto is mixed with beet juice concentrate to mask blue hue of beet pigment while its use as a food colour. A blend of annatto, beat and caramel gives butterscotch colour. Annatto is mixed with FD & C Blue # 1 to produce green colour. Application of annatto blend with other food colours includes all most all kind of food products.
10.0 Suggested Readings


1.0 Introduction

Food additives are defined by the Food and Drug administration as any substance used to provide a technical effect in foods. The use of food additives has become more prominent in recent years due to the increased production of prepared, processed and convenience foods. World health organization and FAO defined food additives as non nutritive substances added intentionally to food, generally in small quantities to improve its appearance, flavour, texture or storage properties. FAO, WHO did not consider in this category, substances added primarily for their nutritive value such as vitamins and minerals.

Recent years have been an intensification of focus upon the safety of our food supply, particularly with respect to intentional and unintentional chemical additives including pesticides. There may have been time when adding chemicals to food was not necessary and not practiced. Prehistoric man added chemicals to foods when he smoked meat. No highly developed society could exist today without food additives. Food additives immediately become necessary when preservation of food became essential for storage and transportation under conditions that can affect spoilage. These food additives are added for preservation and for better functional properties associated with food color, flavour and texture, still some other additives are incorporated as nutritional supplements and as processing aids in manufacturing the thousands of products that we have accustomed to and the consumers demand.

The present day food and food additive laws and regulations are in a direct line of descent from the earliest attempts to ensure wholesomeness of food. At the beginning of the 20th century it is discovered that many diseases are caused by the deficiency of some essential nutrients. This lead to a new era in food additives. The discovery that vitamins, essential amino acids and essential fatty acids must be present in the diet for maintaining an optimum nutritional balance leads to the formulation of fortified foods or neutraceuticals. The trace elements are also essential but how to deal with them in terms of additives, is still unclear. Recent knowledge acquired from the studies has provided the optimum nutritional requirement with regard to major food components. The recommended daily intake (RDI) of most of the essential nutrients is now available.
The Additives can be classified on the basis of their use as mentioned below

1. Antioxidants
2. Preservatives
3. Emulsifiers and stabilizers
4. Food colours
5. Flavours
6. Sequestrants
7. Anticaking agents
8. Acids, buffers and bases
9. Humectants
10. Firming and crisping agents
11. Sweeteners
12. Enzymes
13. Nutritive additives
14. Flour and bread additives
15. Cryoprotectants

Food contains substances, which in certain concentrations and under particular conditions, may threaten or damage health. These may be naturally occurring food ingredients or from external sources. By adding them appropriately and correctly to the food can prevent toxins produced by exogenous substances and greatly improve food safety and acceptability. Many additives are added without prior testing. The absence of adverse effects on the health after consumption of food treated with additives is accepted as adequate proof that they are harmless. At present, because of the development of toxicology as a specialized discipline, additives are now permitted and employed for food purposes only if the toxicological tests approve the safety. The safety limit for each additives must be determined. The following criteria are nowadays regarded as important for assessing the harmlessness of additives:

- Acute toxicity
- Metabolic investigations and toxicokinetics
- Genotoxicity/mutagenicity
- Reproductive toxicity including fertility and tetratogenicity
- Subchrome toxicity
- Chronic toxicity
- Carcinogenicity

Before any substance can be added to food, its safety must be assessed in a stringent approval process. The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) shares responsibility with FDA for the safety of food additives used in meat, poultry, and egg products. FDA initially evaluates all additives for safety.

When an additive is proposed for use in a meat, poultry, or egg product, its safety, technical function, and conditions of use must also be evaluated by the Labeling and Consumer Protection Staff of FSIS, as provided in the Federal Meat Inspection Act, the Poultry Products Inspection Act, the Egg Products Inspection Act, and related regulations. Although FDA has overriding authority regarding additive safety, FSIS may apply even stricter standards that take into account the unique characteristics of meat, poultry, and egg. Additives are never given permanent approval. FDA and FSIS continually review the safety of approved additives, based on the best scientific knowledge, to determine if approvals should be modified or withdrawn.
2.0 Antioxidants

Antioxidants are used to protect unsaturated constituents of foods; namely fats, though other substances, such as vitamins, also need protection. So far as fish is concerned protection must be given to the unsaturated fat it contains, not only because of the obnoxious taints that could otherwise develop but also because of the loss of valuable essential fatty acids and polyunsaturated fatty acids that would follow. In the absence of any antioxidant, the fat may undergo autoxidation and decomposition forming smaller carbonyl compounds such as aldehydes, ketones, alcohol and acids. This process is accelerated by heavy metals ions like Fe and Cu.

2.1 Some of the common antioxidants used are as under:

- Butylated hydroxy toluene (BHT)
- Butylated hydroxy anisol (BHA)
- Propyl gallate
- Dodecyl gallate
- L – ascorbic acid
- Sodium L-ascorbate
- Calcium L-ascorbate
- Ascorbyl palmitate
- Natural tocopherol extract
- Synthetic tocopherols
- Ethoxyquin
- Nor dehydroguaiaretic acid

3.0 Preservatives

The preservation of food by chemical and biological techniques is an important field of research in food science and also a major branch of food industry. Physical, chemical and microbiological processes can adversely affect the quality of foods. Among these, the major cause for food spoilage, is by microorganisms and there are many chemicals that can reduce the rate of microbial spoilage or actually prevent it. The food spoilage by microorganisms cannot take place unless certain extrinsic conditions are met such as:

- Presence of spoilage microorganisms
- Availability of nutrients for the growth of microorganisms
- Existence of favorable environmental condition such as temperature, water activity oxygen, redox potential, and pH
- Sufficiently long food storage time.
- Microorganisms must cause deterioration in the quality of food.

The chemical methods are characterized by the addition of chemicals known as preservatives that inhibit the development of microorganisms or kills them. In biological methods, high purity harmless microorganisms cultures are added to food to inhibit the growth of spoilage microorganisms. Preservatives aim to prevent microbial spoilage.
There are three theories regarding the functioning of the preservatives. One is interference with the cell membrane of the invading microorganisms; the second, interference with genetic mechanism and the third, interference with intracellular enzymic activity. A bacteriocin - nisin is now permitted to use as foods preservative. It is a short chain polypeptide and it is recognized as an antibiotic that is particularly active against clostridia and Lactobacillae. It is now used in canned foods that are prone to bacterial spoilage. Sodium nitrate/nitrite is found to be highly effective in preventing the growth of Clostridium botulinum. Use of these compounds as an additive is being questioned, because nitrite is liable to be converted to nitrosoamine, which is a carcinogen. The preservative action of this is very good. The other preservatives used are benzoic acid, formic acid, propionic acid, acetic acid, sorbic acid, propyl-4-hydroxy benzoate and SO₂.

Of these SO₂ and sulphates are commonly used preservatives. Benzoic acid and their derivatives found wide application in food. SO₂ and benzoic acid are bacteriocides. SO₂ is pungent and it is not always advisable to use it. It is a permitted additive to drinks like wine. The propionic acid acts as a fungistat. It is produced commonly by bacterial metabolism.

4.0 Emulsifiers and Stabilizers

Any substance which is capable of aiding the formation of a stable mixture of two otherwise immiscible substances, say fat and water, is called an emulsifier that helps to maintain an emulsion when it has been formed. The stabilizer may have the same basic characteristic as an emulsifier or it may serve to thicken one or other participant of the emulsion or make it more viscous and hence less likely to separate into its components. Food needs emulsifiers, because all foods contain water and the three main nutrient constituents, protein, carbohydrate and fats. Natural foods have their own in-built emulsifier systems. In fact, this holds for all living organisms and they are essential part of the whole life system. The principle of an emulsifier is that it should have within its molecular structure one grouping with an affinity for water and another with an affinity for fat. For example, water and methanol or ethanol are miscible in all proportions. The whole family of such alcohol can be represented as CH₃(CH₂)ₙOH, where CH₃(CH₂)ₙ is a water hating (hydrophobic) paraffinic chain and OH is a water loving group (hydrophilic). Both act in opposite direction. As the length of the chain increases, the power of repulsion increases until the chain is pushed out. But the affinity between the OH of water and that of alcohol remains and the alcohol is anchored to the surface of the water. If that surface is now covered either with a films of paraffin or one of liquid oil, the alcohol chains will be dissolved in it and so produce cohesion between two surfaces. When it is stirred, one phase will break up into small globules and disperse in the other. This is an emulsion and the long chain alcohol is acting as the emulsifier.

Stabilizers are a selection of gums. They are all naturally occurring carbohydrates or polysaccharides and depend entirely on their polymeric structures for the varying properties they have. The permitted stabilizers are alginic acid or its sodium or calcium salt, carrageenan, locust bean gum, acacia, ghatti gum, karaya gum and xanthan gum. These are all viscous gums. Their use is limited only by good manufacturing practice.

There is one group of emulsifiers that can be claimed to be original. They are based on three groups of compounds, viz polyoxyethylene glycerine, sorbitans, and fatty acids and by varying the composition; a wide range of properties can be produced. Polyoxyethylene is produced from ethylene. Sorbitans are dehydration products of sorbitol. Sorbitans and polyoxyethylenes can be esterified with fatty acids. Sorbitan esters may be condensed with polyoxyethylene. The fatty acids that are used are lauric, palmitic, olive and stearic. Another interesting emulsifier is “Quillaica” an extract of a
South American tree bark, an additive to soft drinks to produce a head of foam. Its active principle is a saponin. The hydrophobic moiety is either a steroid group or a triterpenoid and the hydrophilic moiety is monosaccharide. The selection of an emulsifier or stabilizer for a new product is a matter of both experience and expertise.

5.0 Flavours

Flavour bearing compounds are used in food to give a special taste, which will increase the appeal for the product. Flavours are unique among food additives and it is till now very difficult to bring them under satisfactory statutory control. There are a large number of flavour ingredients. Any natural flavour can be an amalgam of upto 50 chemical components. Because of the large number of flavour substances that have been used as flavour enhancers, there is no toxicological proof of safety for many of the chemicals.

5.1 Classification of Flavour Compounds

- Natural flavour bearing substances, which are from natural constituents of food such as edible fruits or nuts.
- Natural substances consisting of or derived from vegetables, herbs or spices to be used in small quantities as additives to food, provided they are not used in quantities exceeding those occurring naturally in food.
- Natural substances used at present, but whose sources are not at present widely consumed foodstuffs, herbs or spices.
- Flavoring substances for which the evidence suggests possible toxicity and which should therefore be banned.
- Artificial or synthetic substances, which are acceptable for addition to food, subject to stated limits of addition. These substances are further classified on the basis of toxicological evaluation as follows:
  i. No limit for addition except GMP
  ii. Limit on technological grounds where levels must be higher
  iii. Limit on both technological and toxicological level
- Artificial and synthetic substances which may be provisionally accepted for use in food within set limits but on which some of the desirable toxicological data is missing.

6.0 Food Colours

Colour plays an important role in all walks of life and in many of human activities. A desired colour makes food attractive and pleasing. At the introduction of food colours, it was considered as blessing to the manufacturers as a solution for all their colouring problems. It was impossible at that period to give the right kind of judgement, because the toxicological knowledge was lacking. Some of these colouring compounds are suspected as a causative agent for dreaded diseases like cancer.

On the subject of natural colours, naturalness per se does not assure safety. However, a colour isolated in a chemically unmodified form from a recognized food stuff and used in that food stuff at levels normally found in natural food from which it is extracted have been accepted for use without supporting toxicological evidence. This would provide the product as acceptable as the food itself with no requirement for biological testing. But the extracted colour would require test if it has to be put back into
food at a higher level, or put into another food or chemically modified after extraction or if it had been extracted from a non-food source. Nature-identical substances properly purified are generally to be preferred to an extract of the natural material. The basis of the colour is a conjugate system of double bonds and N atoms in the system to intensify colour.

There is a long way to go before all food colour needs will be met from sources other than dyestuffs. The spectrum of present day food colours can be classified into three categories:

- Substances which are safe and be acceptable for use without qualification
- Substances, which are provisionally acceptable.
- Substances on which there is insufficient evidence to express an opinion.

There are certain restrictions and regulations with regard to the use of food colours such as:

- Current regulations forbid the addition of colour to raw or unprocessed meat, poultry, fish, fruit, vegetables, tea, coffee, coffee product, condensed or dried milk.
- It is not general practice to add colour to foods specially prepared for infants and young children.
- Caramel – a form of burnt sugar – has been prepared by a variety of methods and more than 100 different caramel products are available. This makes it impossible to characterize caramel for the purpose of regulation. Limitation of such methods of preparation to a few is proposed to characterize and evaluate each of them.
- Periodical removal of dye stuffs which do not satisfy toxicological tests are needed.

For many dye stuffs many countries restrict the ADI – Allowable Daily Intake (mg/kg body) to safeguard the interest of the consumers.

7.0 Sequestrants

Sequestrants are compounds that are added to food system to inactivate prooxidant metallic catalyst such as copper and iron. These substances have an affinity for the particular metal ion and prevent the metal ion from oxidation reactions. The most effective compound is ethylene diamine tetracetic acid (EDTA)

\[
\text{HOOC—CH}_2—\text{H}_2\text{CCOOH} \\
\text{N—CH=CH—N} \\
\text{HOOC—CH}_2—\text{H}_2\text{CCOOH}
\]

In Food, as calcium salt, it works satisfactorily as a sequestrant without interfering with trace mineral metabolism with an acceptable ADI of 2.5mg/kg body weight. It is used in canned fish and canned crustaceans. It is found very effective to
prevent struvite (magnesium ammonium phosphate) crystals. Other preferred sequestrants are phosphates and complex organic acids. There are a number of phosphates like sodium orthophosphate, sodium pyrophosphate etc, which have sesquestrant activity. Other permitted sesquestrants are citric acid and its salts and tartaric acid and its salts. The amino acid glycine forms coordination complexes with copper and other metal ions and hence can be considered as a sesquestrant.

Polyphosphates are naturally present in freshly slaughtered meat in the form of adenosine triphosphate and diphosphate. Phosphates are chemical compounds that can act as buffering and chelating agents and as polyanions influencing the ionic strength of solutions. The chemical properties and reactions of phosphates with food components and other additives influence water and fat retention, food particle cohesion, texture, flavour, emulsification, coagulation, curing and preservation of food products.

7.1 Types of phosphates

Food grade phosphates are sodium salt of phosphoric acid. Most common different type of polyphosphates used in processed meat is given below:

- Sodium tripolyphosphate (STPP)
- Sodium pyrophosphate (SPP)
- Sodium hexametaphosphate (SHMP)
- Sodium acid pyrophosphate (SAPP)
- Tetrasodium pyrophosphate (TSPP)
- Trisodium phosphate (TSP)

Chemical function of phosphates: All phosphates perform the following three basic chemical functions namely buffering, sequestering metal ion and acting as polyanions to increase the ionic strength of solutions.

7.2 Influence of Phosphates on Various Quality Attributes of Meat Systems

- **Increase binding**: The texture of ground and cubed meat products depends on the rebinding of the pieces of the meat. The salt soluble proteins, myosin, have been found largely responsible for the binding of meat particles in sausage type products. The polyphosphates have been demonstrated to increase the solubility of myosin particularly when combined with salt. The effectiveness of phosphate for binding in restructured meat is TSPP>STPP>SHMP.

- **Water holding capacity**: The moisture retention, often termed water holding capacity (WHC) or water binding ability is a considerable concern to meat industry. The WHC of meat tissue increases as the pH of the tissue either decreases or increases away from the isoelectric point of approx 5.0. Therefore the effect of PP on the increase in WHC in meat products is due to increase in pH and ionic strength, the ability of chelating divalent metal ions, ability to bind meat proteins, the ability of phosphates to dissociate actomyosin into actin and myosin.

- **Increasing tenderness**: The onset of rigor mortis causes a shortening of the muscle fibres as the contractile muscle proteins slide over each other to form
complexes that are highly stabilized by hydrogen bonding. The ability of phosphate to cause the dissociation of actomyosin into actin and myosin has an important effect on the tenderness of all types of meat. The phosphate in combination with the magnesium ion can cause the relaxation of the muscle fibres. This explains the tenderizing effect of PP.

- **Color preservation**: Myoglobin, a deep red pigment found in muscle tissue, is easily oxidized to the bright pigment called oxymyoglobin. Oxymyoglobin can further be oxidized to an undesirable brown pigment metmyoglobin. Factors that affect the color of meat are pH, the presence of reducing substances, curing salts and metal ions and exposure of meat to oxygen. Fresh, aged meat formally has a pH of 6.2 to 6.6. Low pH values have been found to accelerate the oxidation of fresh meat pigments to form brown metmyoglobin pigments. Optimum stabilization of the redness of meat is obtained at the higher pH values of 6.0-6.6. Therefore, the color of meat can be stabilized if the pH is maintained at about 6.5 with polyphosphates.

- **Functional properties**: The phosphates increase the WHC as well as fat emulsifying capacity (EC) of the myofibrillar proteins. The increase in EC is the result of PP solubilizing and dissociating actomyosin into actin and myosin, which in their dissociated form can emulsify more fat.

- **Prevention of off flavours**: The phosphates act as antioxidants, thus prevent oxidation of fats and development of rancid flavor in the meat fats.

8.0 **Anticaking Agents**

Anticaking agents are anhydrous substances that can pick up moisture without themselves becoming wet and they are added to particulate products, such as dry mixtures, to prevent the particles clumping together and to keep the product free flowing. They are either anhydours salts or substances that hold water by surface adhesion yet themselves remain free flowing. About 20 substances of both types are permitted. Some of them are mentioned below:

- Aluminium, ammonium, calcium, potassium and sodium salts of long chain fatty acids such as myristic, palmitic and stearic
- Calcium phosphates
- Potassium and sodium ferorocyanide.
- Magnesium oxide
- Aluminium, Mg, Ca and a mixed Ca-Al salts of silicic acid

Compounds in group 1, 2 and 3 from hydrates and group 4 and 5 adsorb water. Potassium and sodium ferrocyanide have the accepted daily intake of 0.025 mg /kg body weight. Sodium pyrophosphate and bone phosphate, which are adsorptive types can also be permitted in certain countries.
9.0 Humectants

Humectants are used to keep certain products, such as bread and cake moist. Both anticaking agent and humectants pick up water. Anticaking agents immobilize water, whereas as humectants pass it on to the product to compensate for a natural drying out that would make the product unattractive. All humectants are hygroscopic and one of the commonest is glycerol (CH$_2$OH CHOCH$_2$OH). Other permitted humectants are propane 1,2 diol (CH$_3$CHOHCH$_2$OH) and sorbitol (CH$_3$CHOH CHOH CHOH CHOH CHOH CHOH CH$_2$OH), sodium and potassium lactate.

10.0 Firming and Crisping Agents

These are substances that preserve the texture of vegetable tissues by maintaining the water pressure inside them and keep them turgid. They do this by preventing a loss of water from the tissues. The permitted compounds are aluminium potassium sulphate, aluminum sulphate, calcium sulphate and calcium hydroxide. In addition to this sodium chloride, calcium citrate, calcium lactate, calcium phosphate, calcium gluconate and calcium heptonate are also used.

11.0 Sweeteners

Sweeteners are one of the most common constituents of foods. The most common sweetener is sugar or more accurately sucrose. An alternative to sugar is saccharin. People suffering from diabetes and obesity can use this. Another synthetic sweetener is cyclamate and also a dipeptide aspartame. Another natural sweetener isolated is thaumatin isolated from a fruit found in Central and South West Africa. It is proteinaceous in character and anything that affects protein folding such as pH, temperature etc destroy the sweetness. Saccharin is 300 times sweeter than sugar and cyclamate 30 times. Both saccharin and cyclamate are noncaloric. Aspartame has a sweetness rating of 180. Aspartame is the methyl ester of the dipeptide L-aspartyl L-phenylalanine. There has been a new look at monosachharide sweeteners. Sorbitol is now used in confectionary or mannitol if the confectionary is calorie reducing and xylitol in chewing gum.

12.0 Enzymes

Enzymes are used in food for some specific functions or to make a product of consumer’s choice e.g. making leavened bread, cheese and yoghurt. The enzyme "papain" is used to tenderise meat. This attacks the cross links that form with age between muscle fibers and collagen. Two other useful proteases are bromelain (pineapples) and fin (figs). Protein hydrolysates can be prepared from meat scraps and fish waste by means of the digestive enzymes pepsin and trypsin. They break down the muscle proteins to smaller peptide units that may go into colloidal solution. Another enzyme ‘rennin’ prepared from the stomachs of calves is used in cheese making. A number of bacteria such as Bacillus cereus, Mucor michei and Mucor pusillus can yield rennin. The action of rennin is to reduce the water soluble milk protein caseinogen to casein which then interact with Ca present in the milk to form cheese.

Another enzyme, which is not a protease, is Glucose oxidase which is used to prevent Maillard browning caused by reaction between aldehyde group of sugars and amino group of proteins? This is mainly used in dried eggs, dried meat and dehydrated potatoes. The enzyme will also remove oxygen and is used for stabilizing citrus-juice based soft drinks co-stabilizing vitamins B$_{12}$ and C in aqueous solutions, preventing rancidity in oil/water emulsions, or oxidation of beer or vinegarization of wine or the browning of fresh fruit. The enzyme is isolated from the mould Aspergillus niger.
Another enzyme is catalase, a peroxidase which is used in the dairy industry and in the pasteurization of eggs. It acts on $\text{H}_2\text{O}_2$ to release $\text{O}_2$ and $\text{H}_2\text{O}$. In the dairy industry the enzyme is used to remove residual peroxides from milk that is to be used for cheese making. The principle sources of the enzyme are *Aspergillus niger* and *Micrococcus lysodeikticus*.

Another set of enzymes is carbohydrases, particularly amylases used to break polysaccharides to smaller units. The major sources of the enzymes are microbes such as *Arthrobacter*, *Aspergillus niger*, *Aspergillus crysae*, *Bacillus subtilis* etc. Based on the source, the enzymes are identified under five classes.

- Enzymes obtained from edible tissues of animals
- Enzymes derived from edible parts of plants
- Enzymes derived from microorganisms that are traditionally accepted as constituents of food.
- Enzymes derived from non-pathogenic organisms commonly found as contaminants in food. These may be evaluated individually and an ADI must be established
- Enzymes that derived from microorganisms that are less well known. These may be subjected to toxicological studies.

### 13.0 Nutritive Additives

An adequate diet requires proteins (with sufficient amino acids) fats, carbohydrates and minor constituents like vitamins, trace elements and minerals. Depending on the food the fortification with minor constituents vary eg. Margarine is fortified with Vitamin A and D, white flour with Ca, Fe and the vitamins thiamin and nicotinic acid.

### 14.0 Vitamins

Vitamin A is added to margarine to match the average content of Vitamin A in butter and in Eastern countries to vanaspati to match ghee. In place where there is not sufficient sunlight, certain food like margarine is fortified with vitamin D at a higher level than that occurs in butter. Vitamin E, a natural antioxidant, functions efficiently in presence of a seleno-enzymes. This can be incorporated into food products. This vitamin is needed to protect unsaturated essential fatty acids in the cell membrane. Vitamin K is another fat-soluble vitamin. These fat-soluble vitamins require fat in the diet so that they can be properly absorbed.

### 15.0 Conclusions

The increased knowledge about the additives and their functions open a new field of in product development, the so-called fortified/functional/neutraceutical products they have specific functional properties, which may satisfied the needs and nutritional requirements of people such as sportsman, aged and those suffering from diseases.
16.0  Suggested Readings


Codex Alimentarius commission, 1979. Guide to the safe use of food additive, 2nd Ser, Joint FAO/WHO Food standards programme, Rome
<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Additives</th>
<th>Purpose</th>
<th>Permitted level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agar</td>
<td>Thickener</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>2</td>
<td>BHA</td>
<td>Antioxidant</td>
<td>0.03% of total weight of fat content</td>
</tr>
<tr>
<td>3</td>
<td>L-Ascorbic acid</td>
<td>Color enhancer</td>
<td>500 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>Natural flavorings</td>
<td>Flavor enhancer</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>5</td>
<td>Natural smoke solutions</td>
<td>Flavor enhancer</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>6</td>
<td>Sodium citrate</td>
<td>Color enhancer</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>7</td>
<td>Disodium-5-guanylate</td>
<td>Flavor enhancer</td>
<td>500mg/kg as guanylic acid</td>
</tr>
<tr>
<td>8</td>
<td>Disodium inosinate</td>
<td>Flavor enhancer</td>
<td>500mg/kg as inosinic acid</td>
</tr>
<tr>
<td>9</td>
<td>Mono sodium glutamate</td>
<td>Flavor enhancer</td>
<td>2000 mg/kg</td>
</tr>
<tr>
<td>10</td>
<td>Sodium nitrate or potassium nitrate</td>
<td>Preservative</td>
<td>500 mg/kg</td>
</tr>
<tr>
<td>11</td>
<td>Sodium nitrite or potassium nitrite</td>
<td>To fix color</td>
<td>125 mg/kg</td>
</tr>
<tr>
<td>12</td>
<td>Sodium phosphate (e.g. Sodium tripolyphosphate)</td>
<td>Binder</td>
<td>3000 mg/kg as $P_2O_5$</td>
</tr>
<tr>
<td>13</td>
<td>Glucono delta lactone</td>
<td>To accelerate color fixing</td>
<td>3000 mg/kg</td>
</tr>
<tr>
<td>14</td>
<td>Potassium sorbate</td>
<td>Antimould agent</td>
<td>0.1 g /100 g</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Additives</td>
<td>Purpose</td>
<td>Permitted level</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------</td>
<td>--------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>Antioxidant</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>2</td>
<td>BHA</td>
<td>Antioxidant</td>
<td>0.01% of fat</td>
</tr>
<tr>
<td>3</td>
<td>BHT</td>
<td>Antioxidant</td>
<td>0.01% of fat</td>
</tr>
<tr>
<td>4</td>
<td>S0₂</td>
<td>To prevent blackening</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>Benzoic acid</td>
<td>Preservative</td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td>6</td>
<td>Sodium nitrite</td>
<td>To fix color, flavour development, inhibit Cl. botulinum</td>
<td>200 mg/kg in smoked fish</td>
</tr>
<tr>
<td>7</td>
<td>Acetic acid</td>
<td>Produce tenderness, controls the pH and autolytic reaction</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>8</td>
<td>Citric acid</td>
<td>Prevent struvite formation</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>9</td>
<td>Sodium acetate and Potassium sorbate</td>
<td>Antimicrobial</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>10</td>
<td>EDTA</td>
<td>Chelating agent</td>
<td>340 mg/kg</td>
</tr>
<tr>
<td>11</td>
<td>Fast green FCF</td>
<td>Color</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>12</td>
<td>Caramel</td>
<td>Color</td>
<td>Limited by good manufacturing practice (GMP)</td>
</tr>
<tr>
<td>13</td>
<td>Propyl gallate</td>
<td>Antioxidant</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>14</td>
<td>Formic acid</td>
<td>Preservative</td>
<td>200 mg/kg</td>
</tr>
</tbody>
</table>
1.0 Introduction

Fermented foods have long been an important component of nutrition and diet. Over 3,500 traditional, fermented foods exist worldwide. Fermented milks an important category amongst fermented foods was originally developed as a means of preserving nutrients. As per IDF (1969) specifications, fermented milks are defined as the products prepared from milk—whole, partially or fully skimmed, concentrated milk or milk substituted from partially/fully skimmed dried milk, homogenized or not, pasteurised or sterilized and fermented by means of specific organisms.

Cultured dairy products are an excellent medium to generate an array of products that fit into the current consumer demand for health-driven foods. Several technologies associated with culture addition, fermentation or both are available for creating an assortment of flavors and textures in milk products.

2.0 Global Trends and Product Development Strategies

According to a study by global market analyst Euromonitor international global sales of dairy products reached €211.5 billion (Rudrello, 2004). The manufacture of cultured dairy products represents the second most important fermentation industry (after the production of alcoholic drinks). A dynamic category, fermented dairy drinks were reported to grow at six times the rate of total dairy growth between 1998 and 2003 in value terms (Adwan, 2003). A key factor driving value sales growth in developed markets was the increasing demand from consumers for dairy products with 'functional' properties. This led to the promotion of added-value products such as probiotic and other functional yoghurts, reduced-fat and enriched milk products and fermented dairy drinks and organic cheese. Another important trend was the increasing demand for consumer convenience. Scientific and clinical evidence is also mounting to corroborate the consumer perception of health from fermented milks.

Understanding consumer needs and preferences are critical to successful marketing and enhancing marketing value of a product. Nutritionally improved foods with at least one nutritional improvement over the conventional counterpart have been successful in the marketplace. Furthermore, these foods must fit into current lifestyles providing convenience of use, good flavor, and an acceptable price-value ratio. Such foods constitute current and future waves in the evolution of the food development cycle.

Product modification strategies include removal or reduction of fat, cholesterol, sodium and calories and fortification with vitamins, calcium, fiber, live cultures and active ingredients to align with health perceptions of consumers. In addition to basic technologies, modern processes lead to milk fermentation under predictable,
controllable and precise conditions to yield hygienic fermented dairy products of high nutritive value.

3.0 Probiotic, Prebiotic and Synbiotic Fermented Milks

Probiotics, prebiotics and associated ingredients might add an attractive dimension to cultured dairy products for augmenting current demand for functional foods. The importance of these probiotic-containing products, commonly regarded as functional foods, in the maintenance of health and well-being is becoming a key factor affecting consumer choice.

3.1 Development of Probiotic Fermented Milks

Milk is an excellent medium to carry or generate live and active cultured dairy products. Probiotic bacteria, defined as “living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition”, have become a major topic of lactic acid bacteria research over the past 20 years. The potential health-promoting effects achieved by the consumption of dairy products containing probiotic organisms, such as *Lactobacillus* and *Bifidobacterium* spp., have resulted in intensive research efforts in recent years.

Several clinical studies in the recent past have concluded that consuming certain strains of cultures, or their metabolites, or both had various beneficial effects on health including enhanced immune response, balancing of colonic microbiota, vaccine adjuvant effect, reduction of fecal enzymes implicated in cancer initiation, treatment of diarrhea associated with travel, antibiotic therapy, reduction of serum cholesterol, antagonism against food-borne pathogens and tooth decay organisms and amelioration of lactose malabsorption symptoms. The mode of action in most cases seems to involve modulation of ecosystem of the gastrointestinal tract of the host.

3.1.1 Technological challenges for future probiotic fermented milks

The viability and stability of probiotics has been both a marketing and technological challenge for industrial producers. Unless strict demands are set on probiotic product definition and labeling their regulatory definition will remain obscure. Before probiotic strains can be delivered to consumers, they must first be able to be manufactured under industrial conditions and then survive and retain their functionality during storage as frozen or freeze-dried cultures and also in the food products into which they are finally formulated. The probiotic strains should also survive the gastrointestinal stress factors and maintain their functionality within the host. Additionally, they must be able to be incorporated into foods without producing off-flavors or textures and they should be viable but not growing. The packaging materials used and the conditions under which the products are stored are also important for the quality of products. Also, it is usually considered that the acceptable final population of the probiotic organisms in fermented milks at the end of the products shelf life, should be anywhere between 5 and 8 log cfu g⁻¹ (Svensson, 1999).

Many methods including encapsulation have been applied to enhance the survival of probiotic strains during manufacture and storage of fermented milks.

3.2 Prebiotics

Prebiotics is a relatively new concept in the dairy products market. A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity or both of one or a limited number of bacterial species
already resident in the colon (Gibson and Roberfroid, 1995). The health effect of a prebiotic therefore resembles that of a probiotic.

Fructooligosaccharides are the only products presently recognized and used as food ingredients that meet of these criteria. Experimental evidence suggests that transgalactosylated disaccharides and soybean oligosaccharides may also fit this classification. At present, most searches for prebiotics are directed toward the growth of lactic acid–producing microorganisms owing to their purported health-promoting properties.

### 3.3 Synbiotics

Another possibility in microflora management procedures is the use of synbiotics, in which probiotics and prebiotics are used in combination. The live microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth (eg: a fructooligosaccharide in conjunction with a bifidobacterial strain or lactitol in conjunction with a lactobacillus organism). This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation and result in advantages to the host that the live microorganism and prebiotic offer.

### 4.0 Fortification with Physiologically Active Ingredients

Some of the popular ingredients of functional significance being incorporated into cultured dairy products to enhance their market value include

- Calcium, claimed to prevent osteoporosis, some types of cancer and control hypertension.
- Antioxidant vitamins (C and E) to prevent cancer, cardiovascular disease, and cataracts as well as multivitamin-mineral mixes that are being incorporated in fat free cultured milks to provide meal replacements to consumers within a targeted niche.
- Dietary fibers like psyllium, guar gum, gum acacia, oat fiber, and soy components can also be fortified to fermented milks.
- Omega - 3 - fatty acids, the importance of Omega - 3 - fatty acids like α-linolenic has been widely publicized because they are precursors of important long-chain fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, which cannot be synthesized in the human body yet they are vital for the normal functioning and development of the brain and are believed to reduce plaque formation in the arteries (Milner and Alison, 1999). They are further claimed to exert cancer inhibition, anti-allergy effects, and improvement in learning ability.
- Isoflavones that are functional ingredients of a more recent interest, even though their commercial source, soy beans have been consumed for over 5000 yrs. At present, their possible protective action(s) against various cancers (Setchell, 1998), osteoporosis, menopausal symptoms and high levels of blood cholesterol (Mason, 2001) is under investigation.
- Phytosterols, for many people maintaining low-level cholesterol in the bloodstream is a major concern, and one natural way of achieving this goal involves diet-containing phytosterols. Heinemann et al. (1991) suggested that phytosterols interfere with the uptake of cholesterol from the intestinal tract and Jones et al. (1999) indeed showed that the inclusion 1.7 g/day of phytosterols into the diet of hypercholesterolemic men had the effect of lowering blood cholesterol.
Gamma-aminobutyric acid (known as GABA), certain researchers in Japan have reported that a novel fermented milk product containing GABA may lower blood pressure in people with mild hypertension.

5.0 Use of Biothickeners - Eps Cultures

Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) are high molecular weight carbohydrates composed of a backbone of repeated subunits of monosaccharides. EPS derived from food starters are currently being considered as an alternative in the food industry to introduce the desired texture to foods. EPS may act both as texturizers and stabilizers, firstly increasing the viscosity of a final product, and secondly by binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network. As a consequence EPS can decrease syneresis and improve product stability. Furthermore, it has been reported that EPS can positively affect gut health thus exhibiting advantageous biological properties, such as immunostimulation and antitumor and anti-ulcer activities.

EPS producing cultures have been successfully used for the manufacture of Nordic ropy milk, Scandinavian fermented milk drinks which display a firm, thick, slimy consistency and these rely on the souring capacity of mesophilic ropy strains of *Lactococcus lactis* subsp. *lactis* and ssp. *cremoris* and concomitant production of heterotype EPS for texture.

6.0 Low Calorie/ Low Fat Fermented Milks

With billion of dollars spent on dieting each year, consumers’ desire for nutritious low-cal dairy products continues to grow and consumption of low - or nonfat dairy products has increased in recognition of their health benefits, and consumers' health problems. Fat free and low-fat formulations have earned for yogurt highly acceptable place in consumer lifestyles that are seeking fat and cholesterol reduction. Although, the manufacture of low- or nonfat dairy products has been possible for many years, the use of fat replacers in the manufacture of dairy products is still novel.

Fat replacers, which decrease the calorific value of food, can be used to solve some physical and organoleptic problems originating from low-fat levels in the final products. Various fat replacers and replacer blends have been used to produce low fat/low calorie cultured dairy products and the technically developed fat substitutes are divided into 2 main types: modified starches or proteins which have good emulsifying or gel properties along with low calorie values; and modified products that contain bonds resistant to digestion, eg. glycerol esters and complex carbohydrates or fatty acid esters.

Also, non-nutritive sweeteners can be used to impart an attractive calorie reduction in fermented milks. Sweeteners like Aspartame, Acesulfame-K, Sucralose, individually and in combination are being used as sweeteners for the formulation of numerous low-cal and sugar free yoghurts, milk beverages, whey based beverages and cultured milk products.

7.0 Utilisation of Fruits in Fermented Milks

Owing to expanding market share and size of dairy companies, there has been a reduction of clearly structured markets i.e. merging of dairy products and fruit beverage markets with introduction of ‘juiceceuticals’ that include products like fruit-yogurt beverages. Addition of fruit preparations, fruit flavors, and fruit purees have enhanced versatility of flavor, texture, color, and variety.
As consumers connect both these foods with health and wellness, the two categories of fruits and cultured milk products are typical example of hybrid dairy products offering health, flavor and convenience that will drive growth in coming years. Processed fruits are more widely employed, they may be added to cultured milk in various forms namely fruit purees, fruit pieces, fruit syrup/juices, crushed fruit, frozen/osmodehydrofrozen fruits, fruit preserves and other miscellaneous fruit products. Several processes have been outlined for preparation of fruit based fermented milks with fruit concentration mainly ranging from 4-20% (Balasubramanyam and Kulkarni, 1991; Desai et al. 1994).

8.0 Whey Based Fermented Milks

Industrial lactic fermented beverages are formulated products containing yogurt, whey, fruit juice or pulp, flavor, other raw materials and allowed additives however, yogurt microorganisms should be plenty and alive in the final product. Whey used in lactic beverages is a byproduct from cheese and casein production. Through new technologies, whey and its fractions became versatile ingredients and also have high economic value.

Whey products improve textural properties, extend shelf-life, emulsification and stability, improve flow properties, enhance color and taste and have been shown to provide beneficial functionality. Whey products have certain essential amino acids, good digestibility and present protein efficiency index higher than 3.0. Vitamins such as thiamin, riboflavin, pantothenic acid, and vitamin B₆ and B₁₂ are also present. Due to their functional properties, whey solids/ whey as such could be used in conjunction with fermented milks. Several methods for preparation of low cost good quality whey based fermented milks have been reported (Macedo et al.; 1999; Augustin et al. 2003).

9.0 Use of Non Conventional Food Sources

Owing to the worldwide shortage of food, attempts have been made to find alternative sources of protein, particularly for the developing and underdeveloped nations where malnutrition exists. Soybeans particularly are plentiful, relatively inexpensive and rich in protein; efforts have been devoted to exploit them for manufacture of more acceptable and palatable food products. Thus, incorporation of these nonconventional food sources like soybeans and different kinds of millets with fermented milks may help in increasing utilization of these non conventional food sources and producing low cost, nutritious fermented foods apart from extending the variety of fermented milks.

Several attempts have been made to incorporate soy solids to milk by various researchers world over as Soymilk based fermented milks would offer several distinct nutritional advantages to the consumer i.e., reduced level of cholesterol, saturated fat, and lactose. Soymilk is low in fat, carbohydrate, calcium, phosphorus and riboflavin, but high in iron, thiamin and niacin in comparison with cow's milk. Soymilk contains higher amount of protein than buffalo milk and is deficient in sulfur containing amino acids. Replacing a part of milk used in making yogurts with soymilk enriches nutritional value of the product.

Soymilk is characterized by beany or soy flavor which can be modified by lactic acid fermentation. In one study soy yoghurt with improved sensory characteristics was prepared by Nsofor and Chukwu (1992). In another experiment Öner et al. (1993) totally or partially replaced wheat flour with soybean for production of Tarhana, one of the most widely consumed fermented foods in the Middle East and Turkey, traditionally produced from yoghurt and wheat. Protein concentration of the product was doubled with the use
of soybean. Also, beany off flavour problem was diminished following a specific technique of manufacture.

Mugocha et al. (2000) reported that yoghurt type bacterial cultures could be successfully used to produce a composite fermented beverage from finger millet and skim milk. In another study yogurt extended with chickpea (Cicer arietinum), inoculated with *S. thermophilus* and *L. bulgaricus* was prepared by Morales et al. (2000).

10.0 Use of Biopreservatives

Cultured dairy products are generally perishable commodities. Over the years, cultured dairy products have developed different identities and appeals. The recent increase in their popularity has been attributed to the introduction of fruits, stabilizers, flavors, coloring agents and unique starter culture combinations into the manufacture of the product. However, these additives and techniques have also increased processing control demands, thus requiring more stringent manufacturing practices. The low pH of cultured milks offers a selective environment for the growth of acid tolerant yeasts and molds.

Benzoates and sorbates are permitted additives to inhibit yeasts and molds and therefore may be found in yogurt. These acid tolerant organisms, however develop resistance to chemical preservatives and also, in recent years consumers have been demanding a reduction in the use of chemicals in their foods and beverages because of potential health risks. Use of biopreservatives is one important alternative technology that could be used to extend the shelf life of ready to consume fermented milks and preserve the freshness, flavor, texture and nutrient value of these products. The antimicrobial system possessed by LAB and Propionic acid bacteria (PAB) offers scope for the development of an effective natural preservation process.

10.1 Bacteriocins Produced by LAB

The bacteriocins elaborated by LAB are recognized as safe GRAS and have arisen a great deal of attention as a novel approach to control pathogens in foodstuffs. LAB have an essential role in most food and beverage fermentation processes and selective isolated strains can positively have impact on their use as starter cultures for fermented milks, with a view to improving the hygiene and safety of fermented milk so produced.

Nisin is one such polypeptide antibacterial substance or bacteriocin produced by the fermentation of a modified milk medium with *Lactococcus lactis* ssp. *lactis*. Nisin has an inhibitory effect against a wide variety of gram-positive food-borne pathogens and spoilage microorganisms and can also act on several gram-negative bacteria when the integrity of their outer membranes is disrupted. Nisalpin, the commercial product containing 2.5% pure nisin A, is being legally used in more than 50 countries for specific food applications.

10.2 Bacteriocins Produced by PAB

PAB also produce metabolites with antimicrobial activity such as propionic acid, acetic acid and diacetyl. These have also been found to produce antiviral peptides and several bacteriocins.

The biopreservative MicroGARD™ is skim milk pasteurized after fermentation with *P. freudenreichii* ssp. *shermanii*. It has been approved by the FDA for use in products like cottage cheese and yogurt. MicroGARD™ inhibits some fungi and Gram(−) bacteria, but not Gram(+) bacteria. According to Daeschel (1990) about 30% of the
cottage cheese produced in the United States was made with MicroGARD™ as a preservative. Another commercial product is BioProfit, which contains Lactobacillus rhamnosus LC 705 and Propionibacterium freudenreichii JS. Used as a protective culture (107 cells per gram) the product is reported to inhibit yeasts in dairy products, and Bacillus spp. in sourdough bread.

11.0 Conclusion and Future Perspective

The market for fermented milks is booming especially probiotics and those with special added ingredients. Modern consumers are increasingly interested in their personal health and expect the food that they eat to be healthy or even capable of preventing illness. Producers and marketers of cultured milks are making every effort to keep them growing through product development and packaging innovations while delivering a ‘good for you’ flavorful products suited for all occasions of gastronomic indulgence. A major consideration in the continued development and success of ever growing fermented milk market is communication. This is linked to other important factors such as development of supporting scientific documentation; a health claims strategy and successful presentation.

Special emphasis on research in arena of starters and their functionality especially in view of natural biodiversity that still exists in food grade microorganisms is required as starter cultures are the heart of fermented milk industry. It is also very important to preserve this pool for future application. In the field of probiotics more emphasis is required to get a clear understanding of relationship between food, intestinal bacteria and human health and disease. Newer molecular research tools, better process formulation technologies for enhanced probiotic stability and functionality along with biosafety evaluation of probiotics used for human consumption are major thrust areas. New product categories, and thus novel and more difficult raw materials with regard to technology of probiotics, will certainly be the key research and development area for future functional food markets.

12.0 References


1.0 Introduction

Whey and whey products have been used successfully in conventional foods, fortified, enriched or enhanced foods and dietary supplements. Whey products contribute to creaminess, texture, water binding, opacity and adhesion in a variety of food systems. Their high nutritional quality and unique range of functional properties makes them valuable in a wide range of low fat products including soups, sauces, salad dressings and meats. Sweet whey, modified whey, when protein concentrate (34-80% protein), and whey protein isolates (>90% protein) are among the most commonly used whey products. Today, the food industries are looking for ingredients, which can provide good functional and nutritional properties for formulation of value added food products. Whey protein is available to food manufacturers in various form and purities, making it suitable for a variety of applications.

2.0 Whey Protein Concentrates and Functional Enhancements

Functional properties of proteins are those physico chemical properties which govern the performance and behavior of proteins in food systems during their preparation, processing, storage and consumption i.e. properties effecting the final quality of foods. Most of the key protein functional properties may be classified into two main groups: hydration related and surface –related properties. Hydration related functional properties include dispensability, solubility, swelling, viscosity and gelation. Surface related properties included emulsification, foaming, and absorption at air-water interfaces. Other functional properties include diffusion, molecular unfolding (denaturation) and protein- protein, protein-ion, and protein-ligand binding.

A number of unique functionally enhanced WPCs are available for specialized applications.

→ High Gelation: An important function of WPCs is their ability to form heat-induced gels. These gels can hold water and other non-protein components of food systems. Calcium is required for gelling to occur and free calcium concentration is critical in determining gel hardness and water retention properties. Heating time, heating temperature, pH, and NaCl all influence the texture of WPC gels. High gelling WPCs function particularly well in reduced fat meats. Studies have shown that high gelling WPCs when combined with a gum and starch, yield a low fat (3%) pork sausage that has similar textural characteristics to a full fat (20%) control.
**Cold Gelation:** Studies have demonstrated that whey proteins have to be pretreated by heating to at least 70°C to achieve cold gelling ability. Cold gelling WPCs are now commercially available, and they can find good applications are salad dressings and mayonnaise type products. In meat applications using vacuum tumbling, cold-gelling WPCs can be added until the protein is in the meat, to be followed by the addition of salt to gel the WPC. Tests have shown that this method improves meat quality and yields.

**Heat Stability:** Heating WPCs leads to the unfolding of globular proteins followed by association to form aggregates. Researchers have shown that a lactalbumin heated alone did not form aggregates while B-lactoglobulin formed large aggregates with no evidence of intermediates. However, the two proteins interact to form soluble aggregates, as well as larger particles, by means of both disulfide bonds, and hydrophobic interaction. This step can lead to the formation of small aggregate precipitates or gel lattice structures. Small aggregates remain soluble; they bind increased amounts of water, increase viscosity and add body or improve the product texture. However, gelation and some flocculating can occur. Lowering the pH of WPCs has been shown to inhibit unfolding of the protein and to increase their stability to thermal Denaturation. Controlling the heat Denaturation process in low fat systems to prevent defects is important. Some precautions to follow include keeping the temperature below 75°C while the system is dilute. Denaturation of WPCs decrease from 80% to 40% (with heating at 80°C for 20 minutes). Finally, adjusting calcium levels with chelators can be effective in some systems.

**Water Binding:** Efficient water binding is an important function provided by WPCs in reduced and fat free salad dressings. The retained water improves texture and reduces cost by replacing oil with water. This function is also valuable in reduced fat chopped meat and seafood products, providing improved texture and mouthful.

### 3.0 A Range of Functional Ingredients

The UF whey products most often used in frozen desserts include:

- **Sweet Whey**
- **Reduced lactose whey.**
- **Demineralized whey**
- **Modified Whey**
- **Acid Whey** (acid flavored sherbet, sorbet only)
- **Whey Protein Concentrate** (WPC 34% protein; WPC34)
- **Whey Protein Concentrate** (WPC 50% protein; WPC50)
- **Whey Protein Concentrate** (WPC 80% protein; WPC80)
- **Whey protein Isolate** (90% Protein: WPI)
4.0 **Bakery Products**

Bakery products available on Indian shelves and a study of their ingredients will reveal that the country is not far behind the world as far as application of nutraceutical is concerned; whey protein have been considered a potential ingredient for the bakery industry, in view of their desirable functional characteristics and nutritive value.

The physical structural of bread reflects the unique properties of the major proteins of wheat flour. Upon hydration, gluten forms a stretchable viscoelastic network that can entrap gas produced by yeasts. Bread, with milk proteins added in one form or another shows a good crumb structure, bread yield, flavour and keeping quality. In bread making, some denaturation of whey proteins is necessary to avoid adverse reaction between whey proteins and other components of the system. Addition of denature WPC results in weaker, less elastic dough’s, which after baking yields loaves of reduced volume. In the manufacture of high proteins biscuits, milks play an important role as they increase the nutritive value and also the texture.

Whey proteins in the undenatured form when added to the flour at 5% level, in the preparation of noodles, little changes was observed apart from some weakening of the cooked noodles, addition of 10% WPC gave a very sticky dough, the noodles were significantly harder to dry, but the colour was enhanced.

5.0 **Meat Products**

Whey protein concentrates may find better applications as ingredients in minced and ground meat products such as Wiener, Frankfurter, Hotdogs, Bologna (large sausages) and meat loaves or luncheon meat. Minced meat products often contain large amount, of fat and the mince is considered to be an emulsion. During processing and cooking, a protein product, which is less expensive than meat is often added. It has been observed that WPC work as emulsion stabilizers in comminuted meat products. In comminuted meat products, WPC contributes to fat emulsification, water binding and improved consistency; as it releases meat proteins for gel formation and water binding.

Researchers conducted studies on the development of a textured whey protein meatless patty using WPC-80 and reported that mushroom flavored and vegetable flavored textured whey protein patties were as acceptable as commercial soy patty.

WPC and WPI can be used effectively to improve the texture properties of surimi-based seafood, fish balls, fish and shellfish and other similar products.

6.0 **Traditional Food**

Traditional ragi based (Finger millet) products like ragi-malt and ragi dosa can be prepared by incorporating with WPC incorporation of WPC up to 30% doesn’t have any adverse effect on the sensory attributes when compared to control.

7.0 **Confectionary**

Dairy products have been used as valued ingredients by the confectionery industry for many years as they help achieve the required flavour, colour and texture in many products including chocolate coating, caramels, aerated confections, and toffee. Milk
protein in sweetened condensed milk contributes significantly to the emulsification of fat and give body, texture and mouth feel to the final products.

In Chocolate manufacture whey powders are generally used as replacer for skim milk powder to reduce cost.

Demineralized whey, WPC and blend are used as total or partial replacement for milk powders in coating formulation, polishing and glazing provides a brilliant surface and a moisture barrier coating to high quality confectionaries

8.0 Low Fat Application

Fat replacers are generally categorized into two groups: fat substitutes and fat mimetics. Fat substitutes are ingredients that have a chemical structure somewhat close to fats and have similar physico-chemical properties.

Fat mimetics are ingredients that have distinctly different chemical structures from fat. They are usually carbohydrate and / or protein based. Fat mimetics are the most widely used ingredients for producing emulsion based reduced fat products.

Micro particulate WPC provides fat like mouth feel and allows consumers to lower dieting fat without sacrificing sensing qualities. FDA approves this protein based fat substitute. WPC is a fat replacer in meat products.

9.0 Acid Foods and Beverages

Solubility of WPC at low pH, a unique property among proteins, allows it to function in acid foods and beverages, where non-fat dry milk and casein cannot. So WPC as a source of protein in such products as fruit jams, jellies, fruit juices, carbonated beverages and other soft drinks. The unique solubility of WPC enables them to be used in milk-based beverages, fruit juices, soft drinks, and cream liqueurs, wine aperitifs etc.

10.0 Nutrition

Whey protein concentrate is a great “Nutrient buy”. The price value relationship is such that there are few equivalent sources of key nutrients such as high quality protein. Calcium and a variety of health enhancing components such as whey protein fractions. Indirect impact on the nutrient content of mixes such as in “reduced” or “low” fat products also adds value.

11.0 Conclusions

WPC is not only ingredient but also used as additives and fulfill more than one function. It has different functional properties as, thickness, emulsifiers and stabilizers, flavour enhancers, coloring agent (through millard reaction), gelling agents and antioxidant activities. In addition to this, it also provides nutrient supplement in various product formulation. WPC also act as fat replacers in ice Cream. Whey proteins provide fat like function of lubricity and significant by improve the mouth feel of low fat products. WPC –34 adds a clean dairy flavour and higher protein WPCs has a clean, bland flavour that blends well with other flavour present in the system.
1.0 Introduction

There has been increasing concern of the consumers about foods free or with lower level of chemical preservatives because these could be toxic for humans. Concomitantly, consumers have also demanded for foods with long shelf-life and absence of risk of causing food borne diseases. This perspective has put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its goals concerning microbial safety. This resulted in increasing search for new technologies for use in food conservation systems.

Recently, there has been increasing interest in discovering new natural antimicrobials. Plant products with natural antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth.

Being plant natural foodstuffs, spices appeal to consumers who tend to question the safety of synthetic additives. Antimicrobial properties of spices have been documented in recent years and interest continues to the present. Still little information is available emphasizing the preservative and antimicrobial role of spices in the prevention of foods from the microbial spoilage (Arora and Kaur, 1999).

Antimicrobial activity of spices depend on several factors (Shelef, 1983), which includes:

- Kind of spice
- Composition and concentration of spice
- Microbial species and its occurrence level
- Substrate composition and
- Processing conditions and storage

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon), rhizomes (ginger) and other plant parts (Shelef, 1983). Although, spices have been well known for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf life.
### Table 1: Antimicrobial Effectiveness of Spices and Herbs*

<table>
<thead>
<tr>
<th>Spices and Herbs</th>
<th>Inhibitory Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon, cloves, mustard</td>
<td>Strong</td>
</tr>
<tr>
<td>Allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage, thyme</td>
<td>Medium</td>
</tr>
<tr>
<td>Black pepper, red pepper, ginger</td>
<td>Weak</td>
</tr>
</tbody>
</table>

*Adapted from Zaika (1988)

### Table 2: Inhibitory Effects of Spices and Herbs*

<table>
<thead>
<tr>
<th>Spice / Herb</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>Salmonella typhymurium, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, mycotoxigenic Aspergillus, Candida albicans</td>
</tr>
<tr>
<td>Onion</td>
<td>Aspergillus flavis, Aspergillus parasiticus</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Mycotoxigenic Aspergillus, Aspergillus parasiticus</td>
</tr>
<tr>
<td>Cloves</td>
<td>Mycotoxigenic Aspergillus</td>
</tr>
<tr>
<td>Mustard</td>
<td>Mycotoxigenic Aspergillus</td>
</tr>
<tr>
<td>Allspice</td>
<td>Mycotoxigenic Aspergillus</td>
</tr>
<tr>
<td>Oregano</td>
<td>Mycotoxigenic Aspergillus, Salmonella spp., Vibrio parahaemolyticus</td>
</tr>
<tr>
<td>Rosemary</td>
<td>Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus</td>
</tr>
<tr>
<td>Bay leaf</td>
<td>Clostridium botulinum</td>
</tr>
<tr>
<td>Sage</td>
<td>Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus</td>
</tr>
<tr>
<td>Thyme</td>
<td>Vibrio parahaemolyticus</td>
</tr>
</tbody>
</table>

* Adapted from Shelef (1983)
2.0 Antimicrobial Potency of Spices

Essential oils extracted from spices and herbs are generally recognized as containing the active antimicrobial compounds. Table 3 is a list of the proximate essential oil content of some spices and herbs and their antimicrobial components.

Table 3: Antimicrobial Components of Spices and Herbs*

<table>
<thead>
<tr>
<th>Spice / Herb</th>
<th>Proximate Essential Oil Content (%)</th>
<th>Antimicrobial Component(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>0.3 - 0.5</td>
<td>Allicin</td>
</tr>
<tr>
<td>Mustard</td>
<td>0.5 - 1.0</td>
<td>Allyl isothiocyanate</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.5 - 2.0</td>
<td>Cinnamaldehyde, Eugenol</td>
</tr>
<tr>
<td>Cloves</td>
<td>16 - 18</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Sage</td>
<td>0.7 - 2.0</td>
<td>Thymol, Eugenol</td>
</tr>
<tr>
<td>Oregano</td>
<td>0.8 - 0.9</td>
<td>Thymol, Carvacrol</td>
</tr>
</tbody>
</table>

* Adapted from Shelef (1983)

Allicin and allyl isothiocyanate are sulfur-containing compounds. Allicin, isolated from garlic oil, inhibits the growth of both gram-negative and gram-positive bacteria. Sulfur-containing compounds are also present in onions, leeks, and chives. Eugenol, carvacrol, and thymol are phenol compounds and, as Table 3 indicates, are found in cinnamon, cloves, sage, and oregano. The essential oil fraction is particularly high in cloves, and eugenol comprises 95% of the fraction. The presence of these compounds in cinnamon and cloves, when added to bakery items, function as mold inhibitors in addition to adding flavor and aroma to baked products. Paster et al. have shown that essential oils of oregano and thyme (which contain carvacrol and thymol) are effective as fumigants against fungi on stored grain. These investigators have proposed using them as an alternative to chemicals for preserving stored grains.

3.0 Antioxidant Activity of Spices

Spice extractives, such as oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavor in some products. Thus, some spices not only provide flavor and aroma to food and retard microbial growth, but are also beneficial in prevention of some off-flavor development. These attributes are useful in the development of snack foods and meat products.

Antioxidant extract from spices (usually rosemary) commercially available are usually fine powders. Depending on their content of active substances, it is recommended they be used at levels between 200 and 1000mg/Kg of finished product to be stabilized. Generally, the powders are dispersible in oils or fats, insoluble in water, but soluble in organic solvents. Due to their powder characteristics they can also be used in dry mixes.
4.0 Applications of Herbs and Spices in Dairy Products

4.1 Cheeses

Investigations were carried out to assess the efficiency of four plant essential oils; bay, clove, cinnamon and thyme as natural food preservatives. The effect of the plant essential oils at concentrations of 0.1, 0.5 and 1% was studied in low-fat and full-fat soft cheese against *Listeria monocytogenes* and *Salmonella enteritidis* at 4°C and 10°C respectively, over a 14-day period. The composition of the cheese was shown to be an important factor in determining the effectiveness of the plant essential oils. In the low-fat cheese, all four oils at 1% reduced *L. monocytogenes* to ≤1.0 log_{10} cfu/ml. In contrast, in the full-fat cheese, oil of clove was the only oil to achieve this reduction. Oil of thyme proved ineffective against *S. enteritidis* in the full-fat cheese, yet was equally as effective as the other three oils in the low-fat cheese, reducing *S. enteritidis* to ≤1.0 log_{10} cfu/ml from day 4 onwards. It is concluded that selected plant essential oils can act as potent inhibitors of *L. monocytogenes* and *S. enteritidis* in a food product.

Antimicrobial activity of 1% (w/v) fresh garlic, ground clove and red dried chilli on *Listeria monocytogenes* was tested in broth systems at 37°C and at 4°C for 7 h. The initial cell concentration in the broth systems was between 2×10⁶ and 4×10⁶ CFU/ml. At 37°C, growth to viable numbers of 3×10⁸ CFU/ml in 7 h was measured. Clove had bacteriocidal activity and reduced the count to 1 CFU/ml. Garlic displayed bacteriostatic properties, and a count of 4×10⁶ CFU/ml was maintained. Red chilli displayed an inhibitory effect and resulted in 50% lower counts than the control. *L. monocytogenes* had a slow growth rate at 4°C and increased from an initial value of 3×10⁶ to 5×10⁶ CFU/ml during 7 h. The addition of garlic resulted in 3×10⁶ CFU/ml, and clove reduced the viable cell concentration to 1×10³ CFU/ml after 7 h. Two batches of soft cheese were produced in the laboratory using milk that was supplemented with *L. monocytogenes*. The final cheese containing *L. monocytogenes* with about 1×10⁵ CFU/g. Half of each cheese batch was supplemented with either 1% garlic or 1% clove, whereby the other half served as a control. After 7 or 11 days incubation at 4°C, the cheese was incubated at abuse temperature of 25°C for 7 or 3 days, respectively. No antimicrobial effects of 1% (w/w) fresh garlic or clove powder on *L. monocytogenes* were observed in cheese after 1 or 2 weeks at the lower or higher temperature.

4.2 Traditional Indian Dairy Products

Sen and Rajorhia (1996) conducted a study to find out the efficacy of cardamom powder in preservation of sandesh. They found that cardmom powder added @ 0.1% enhanced the shelf-life of sandesh to 90 days as against 60 days for control sample at 7°C. This can be attributed to the presence of several antimicrobial phenolics such as limonene, nerolidon, cineol, terpene, sabinine, pinene, etc.

Also the effect of saffron on the shelf life extension of sandesh was studied. Addition of 0.015% saffron (w/w of chhana) at the end of cooking stage improved the shelf life of sandesh upto 9 and 68 days at 30°C and 7°C as against 3 and 51 days for untreated sandesh samples.
4.3 Microbial Cultures

The effects of some herbs (Allium sp., Thymus sp., Anhriscus sp. and Ferule sp.) on growth and acid production of thermophilic cultures (Streptococcus thermophilus and Lactobacillus bulgaricus) have been studied. The herbs were sterilized with gamma radiation and added to fermentation media at concentrations of 0.5, 1, 2 and 3% (w/w). Control medium was without herb. Results showed that increasing herb concentrations stimulated acid production by Streptococcus thermophilus and Lactobacillus bulgaricus.

5.0 Concluding Remarks

Use of spices as microbial growth inhibitor in foods is often limited because of flavor considerations as effective antimicrobial dose may exceed the organoleptically accepted level (Pandit and Shelef 1994; Brull and Coote, 1999). Nonetheless, combinations of spices and other antimicrobial barriers could enhance the food shelf stability and microbial safety even in moderated levels. Due to this and due to the fact that spices are as GRAS, the antimicrobial properties of spices continue to be of interest (Pandit and Shelef, 1994). It is established that spices and their derivatives could be suitable alternatives for inclusion in food conservation systems and could act sometimes as main or adjuvant antimicrobial compounds. Before including spices and/or their derivatives in food conservation systems, some evaluations about microbiological quality, economic feasibility, antimicrobial effect for a long time and toxicity should be carried out.

6.0 Suggested Readings

Antimicrobial effects of spices and herbs. Found at site: http://www.hi-tm.com/


