Lecture Compendium

RECENT DEVELOPMENTS IN HEALTH FOODS AND NUTRACEUTICALS

18th short Course

February, 8- February 28, 2005

Centre of Advanced Studies

Dairy Technology Division
National Dairy Research Institute
(Deemed University)
Karnal-132001
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Lecture Compendium

Recent Development in Health Foods and Nutraceuticals

February 8, 2005 – February 28, 2005

Dairy Technology Division
National Dairy Research Institute
Karnal – 132 001
Dairy Technology Division had made significant contributions towards the “White Revolution” of the country thorough human resource development, technological innovation for manufacturing a wide array of nutritious and quality products and transfer of technology as per the need of Indian dairy industry. Since the inception of Centre of Advanced Studies in Dairy Technology Division has organized 17 short-term courses for the teachers and scientists of State Agriculture Universities and ICAR Institutes. The 18th short course on “Recent Development in Health Foods and Nutraceuticals” will definitely pave the way for newer dimensions in the area of health foods.

Milk has an excellent nutrient profile, providing significant amounts of high-quality proteins, minerals, vitamins as well as several other essential nutrients. In addition, milk and milk products are nutrient-dense foods that are known to contain various therapeutic agents. Consumption of fermented dairy products might fend off certain specific diseases has been well established in the form of “Probiotics”. Recent thorough scientific investigations have supported many of the traditional views thus turning myth into reality. In the 1990s consumers began to consider food from a radically different viewpoint. This new changing classification of foods can be defined as those containing physiologically active molecules providing specific health benefits i.e. “functional foods”. Functional foods include wholesome, fortified, enriched, nutritionally modified or designer foods, which exhibit disease prevention or therapy when consumed as a part of daily diet on regular basis at effective levels. This interest in functional foods has resulted in an increasing number of new food products on shelf, designed to cater the specific health concerns. It is one of the areas where the demarcation line between food and pharma industry is almost diminished. Many traditional herbs used in ‘Ayurvedic’ system of medicines have potential to be incorporated in processed food products for improving the various body functions. India is endowed with a rich diversity of flora and fauna. These are believed to contain ever-expanding array of previously unknown molecules with hard-to-pronounce names and possess ability to prevent diseases like cancer, coronary heart diseases, diabetes and hypertension in addition to delaying the ageing process.

National Dairy Research Institute has given a lot of impetus on developing functional foods and nutraceuticals through interdisciplinary scientific investigations for fulfilling the diverse dietary needs of Indian consumers. Several research projects are in progress in this direction. The R&D efforts in these areas will help the Indian food industry to deliver nutritional and therapeutic products to consumers and also diversify their product profile to sustain in this competitive world. I am certain that the information compiled by the faculty of the course based on intensive literature survey and their own rich experience will be of great benefit to the participants in their teaching and research endeavours.

Director
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Title</th>
<th>Faculty Members</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Functional foods and nutraceuticals- an overview</td>
<td>Dr. G.R. Patil</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Contribution of milk and milk products to nutrient intake</td>
<td>Dr. Rajan Sharma &amp; Dr. Y.S. Rajput</td>
<td>9</td>
</tr>
<tr>
<td>3.</td>
<td>Therapeutic potential of milk and milk products</td>
<td>Dr. Rajan Sharma &amp; Dr. Y.S. Rajput</td>
<td>18</td>
</tr>
<tr>
<td>4.</td>
<td>Feeding strategies to improve the nutritional status of milk</td>
<td>Dr. Amrish Tyagi</td>
<td>27</td>
</tr>
<tr>
<td>5.</td>
<td>A new prospect for dairy biotechnology - designer milk</td>
<td>Dr. Latha Sabikhi</td>
<td>34</td>
</tr>
<tr>
<td>6.</td>
<td>Mineral and vitamin fortification of milk and milk products</td>
<td>Dr. Sumit Arora</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>Health benefits of specific phytochemicals</td>
<td>Dr. K. R. Anilakumar</td>
<td>47</td>
</tr>
<tr>
<td>8.</td>
<td>Strategies for fiber fortification in dairy products</td>
<td>Dr. A. A. Patel &amp; Ms. Simran Kaur Arora</td>
<td>55</td>
</tr>
<tr>
<td>9.</td>
<td>Role of casein and caseinates in speciality food products</td>
<td>Dr. Vijay Kumar Gupta</td>
<td>62</td>
</tr>
<tr>
<td>10.</td>
<td>Ghee- a functional and nutraceutical product</td>
<td>Dr. S. K. Kanawjia &amp; Mr. S. Makhal</td>
<td>68</td>
</tr>
<tr>
<td>11.</td>
<td>Geriatric nutrition: challenges and opportunities</td>
<td>Dr. Sudhir Kumar Tomar</td>
<td>76</td>
</tr>
<tr>
<td>12.</td>
<td>Formulation of food products for cardiovascular health</td>
<td>Dr. D. K. Thompkinson</td>
<td>84</td>
</tr>
<tr>
<td>13.</td>
<td>Technological developments for Cereal-milk based convenience foods</td>
<td>Dr. Alok Jha</td>
<td>90</td>
</tr>
<tr>
<td>14.</td>
<td>Selection of culture strains for making probiotic fermented milks</td>
<td>Dr. Rameshwar Singh &amp; Dr. R. P. Singh</td>
<td>95</td>
</tr>
<tr>
<td>15.</td>
<td>Developments in probiotic cheese</td>
<td>Dr. Latha Sabikhi</td>
<td>100</td>
</tr>
<tr>
<td>16.</td>
<td>Utilization of <em>lactobacillus acidophilus</em> in development of health foods</td>
<td>Dr. D.N. Gandhi</td>
<td>105</td>
</tr>
<tr>
<td>17.</td>
<td>Fat replacers for healthy foods</td>
<td>DR. B. D. Tiwari</td>
<td>110</td>
</tr>
<tr>
<td>18.</td>
<td>Technological aspects of low fat and low sugar dairy products</td>
<td>Dr. Dharam Pal &amp; Ms. Shashi Prabha</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>Title</td>
<td>Author(s)</td>
<td>Page</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>19.</td>
<td>Technology for indigenous dairy products Using artificial sweeteners</td>
<td>Mr. F.C. Garg</td>
<td>122</td>
</tr>
<tr>
<td>20.</td>
<td>Specialty frozen dairy products</td>
<td>Dr. R.S. Mann &amp; Mr. P.K. Singh</td>
<td>127</td>
</tr>
<tr>
<td>21.</td>
<td>Specialized processed foods for metabolic disorders</td>
<td>Dr. Satish Kulkarni</td>
<td>133</td>
</tr>
<tr>
<td>22.</td>
<td>Packaging of health beverages</td>
<td>Dr. G.K. Goyal</td>
<td>137</td>
</tr>
<tr>
<td>23.</td>
<td>Dietary significance of conjugated linoleic acid (cla)</td>
<td>Dr. Vinod K. Kansal</td>
<td>145</td>
</tr>
<tr>
<td>24.</td>
<td>Safety and stability of artificial sweeteners</td>
<td>Dr Sumit Arora</td>
<td>151</td>
</tr>
<tr>
<td>25.</td>
<td>Estimation of conjugated linoleic acid content in dairy products</td>
<td>Dr. Vivek Sharma</td>
<td>160</td>
</tr>
<tr>
<td>26.</td>
<td>Thermogenic and lipotropic foods: An embryonic dietary approach</td>
<td>Dr. S.K. Kanawjia &amp; Mr. S. Makhal</td>
<td>165</td>
</tr>
<tr>
<td>27.</td>
<td>Opportunities for product development from chhana and chhana by-products</td>
<td>Dr. B.B. Verma &amp; Ms. Kirti Sharma</td>
<td>174</td>
</tr>
<tr>
<td>28.</td>
<td>Prospects of developing therapeutic products using whey proteins</td>
<td>Dr. Ashwani Kumar Rathour</td>
<td>179</td>
</tr>
<tr>
<td>29.</td>
<td>Bioactive peptides - as nutraceuticals</td>
<td>Dr. Bimlesh Mann, Dr. Rajesh Bajaj &amp; Dr. R.B. Sangwan</td>
<td>184</td>
</tr>
<tr>
<td>30.</td>
<td>Natural antioxidants: functionality and changes during processing</td>
<td>Dr. R. R. B. Singh</td>
<td>190</td>
</tr>
<tr>
<td>31.</td>
<td>Nutritional metabolism - biochemical aspects</td>
<td>Dr. Surjeet Singh</td>
<td>198</td>
</tr>
<tr>
<td>32.</td>
<td>Oligosaccharide as ingredient for functional foods</td>
<td>Dr. Ashish Kr. Singh &amp; Dr. R.R.B. Singh</td>
<td>206</td>
</tr>
<tr>
<td>33.</td>
<td>Therapeutic values of fermented milk products – An Overview</td>
<td>Dr. S. Singh, Dr. S.K. Kanawjia</td>
<td>215</td>
</tr>
</tbody>
</table>
FUNCTIONAL FOODS & NUTRACEUTICALS - AN OVERVIEW

Dr. G.R. Patil
Head Dairy Technology Division
NDRI Karnal

1.0 Introduction

There were revitalized interests among the consumers about the nutritional and therapeutic aspects of food they eat. This has led to a paradigm shift to explore the missing linkage between the food and health. There is basic temptation in human beings to move towards the nature and the products, which are “natural”. All such developments over the last one decade resulted in increasing number of potential nutritional products with medicinal and health benefit termed as “Functional foods”. Functional foods, designer foods, pharma foods and nutraceuticals are synonymous for the components of diet that possess unique disease preventing/curing ability. Functional foods have been defined as foods that, by virtue of the presence of physiologically-active components, provide a health benefit beyond basic nutrition. In a strict sense, health claims should be both implicit and explicit and scientific validation is increasingly the litmus test. There are three broad categories: inherently/naturally nutritious (e.g. wholemeal bread, soyamilk), fortified (e.g. added calcium orange juice, energy drinks) and engineered (e.g. probiotics, cholesterol-lowering spreads).

2.0 Why the sudden worldwide interest in nutraceuticals?

In recent years, there has been a vast and rapidly growing body of scientific data showing that diet plays an important part in diseases. Diet is thought to contribute to six of the 10 leading causes of death. Nutrients and nonnutritive food components have been associated with the prevention and/or treatment of chronic diseases such as cancer, coronary heart disease, diabetes, hypertension, and osteoporosis. Up to 70% of certain cancers may be attributed to diet. As the data supporting the role of diet in health promotion and disease prevention continue to mount, it is likely that the quantity of enhanced foods will expand substantially. There is an increasing demand by consumers for quality of life, which is fueling the nutraceutical revolution. Functional foods are viewed as one option available for seeking cost-effective health care and improved health status. Moreover, the large segment of the population is aging and considerable health care budget in most country is focused on treatment rather than prevention. Thus, the use of nutraceuticals in daily diets can be seen as means to reduce escalating health care costs that will contribute not only to a longer lifespan, but also more importantly, to a longer health span. Development of functional food products will continue to grow throughout the 21st century as consumer demand for healthful products grows.

3.0 Trends in functional foods & nutraceuticals

Companies involved in investigating and developing nutraceutical products mainly belong to the food (55%) and pharmaceutical (35%) industries. Strategic alliances are already in place among pharmaceutical and food companies. The behaviour of the Asian market is
usually considered to be a success. Japan since 1969 not only allows more than 24 foods for specified health use (FOSHU) but also encourages the use of functional foods. The European Union grants exclusively for innovative medical and health claims, and Brazil has recently opened for discussion a new proposal for legislation on functional foods. In Canada, however, there are many constraints and challenges faced by this new industry. No approval process exists for health claims on food, as they are currently prohibited, and no patent or equivalent protection is provided by mandatory pre-market clearance. There are no specific regulations dealing with such products, even though some have appeared on the Canadian market. In India, neutraceuticals are marketed as Indian System of Medicine (ISM) drugs under the OTC category. No clinical validation of their safety or efficacy is required if therapeutically usefulness is mentioned in the text of ISM. Sloan (2000) discussed market trends in functional foods. Some prominent trends are given here:

**Trend 1: Nutrient Horsepower**: Foods containing naturally occurring substances such as fruit and vegetable extracts and fortified foods.

**Trend 2: New youth market**: Foods enriched with calcium, zinc, iron, B vitamins, and fibre.

**Trend 3: Upgrading fun and favourite foods**: Enrichment of fun foods like pizza, cheesecake, snacks, vitamin-fortified candies, candies for dental and oral health,

**Trend 4: Tailor made**: Customized foods such as male and female personalized foods, products targeted towards preventing/tackling specific condition e.g. CVD; probiotic foods, etc.

**Trend 5: Living well**: Foods based on performance (Energy, sleeplessness, anxiety, etc.) and quality of life (weight loss, joint pain, immunity, menopause, vision, etc.)

**Trend 6: Plant performers, sea urchins, and flowers**: Foods based on whole grains, Omega 3s, herbals, lycopene, lutein, etc.

**Trend 7: The Rx/OTC interface**: Products offering symptoms relief, risk factor reduction or aid in disease management e.g. foods for relief of digestive upsets; reducing cholesterol/triglyceride, allergy symptoms, etc. The functional components used in formulation of these formulated foods are given in Table 1.

**Table 1: Examples of Functional Components**

<table>
<thead>
<tr>
<th>Class/Components</th>
<th>Source*</th>
<th>Potential Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carotenoids</strong></td>
<td></td>
<td></td>
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<tr>
<td>Beta-carotene</td>
<td>carrots, various fruits</td>
<td>neutralizes free radicals which may damage cells; bolsters cellular antioxidant defenses</td>
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<tr>
<td>Lutein, Zeaxanthin</td>
<td>kale, collards, spinach, corn, eggs, citrus</td>
<td>may contribute to maintenance of healthy vision</td>
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<tr>
<td>Lycopene</td>
<td>tomatoes and processed tomato products</td>
<td>may contribute to maintenance of prostate health</td>
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<tr>
<td><strong>Dietary (functional and total) Fiber</strong></td>
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<tr>
<td>Insoluble fiber</td>
<td>wheat bran</td>
<td>may contribute to maintenance of a healthy digestive tract</td>
</tr>
<tr>
<td>Beta glucan</td>
<td>oat bran, rolled oats, oat flour</td>
<td>may reduce risk of coronary heart disease (CHD)</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>psyllium seed husk</td>
<td>may reduce risk of CHD</td>
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<tr>
<td>Whole grains</td>
<td>cereal grains</td>
<td>may reduce risk of CHD and cancer; may contribute to maintenance of healthy blood glucose levels</td>
</tr>
</tbody>
</table>

**Fatty Acids**

<table>
<thead>
<tr>
<th>Monounsaturated fatty acids (MUFAs)</th>
<th>tree nuts</th>
<th>may reduce risk of CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyunsaturated fatty acids—Omega-3 fatty acids—ALA</td>
<td>walnuts, flax</td>
<td>may contribute to maintenance of mental and visual function</td>
</tr>
<tr>
<td>PUFAs - Omega-3 fatty acids—DHA/EPA</td>
<td>salmon, tuna, marine and other fish oils</td>
<td>may reduce risk of CHD; may contribute to maintenance of mental and visual function</td>
</tr>
<tr>
<td>PUFAs - Conjugated linoleic acid (CLA)</td>
<td>beef and lamb; some cheese</td>
<td>may contribute to maintenance of desirable body composition and healthy immune function</td>
</tr>
</tbody>
</table>

**Flavonoids**

<table>
<thead>
<tr>
<th>Anthocyanidins</th>
<th>berries, cherries, red grapes</th>
<th>bolster cellular antioxidant defenses; may contribute to maintenance of brain function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavanols—Catechins, Epicatechins, Procyanidins</td>
<td>tea, cocoa, chocolate, apples, grapes</td>
<td>may contribute to maintenance of heart health</td>
</tr>
<tr>
<td>Flavanones</td>
<td>citrus foods</td>
<td>neutralize free radicals which may damage cells; bolster cellular antioxidant defenses</td>
</tr>
<tr>
<td>Flavonols</td>
<td>onions, apples, tea, broccoli</td>
<td>neutralize free radicals which may damage cells; bolster cellular antioxidant defenses</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>cranberries, cocoa, apples, strawberries, grapes, wine, peanuts, cinnamon</td>
<td>may contribute to maintenance of urinary tract health and heart health</td>
</tr>
</tbody>
</table>

**Isothiocyanates**

| Sulforaphane | cauliflower, broccoli, broccoli sprouts, cabbage, kale, horseradish | may enhance detoxification of undesirable compounds and bolster cellular antioxidant defenses |

**Phenols**

| Caffeic acid, Ferulic acid | apples, pears, citrus fruits, some vegetables | may bolster cellular antioxidant defenses; may contribute to maintenance of healthy vision and heart health |

**Plant Stanols/Sterols**

<table>
<thead>
<tr>
<th>Free Stanols/Sterols</th>
<th>corn, soy, wheat, wood oils, fortified foods and beverages</th>
<th>may reduce risk of CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stanol/Sterol esters</td>
<td>fortified table spreads, stanol ester dietary supplements</td>
<td>may reduce risk of CHD</td>
</tr>
</tbody>
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**Polyols**

| Sugar alcohols—xylitol, sorbitol, mannitol, lactitol | some chewing gums and other food applications | may reduce risk of dental caries |

**Prebiotic/Probiotics**

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<thead>
<tr>
<th>Inulin, Fructo-oligosaccharides (FOS), Polydextrose</th>
<th>whole grains, onions, some fruits, garlic, honey, leeks, fortified foods and beverages</th>
<th>may improve gastrointestinal health; may improve calcium absorption</th>
</tr>
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<tbody>
<tr>
<td>Lactobacilli, Bifidobacteria</td>
<td>yogurt, other dairy and nondairy applications</td>
<td>may improve gastrointestinal health and systemic immunity</td>
</tr>
</tbody>
</table>

**Phytoestrogens**

<table>
<thead>
<tr>
<th>Isoflavones—Daidzein, Genistein</th>
<th>soybeans and soy-based foods</th>
<th>contribute to maintenance of bone health, healthy brain and immune function; for women, maintenance of menopausal health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignans</td>
<td>flax, rye, some vegetables</td>
<td>may contribute to maintenance of heart health and healthy immune function</td>
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<tr>
<td>Soy Protein</td>
<td>Soy Protein</td>
<td>soybeans and soy-based foods</td>
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<tr>
<td><strong>Sulfides/Thiols</strong></td>
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<td></td>
</tr>
<tr>
<td>Diallyl sulfide, Allyl methyl trisulfide</td>
<td>garlic, onions, leeks, scallions</td>
<td>may enhance detoxification of undesirable compounds; may contribute to maintenance of heart health and healthy immune function</td>
</tr>
<tr>
<td>Dithiolthiones</td>
<td>cruciferous vegetables</td>
<td>contribute to maintenance of healthy immune function</td>
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</table>

Examples are not an all-inclusive list.

### 4.0 Scientific validation of functional foods:

The scientific evidence for functional foods and their physiologically active components can be categorized into four distinct areas: (a) clinical trials, (b) animal studies, (c) experimental in vitro laboratory studies, and (d) epidemiologic studies. Much of the current evidence for functional foods lacks well-designed clinical trials; however, the foundational evidence provided through the other types of scientific investigation is substantial for several of the functional foods and their health-promoting components.

Basic examples of functional foods that fall into this realm are foods naturally rich in soluble fiber, such as oat bran or psyllium, which have been associated with reduced incidence of coronary heart disease. Other examples would be fruits and vegetables, soy protein, serols and stanol esters for which health claim regarding cholesterol reduction of coronary heart diseases (CHDs) have been approve.

Other functional foods may have a qualified health claim such as nuts or have substantial scientific support but currently lack an FDA-approved health claim. Examples include n-3 fatty acids found in fish, which have been shown in clinical trials to reduce serum cholesterol levels in subjects with elevated levels. The 2000 American Heart Association Dietary Guidelines recommend two servings of fatty fish per week for a healthy heart, and a “qualified” health claim on dietary supplements linking the consumption of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) n-3 fatty acids to a reduction of CHDs risk was recently authorized by the FDA. It states the following: “Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease. FDA evaluated the data and determined that, although there is scientific evidence supporting the claim, the evidence is not conclusive.” A “qualified” claim was authorized because of certain safety concerns regarding the consumption of high levels of n-3 fatty acids, including the following: (a) increased bleeding times, (b) increased risk for hemorrhagic stroke, (c) the formation of biologically active oxidation products from the oxidation of n-3 fatty acids, (d) increased levels of low-density lipoprotein (LDL) cholesterol, and (e) reduced glycemic control among people with diabetes. The FDA has concluded that use of n-3 fatty acid supplements is safe, provided daily intakes of EPA and DHA from food and supplements do not exceed 3 g per day.

A third category of functional foods are those that have been fortified to enhance the level of a specific nutrient or food component that has been associated with the prevention or treatment of a disease or other clinical condition. Many of these products bear authorized
health claims for product marketing. This category would include products such as calcium-fortified orange juice, pasta, or rice marketed to maintain good bone health and reduce osteoporosis risk. Many other functional foods in this category may lack sufficient evidence to warrant an authorized health claim at this time.

A fourth category of functional foods includes whole foods that have been associated with reduced risk of disease. For these whole foods, *In-vitro, in-vivo*, or epidemiological research is available to support their health benefits; however, no health claim exists, partially because of the limited or improperly designed clinical trial data or lack of scientific agreement about the strength of the evidence. This category includes the tomato products rich in lycopene, a carotenoid, whose consumption is associated with reduced cancer rates in epidemiological studies. Other examples can be black and green teas, which are rich in polyphenols, nondigestible oligosaccharides (prebiotics), fermented dairy products (probiotics) etc. For each of these, an association with reduced disease risk has been observed but has not reached scientific consensus.

Finally, there exists a growing selection of functional food components marketed under the umbrella of dietary supplements. For the majority of these products, evidence for their structure/function claims is currently limited, incomplete, or unsubstantiated. Examples include antioxidant-enriched beverages or candies, chewing gum with phosphatidylserine, and snack bars with chromium. This category also includes a large Both claims have support in controlled clinical trials. Other clinical trials on botanical-enriched products have shown conflicting results, such as the use of echinacea to reduce cold and flu symptoms or kava to reduce anxiety. Still other structure/function claims have no clear therapeutic efficacy, such as the use of ginseng for energy or enhanced physical performance. Others, such as ma huang, may be harmful. Historically, evidence for the clinical efficacy of select botanicals was limited primarily because of poor research design (e.g. inconsistency in dosage form or amount, small sample size, and frequently the lack of a placebo control, in part resulting from insufficient funding for research in this area). Yet many of these botanicals are being introduced into our food supply—sometimes irresponsibly—in the form of functional foods. Ideally, the evaluation of the efficacy of individual functional foods must be completed using a scientifically valid risk-benefit model that clearly assesses all physiologic effects, both positive and negative. Review of the in vitro, animal, epidemiologic, and clinical data is essential before functional foods or food components are marketed to consumers for their health-promoting qualities (ADA, 2004).

### 5.0 What is driving the interest in functional foods in developed countries?

Currently, functional foods market is estimated at $70 billion or 4% of processed food market and is growing at three times the rate. In developed markets, higher consumer awareness on health and wellness is being addressed through product innovations and marketing prowess of large players. While the ageing population needs more engineered foods the younger population is demanding more fortified foods to get extra energy. Health-related issues -obesity and coronary heart disease -are forcing food processors to launch campaigns to promote low carbohydrate diets or other such foods. Japan is the single largest market with per capita consumption of $140+ with the US and Europe following at $95 and
$60 respectively. Despite static or reducing population, functional foods will continue to retain the market (or grow) due to growing health concerns. Regulations have ensured the segment's organized growth.

6.0 Could India become a major supplier of nutraceuticals to the world?

India presents the biggest long-term opportunity with a minuscule per capita consumption of less than $1 per capita. Population growth, rising incomes, increasing awareness on health, urbanization, lifestyle changes ("on-the-go" eating) and growing organized retailing are contributing to the potential for functional foods. Just as for processed foods in general, India will be the largest potential markets for functional foods with their GDP growth, demographics and burgeoning consumption (with over 50% in food spend). In India, soya foods and energy drinks are starting from a zero base and will be a major category in due time. Increasing brand awareness and organized retailing will further lead customers to "real" functional foods.

Thanks to the growing acceptance of nutraceuticals, India could hope to leverage the country's key resources in this area to gain a foothold in the global market. Nutraceuticals are among the New Age drugs that are being developed to provide better health. Nutraceuticals are gaining public acceptance in many developed countries in recent times. Looking at the changing trends, the market of nutraceuticals has huge potential. These days, industries are showing Interest in the nutraceutical area. Within few years this potential can turn into a healthy growing market. The nutraceuticals or the functional foods are majorly plant-based products and most of them being predominantly herbal. Hence clues to these nutraceutical products could be got from our ancient and traditional systems of medicine like Ayurveda, Siddha and Unani. The 'Rasayan' and 'Vajikarna' therapeutics of Ayurveda are essentially nutraceuticals and therefore there is ample scope for India to develop a range of nutraceutical/health food products. And to succeed, these products have to be standardized and with scientific validation to ensure safety and efficacy so as to instill confidence in the customers to use them not as an alternative medicine but as a well defined system of medicine. For this to happen, there has to be research carried out on these products. Thus India's own traditional knowledge base gathered from Unani, Ayurveda and Siddha can help out in research work on nutraceuticals. And we can take a lead on this from the western world.

There are a lot of products sold in the name of nutraceuticals in the Indian market. Close to around 100 products are even listed on the Internet along with the global companies and around 20 Indian companies have a record of producing nutraceuticals and marketing them globally. India is relatively a new market. The size of the Indian nutraceutical market is estimated to be about Rs 1,600 crore in 2001. All major pharma players are in the process of entering this market. The level of exports from India is still small, estimated to be perhaps less than Rs 750 crore, if one excludes Psyllium. The major markets for India are the US, Europe and Japan. India can become leader in this field as we holds key expertise as well as we are rich with the biodiversity.
7.0 Key challenges for functional foods in India

In India, we have traditional products touted as functional but have little scientific validation. Regulations will thus have to evolve to weigh R&D, ensure validation and prevent exploitation of consumers. Companies will also have to be sincere and honest in their claims while marketing and communicating with consumers till appropriate regulations for scientific validation are evolved. Processors will need to provide an optimal merger between taste, convenience and health attributes. Companies will require expert knowledge in flavour masking (an example of successful flavour masking is soya flour mixed with wheat flour in India) fortification know-how and delivery systems.

8.0 Safety Issues

Although increasing the availability of healthful foods including functional foods in the diet is critical to ensuring a healthier population, safety is a critical issue. 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 isothiocyanate) highlighted in this review for their cancer-preventing properties have been shown to be carcinogenic at high concentrations (Ames et al., 1990). Thus, Paracelsus' 15th century doctrine that "All substances are poisons - the right dose differentiates a poison from a remedy" is even more pertinent today given the proclivity for dietary supplements.

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 (Rao et al., 1997). Knowledge of toxicity of functional food components is crucial to decrease the risk: benefit ratio.

9.0 Conclusion

Consumer interest in the relationship between diet and health has increased the demand for information on functional foods. Rapid advances in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims, an aging population, and rising interest in attaining wellness through diet are among the factors fueling interest in functional foods. Credible scientific research indicates many potential health benefits from food components. 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.

Health-conscious consumers are increasingly seeking functional foods in an effort to control their own health and well-being. The field of functional foods, however, is in its infancy. Claims about health benefits of functional foods must be based on sound scientific
criteria. Additional research is necessary to substantiate the potential health benefits of those foods for which the diet-health relationships are not sufficiently scientifically validated.

Research into functional foods will not advance public health unless the benefits of the foods are effectively communicated to the consumer. The communication guidelines needs to be established to ensure that research results about nutrition, food safety, and health are communicated in a clear, balanced, and no misleading manner.

10.0 Suggested Readings

1.0 Introduction

Milk is a complex biological fluid containing many components in several states of dispersion. The composition of milk determines its nutritive quality, its value as a raw material for making food products and many of its properties. Milk occupies a special position among foods, being an animal food that has a vegetarian connotation. Milk is composed of water, carbohydrate (lactose), fat, protein, minerals and vitamins. While each component can be discussed separately, it is important to remember that milk is secreted as a complex mixture of these components. The properties and importance of milk are greater and more complex than the sum of its individual component parts. The basic components of milk from different species are shown in Table 1. Since milk is secreted by the female of all mammalian species primarily to meet the complete nutritional requirements of the neonate, the gross composition of milk shows large inter-species differences which reflect these requirements. Significant variations from the average values can be observed and these are influenced by the breed and species of the milk animals, feeding, maintenance, lactation period as well as health and age of the animals.

Table 1. Chemical composition of milk of different species.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Species</th>
<th>Cow</th>
<th>Buffalo</th>
<th>Goat</th>
<th>Sheep</th>
<th>Camel</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/100 g)</td>
<td></td>
<td>75</td>
<td>105</td>
<td>74</td>
<td>118</td>
<td>81</td>
<td>68</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td></td>
<td>13.0</td>
<td>16.5</td>
<td>12.3</td>
<td>18.8</td>
<td>13.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Fat (%)</td>
<td></td>
<td>4.0</td>
<td>6.7</td>
<td>4.5</td>
<td>7.4</td>
<td>4.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Total Protein (%)</td>
<td></td>
<td>3.4</td>
<td>4.5</td>
<td>2.9</td>
<td>5.5</td>
<td>3.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Casein (%)</td>
<td></td>
<td>2.8</td>
<td>3.9</td>
<td>2.5</td>
<td>4.6</td>
<td>2.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Whey Protein (%)</td>
<td></td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.9</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td></td>
<td>4.8</td>
<td>4.5</td>
<td>4.1</td>
<td>4.8</td>
<td>5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td></td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>1.0</td>
<td>0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Adapted from: Spreer, 1995; Miller et. al., 1999 and Sahai, 1996.

Milk also serves a number of physiological functions, probably the most important of which are protective. Proteins and peptides serve most of these functions. The physiologically important proteins and peptides include immunoglobulins, enzymes, enzyme inhibitors, growth factors, hormones and anti-bacterial agents. The nutritional value of milk as a whole is greater than the value of its individual nutrient because of its unique nutritional balance. The
nutritive value of milk can be assessed by (1) the energy it supplies, (2) its content of essential nutrients (fatty acids, amino acids, minerals, and vitamins), (3) the digestibility and absorbability of its nutrients, and (4) its content of harmful allergens, protease inhibitors, and toxins. Nutritive value can be evaluated by analysis for constituents or by feeding trials to determine the extent to which growth is supported, nutrients are retained or health is maintained.

2.0 Energy

The energy content (calorie) of milk and other dairy foods varies widely and depends mostly on the fat content of the milk. Gross energy supplied by milk can be calculated from its fat, protein, and lactose content. For example, full cream (6.0% fat) milk provides 95 kcal per 100 g serving; toned (3.0% fat) milk provides 65 kcal per 100 g serving; double toned (1.5% fat) milk provides 50 kcal/100 g servings; and non fat (skim) milk provides 40 kcal/100 g servings. Milk is considered to be a food of high nutrient density, providing a high concentration of nutrients in relation to its energy content, thus, making it especially valuable for people who do not eat large amounts of food, including young children, elderly people and slimmer. There is no scientific evidence that the intake of dairy foods contributes to overweight.

3.0 Milk Fat

Milk fat is composed of a number of different lipid classes, but triacylglycerol represent about 97 to 98% of the total lipid. Phospholipids, cholesterol esters, diacylglycerols, monoacylglycerols and free fatty acids make up the remaining 2% to 3%. In addition, there are trace amounts of ether lipids, hydrocarbons, fat-soluble vitamins, flavour compounds (lactones and hydroxyl- and keto-acids) and compounds introduced from feed such as β-ionone and gossypol, which have physiological properties. Milk fat triacylglycerols are synthesized from more than 400 different fatty acids, which makes milk fat the most complex of all natural fats. The fatty acid composition of bovine milk fat is characterized by a high proportion of saturated fatty acids (60 to 70%), appreciable amounts of monounsaturated fatty acids (25 to 35%), and small amounts of polyunsaturated fatty acids (4%). Milk fat has a relatively high content of short chain fatty acids with four to eight carbon atoms (e.g. butyric and caprylic), many of which are not found in other natural food fats. It is characterized not only by the kind and amount of fatty acids, but also by the distribution of fatty acids on the glycerol moiety. The fatty acids in milk fat triglycerides are not haphazardly distributed, but are arranged so that the short chain fatty acids, mainly butyric and caproic, occur in outer position, and long chain fatty acids such as myristic acid are found in position two. About 95% of the short chain fatty acid, butyric acid, occupies position one and three, whereas 54% of the long chain fatty acid, myristic acid, is found in position 2 in the triacylglycerol. This arrangement is thought to contribute to the ease of digestibility of milk fat compared with some other fats, especially those with a large proportion of triacylglycerols, each containing three fatty acid with 18 carbons. The short chain fatty acids present in milk fat, but virtually absent in most vegetable oils, are absorbed through the intestinal wall without being resynthesized to glycerides. They are then
transported in the portal vein directly to the liver, where they are immediately converted to utilizable forms of energy. They serve, therefore, as a quick source of energy, which may be important, especially in early life. In contrast, the long chain fatty acids undergo a much more complex chain of reactions during digestion, absorption, and transport.

4.0 Milk Protein

The proteins supply the amino acids, the building blocks from which tissue proteins are synthesized. This assists in the growth of children and makes possible the replacement of protein, used in metabolism or lost by injury. Fluid milk contains approximately 3.5% protein. Casein, found only in milk, comprises about 82% of the total milk protein. Whey proteins, principally β-lactoglobulin and α-lactalbumin, constitute the remaining 18%. Casein, because of its excellent nutritional value, is used routinely as a reference protein to evaluate the quality of protein in other foods. Proteins in cow’s milk are not only present in significant amounts but are of exceptionally high quality. The nutritional quality of dietary proteins depends largely on the pattern and concentration of essential amino acids provided for the synthesis of nitrogen-containing compounds within the body. The high quality of milk protein stems from the fact that it contains, in varying quantities, all of the amino acids required by humans. Moreover, the pattern of distribution of amino acids in milk protein resembles that needed by humans. As shown in Table 2, there are numerous methods of evaluating the quality of proteins under experimental conditions, all of which indicate milk proteins to be of high quality.

| Table 2: Average measure of protein quality form milk and milk proteins |
|-------------------------------+-------+-------+-------+-------+-------|
|                               | B     | PD    | NPU   | PER   | PDC   |
| -----------------------------+-------+-------+-------+-------+-------|
| Milk                         | 9     | 95    | 86.45 | 3.1   | 1.21  |
| Casein                       | 7     | 100   | 76    | 2.9   | 1.23  |
| Whey Proteins                | 1     | 100   | 92    | 3.6   | 1.15  |

*The BV of egg protein is defined as 100
(Adapted from: Miller et.al., 1999)

Whey proteins are slightly superior to casein because of the limiting quantity of the total sulfur-containing amino acids (methionine and cysteine) in casein. However, because whey proteins have a relative surplus of these amino acids, casein and whey proteins, as found in milk complement each other. Both casein and whey proteins have a relative surplus of the essential amino acids lysine, threonine, methionine, and isoleucine, which make milk proteins valuable in supplementing vegetable proteins, particularly those of cereals, which are limiting in these amino acids.

5.0 Milk Carbohydrates
Lactose accounts for about 54% of the total solids-not-fat content of milk and contributes about 30% of the energy (calories) of whole milk. Cow’s milk contains about 4.8% lactose compared to 7% in human milk. Lactose is not transported across the intestinal membranes unless hydrolysed to its monosaccharide components; glucose is taken up more readily than galactose. Lactose is cleaved to glucose and galactose in the intestine of the neonate by an enzyme called lactase (or β-galactosidase). The galactose is then converted to glucose by a different enzyme. Lactose is a major, readily digestible source of glucose, which provides energy for the neonate. In infants, some lactose enters the distal bowel (colon) where it promotes the growth of certain beneficial lactic-acid-producing bacteria which may help combat gastrointestinal disturbances resulting from undesirable putrefactive bacteria. In addition lactose enhances the passage of certain metallic elements across the brush border membrane of the intestine, especially the ileum. Monosaccharides and disaccharides that are hydrolysed before reaching the small intestine do not enhance absorption. The enhancing effect of lactose has been noted in vivo and in vitro experiments, mostly with laboratory rats and gut preparations from them. The effect has been observed with Ca, Ba, Sr, Rb, Mg, Fe, Co, Zn and Pb. It is considered to be an important supplement to vitamin D in facilitating absorption of Ca. The mechanism by which lactose operates in this phenomenon is not known but probably involves an alteration in structure of the brush border membrane rather than formation of lactose-metal complexes. Although, it is well documented that lactose favours the absorption of calcium and perhaps phosphorus in infants, there is no scientific evidence that lactose improves calcium absorption in adults.

Minor quantities of glucose, galactose, and oligosaccharides are also present in milk. One outstanding difference between human and cow’s milk is the much greater content of oligosaccharides in the former. About thirteen of them have been isolated from human milk, ranging from three to fourteen saccharide units per molecule. All contain reducing terminal glucose; the other monosaccharide components are galactose, fucose, N-acetyl glucose, N-acetyl galactose, and N-acetyl neuramic acid. The total concentration of these oligosaccharides is of the order of 10 g/L in mid lactation and 25 g/L in colostrums. By contrast cow’s milk contains about 1 g of oligosaccharide per litre. Several of the human milk oligosaccharides, especially certain N-containing tetra- and pentasaccharides, promote the growth of some strains of Bifidobacterium bifidum. They often are cited as possible controllers of the intestinal flora of breastfed infants.

### 6.0 Minerals

Minerals are important buffer constituents and contribute strongly to maintenance of pH, ionic strength, and osmotic pressure in body fluids and tissues. Milk and dairy products are a good source of most of these minerals, but particularly of calcium and phosphorus, which are important in the formation of teeth and bones. Concentrations of minerals in human and cow’s milks and human daily requirements are given in Table 3 and Table 4. For optimum utilization by the body, calcium and phosphorus must be present in a ratio of 1.3:1. Milk contains these minerals in this ideal ratio and can meet almost the entire requirements of a growing child, and for most other age groups (Mathur et al., 1999). Milk and milk products
are a useful source of zinc, particularly for those who do not eat meat. Zinc is essential for general good health and boosting the immune system (Bourne, 1999).

Table 3. Macrominerals of milk of different species

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Contents in milk (mg/100 g fluid milk)</th>
<th>Requirements for an adult (mg/day)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow milk</td>
<td>Buffalo milk</td>
</tr>
<tr>
<td>Calcium</td>
<td>119</td>
<td>169</td>
</tr>
<tr>
<td>Chlorine</td>
<td>105</td>
<td>75</td>
</tr>
<tr>
<td>Magnesium</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>93</td>
<td>117</td>
</tr>
<tr>
<td>Potassium</td>
<td>152</td>
<td>178</td>
</tr>
<tr>
<td>Sodium</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>Citrate</td>
<td>175</td>
<td>180</td>
</tr>
</tbody>
</table>

* Adapted from Gopalan et. al.,(1993) .
**US recommended allowance, Adapted from ( Walstra and Jenness,1984)

Apart from the major minerals like calcium, magnesium, phosphorus, potassium, sodium, chloride and sulphur present in relatively high proportion, milk also contains number of trace elements present in ppm quantities. The role played by these trace elements should not be underestimated, since nutritionally, they are essential at low levels. Cobalt forms the centre of vitamins B_{12} molecules and is essential for its synthesis. Iron is essential for the hemoglobin and hence its deficiency could cause anaemia. Iodine is a constituent of thyroxine and plays an important physiological role. Iodine deficiency causes enlargement of the thyroid gland resulting in goiter. Selenium is required in trace quantities but high concentrations are toxic. Milk is a good source of zinc, nickel and molybdenum which are essential for certain specific enzyme activities. Milk is poor in Fe and neither cow’s nor human milk furnishes enough for human infants. Fortunately, infants have a considerable liver store of Fe at birth. Intestinal absorption of Fe is greater from human that from cow’s milk although variations among individual infants are large. The superior absorption may be related to the higher contents of lactoferrin, lactose and ascorbate and the lower phosphate content of human milk.

Table 4. Trace elements of milk of different species

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Contents in milk (µg/100 g fluid milk)</th>
<th>Requirements for an adult (µg/day)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow milk</td>
<td>Buffalo milk</td>
</tr>
<tr>
<td>Aluminum</td>
<td>46</td>
<td>-</td>
</tr>
<tr>
<td>Boron</td>
<td>27</td>
<td>52 – 145</td>
</tr>
<tr>
<td>Bromine</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>Chromium</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.06</td>
<td>0.69 – 1.61</td>
</tr>
<tr>
<td>Copper</td>
<td>13</td>
<td>7 – 21</td>
</tr>
<tr>
<td>Fluorine</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Iodine</td>
<td>4.3</td>
<td>-</td>
</tr>
<tr>
<td>Iron</td>
<td>20</td>
<td>42 – 152</td>
</tr>
</tbody>
</table>
### 7.0 Vitamins

Vitamins play an important role in intermediary metabolism as cofactors in numerous enzymatic or non-enzymatic physiological functions such as the visual process (vitamin A), as antioxidants (carotenoids, vitamin E, vitamin C and riboflavin), in regulation of calcium metabolism (vitamin D) and in haematopoiesis (vitamin B₁₂, folate and vitamin B₆). Milk is a very rich source of all the vitamins (Table 5) and majority of the vitamins appear to have high nutrient density (the ratio between nutrient content per joule and recommended intake per joule), which exceeds 100%. This means that these vitamins in milk have the potential to compensate for low concentration in other (non dairy) foods. For vegetarian people milk is the only source of vitamin B₁₂ as it is found exclusively in foods of animal origin. Certain vitamins (folic acid, vitamin B₁₂ and riboflavin) in milk are bound to special proteins and studies have shown that such arrangement increases the bioavailability of these vitamins.

**Table 5. Levels of vitamins in milk and their recommended dietary allowances (RDA)**

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Levels in milk (per litre)</th>
<th>RDA per day for Indians*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow milk</td>
<td>Buffalo milk</td>
</tr>
<tr>
<td>Vitamin A (I.U.)</td>
<td>1590</td>
<td>2000</td>
</tr>
<tr>
<td>Vitamin D (I.U.)</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>1.0</td>
<td>0.33</td>
</tr>
<tr>
<td>Vitamin K (µg)</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B₁ (µg)</td>
<td>450</td>
<td>580</td>
</tr>
<tr>
<td>Vitamin B₂ (µg)</td>
<td>1750</td>
<td>1430</td>
</tr>
<tr>
<td>Niacin (µg)</td>
<td>900</td>
<td>1280</td>
</tr>
<tr>
<td>Vitamin B₆ (µg)</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>Pantothenic acid (µg)</td>
<td>3500</td>
<td>2400</td>
</tr>
<tr>
<td>Biotin (µg)</td>
<td>35</td>
<td>106</td>
</tr>
<tr>
<td>Folic acid (µg)</td>
<td>55</td>
<td>42</td>
</tr>
</tbody>
</table>

Note: P = Poisonous; 0 = no physiological role; ? = physiological role questionable; +? = probably has physiological role; + = indispensable but requirements not known

* Adapted from Gopalan et al., (1993)
** US Recommended intakes for adults (Adapted from Gurr, 1988)
Vitamin B\textsubscript{12} (\(\mu\)g) & 4.5 & 3.6 & 0.4 & 1 \(\mu\)g  
Vitamin C (mg) & 20 & 21 & 45 & 40 mg  

* Adapted from Gopalan et. al., 1993  
* Not fully established. Given values are considered adequate.

### 8.0 Milk Products in human nutrition

Milk is probably the most adaptable and flexible of all food material and the manufacture of milk products influences nutritive value by separation, dilution, or concentration of the components of milk, by addition of nonmilk ingredients, and by some effects of processing treatments. Many of the processes to which milk is subjected cause major changes in the composition (Table 6), physical state and sensory attributes of the product. The main processing operation that may cause loss of nutrient is heat treatment. To be sure, mild heat treatments like pasteurization or even UHT heating cause very little change in nutritive value. The proteins can be affected in that serum proteins are denatured (which has no effect on nutritional quality), and casein loses its propensity to clot in stomach (which loss may be advantageous in, at least to infants). There is some loss of lysine from Maillard reaction, but this has little effect on the nutritional quality because milk protein is comparatively rich in lysine; the same holds for Maillard reactions during long storage of sterilized products. Very intensive heating causes some loss of digestibility and biological value, presumably because of cross-linking reactions. Though it has been considered possible that some of the reaction products may be toxic to humans, no such toxicity has ever been ascertained, at least for heat treatments as occur during the manufacture of milk products.

#### Table 6. Approximate composition (%) of some dairy products

<table>
<thead>
<tr>
<th>Product</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Sugars*</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream</td>
<td>45.45</td>
<td>1.69</td>
<td>50.00</td>
<td>2.47</td>
<td>0.37</td>
</tr>
<tr>
<td>Butter</td>
<td>15.9</td>
<td>0.85</td>
<td>81.11</td>
<td>0.06</td>
<td>2.1</td>
</tr>
<tr>
<td>Anhydrous butter oil (ghee)</td>
<td>0.2</td>
<td>0.3</td>
<td>99.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ice cream</td>
<td>60.8</td>
<td>3.6</td>
<td>10.8</td>
<td>23.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Evaporated whole milk</td>
<td>74.0</td>
<td>6.8</td>
<td>7.6</td>
<td>10.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Sweetened condensed milk</td>
<td>27.1</td>
<td>7.9</td>
<td>8.7</td>
<td>54.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Whole milk powder</td>
<td>2.5</td>
<td>26.3</td>
<td>26.7</td>
<td>38.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Skimmed milk powder</td>
<td>3.2</td>
<td>36.2</td>
<td>0.8</td>
<td>52.0</td>
<td>7.9</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>36.7</td>
<td>24.9</td>
<td>33.1</td>
<td>1.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>40.0</td>
<td>23.2</td>
<td>30.0</td>
<td>1.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Khoa</td>
<td>20.2</td>
<td>16.70</td>
<td>29.00</td>
<td>30.10</td>
<td>5.20</td>
</tr>
<tr>
<td>Burfi</td>
<td>15.10</td>
<td>16.20</td>
<td>20.50</td>
<td>45.80</td>
<td>2.40</td>
</tr>
<tr>
<td>Channa</td>
<td>53.4</td>
<td>17.4</td>
<td>24.8</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Paneer</td>
<td>50.72</td>
<td>17.99</td>
<td>27.13</td>
<td>2.29</td>
<td>1.87</td>
</tr>
<tr>
<td>Shrikhand</td>
<td>40.52</td>
<td>5.19</td>
<td>4.50</td>
<td>43.57</td>
<td>0.530</td>
</tr>
</tbody>
</table>

*Total carbohydrate.
Some vitamins can be destroyed partly by heating (Table 7). Experimental data on such losses are widely variable. The variation is partly the result of the conditions during heating being different, especially oxygen content as many of the reactions involved are oxidations. Also the presence of catalysts, like copper for vitamin C oxidation, affect the result. Changes during storage show similar variations. The oxidation of ascorbic acid plays a key role. Vitamin C itself is more heat-labile in the dehydroascorbic acid form. Folic acid is protected by vitamin C. Oxidation products of vitamin C seem to attack vitamin B₁₂. Keeping the oxygen level low, particularly during heating but also during storage, minimizes loss of vitamins C, B₁, B₁₂ and folic acid. Protecting milk from exposure to light prevents loss of vitamins B₂.

Table 7. Approximate loss (%) of some nutrients in milk by heating

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Pasteurization</th>
<th>Boiling</th>
<th>UHT Heating</th>
<th>Sterilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available lysine</td>
<td>&lt; 1</td>
<td>~ 5</td>
<td>&lt; 1</td>
<td>2 -10</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>~ 5</td>
<td>5 – 10</td>
<td>5 -10</td>
<td>20 – 50</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>~ 0</td>
<td>&lt; 5</td>
<td>&lt; 10</td>
<td>5 – 20</td>
</tr>
<tr>
<td>Folic acid</td>
<td>&lt; 5</td>
<td>~ 5</td>
<td>&lt; 20</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0 -10</td>
<td>5 – 20</td>
<td>5 – 20</td>
<td>20 – 80</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5 – 20</td>
<td>5 – 50</td>
<td>5 – 30</td>
<td>&gt; 50</td>
</tr>
</tbody>
</table>

Dried milk products can be stored for a long time without much loss of nutrients. There may be loss of vitamins because of oxidation. Maillard reaction may proceed to a considerable extent if the powder is allowed to take up water.

Fermented milks of numerous types and names exist. Most result from the action of lactic acid bacteria on milk (e.g. dahi, cultured yoghurt) or concentrated milk (yoghurt, shrikhand misti dahi); buttermilk derives from the churning of cultured cream. Some fermented milks contain other microorganisms: kefir and kumiss contain yeasts producing alcohol and some may contain molds. Fermented milks often are claimed to be particularly healthful. In some conditions, fermented milks may be preferable because they do not readily propagate infectious diseases; the low pH prevents growth or causes killing of pathogenic microorganisms. It often has been suggested that bacteria fermenting the milk may be implanted in the large intestine after ingesting the fermented milk. The low pH of fermented milks may be advantageous, particularly to infants, as it diminishes the buffer action in the stomach. Probably the absorption of calcium is also enhanced. Fermented milks have lower lactose content, and this is advantageous to lactose intolerant consumers. Fermentation affects the content of nutrients, notably B vitamins; some may increase (e.g. niacin, folic acid), others decreases (e.g. vitamin B₁₂), but the changes are rather variable. Changes in energy content or essential amino acids because of fermentation are negligible.

9.0 Conclusion

Milk occupies a special position among the many foods of vegetable and animal origin because it is the sole food for human (as for all mammals) during the first part of their
lives. Milk has therefore to contain everything the young individual needs for the growth and development, in particular a sufficient concentrations of proteins and minerals.

Although milk is no longer the only food for the pre-school child, the school child and the adolescent, it still has important place in the diet of the young because, together with milk products, it contributes significant to the supply of nutrients necessary during this period of development. Milk and milk products are also an important part of a balanced diet for adults not only because milk contains all the nutrients, but because it is rich in those constituents which are important for adult nutrition as well. Without milk and milk products the requirement for some of these nutrients would be difficult, if not impossible to meet. It may be mentioned in this connection that different foods, when combined in the right proportion, can complement each other to furnish the ideal balanced diet. Milk is, of instance, a good complement to vegetable foods.

It may well be that the importance of milk and milk products for nutrition is generally well known, but consumers often do not make use of this knowledge for reasons quite unconnected with any views on nutrition. The great differences in milk consumption which exist from one country to another stem from psychological sources apart from marketing policies. Organoleptic criteria may override nutritional knowledge in determining consumption of milk and milk products and it is therefore that these aspects of milk foods be considered in addition to their nutritional value.

10. Suggested Readings

1.0 Introduction

An increasing amount of scientific evidence confirms that many chronic diseases such as cancer, osteoporosis, coronary heart disease and hypertension are connected to unbalanced diet. Among the main foodstuffs, milk has been identified to be of particular interest with regard to alimentation and health. Milk is a complete food containing protein, fat, lactose, vitamins and minerals, as well as enzymes, cells, hormones and immunoglobulins. It is the exclusive source of nutrients for the young and it also represents a high-grade source of dietary nitrogen and indispensable amino acids for adults. Apart from the nutrition, various physiological roles of various milk components have been identified. Milk components including lactoferrin, vitamin B12-binding protein, folate-binding protein, β-lactoglobulin, α-lactalbumin and casein phosphopeptides are assumed to interact with either minerals or vitamins absorption. Immunoglobulins, enzymes (lysozyme, lactoperoxidase) and other proteins or derived peptides can also contribute to passive protection against infection by a growth or inhibiting activity on bacterial strains and by an antiviral effect. Moreover, some milk substances can modulate digestive and metabolic processes by modulating gastrointestinal hormones and enzyme secretions or by acting on gastrointestinal motility as opioid or anti-opioid substances. Some casein-derived peptides have been identified as angiotensin-converting-enzyme (ACE) inhibitors that could result in an anti-hypertensive effect. Other peptides isolated from bovine κ-casein and human lactoferrin have shown to inhibit platelet function and could have antithrombotic properties. The other milk components including lactoferrin, prolactin, IGF-1 or casein derived peptides can contribute to active immunity and can act as growth factors on different cell types, tissues and organs. This article delineates the current thoughts on therapeutic potential of various milk components.

2.0 Biological Functional Proteins Of Milk

The primary function of the protein in milk is to provide essential amino acids, the balance of which is nutritionally fit for the newborn of each species. Furthermore, some milk proteins facilitate uptake of several important nutrients such as trace elements and vitamins. Lactoferrin, the major iron-binding protein of human milk, effectively delivers iron for intestinal absorption via a brush border membrane receptor. Vitamin B12-and folate-binding proteins are assumed to deliver these vitamins to the infant by a specific mechanism also. The biological significance of milk proteins has been summarized in Table 1.

Many bioactive milk proteins appear to have evolved to facilitate gastrointestinal health. By using a wide array of mechanism, various milk proteins contribute to the different aspects of gastrointestinal health. Kappa-casein, glycomacroepitide and lactoferrin provide oligosaccharide “decoy” that mimic structures in the intestinal mucosa and thereby prevent...
bacterial adhesion. Immunoglobulins can directly bind and neutralize bacteria and viruses and make them non-infectious. Lysozyme can directly degrade the cell wall of bacteria and lactoperoxidase can kill bacteria by the production of hypochlorite. Haptocorrin, the vitamin B₁₂-binding protein, and lactoferrin, which binds iron, can inhibit the growth of bacteria by making these micronutrients, which are essential for many bacteria, unavailable by binding them very tightly. α-Lactalbumin may also protect against pathogens, possibly by the release of peptides affecting immune function (Lonnerdal, 2002). The function of β-lactoglobulin is thought to be related to the binding and transport of retinol because it shows structural homology to serum retinol-binding protein (Hambreus and Lonnerdal, 2003). It has also been reported that β-lactoglobulin is also able to bind hydrophobic molecules, such as palmitic, oleic and myristic acid (Perez et al., 1989).

Table 1. Approximate protein composition of cow’s milk and human milk

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration (g/L)</th>
<th>Biological functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow milk</td>
<td>Human milk</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>26.0</td>
<td>Formation of micelle and curd. Precursor of various bioactive peptides</td>
</tr>
<tr>
<td>Fat Globule membrane</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Whey proteins</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>1.2</td>
<td>B-subunit of lactose synthatase complex</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>3.2</td>
<td>Aids the absorption of retinol</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.4</td>
<td>Binds Fatty acids</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>0.7</td>
<td>Antigen-antibody reaction</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>&lt; 0.1</td>
<td>Antimicrobial action, promoter of iron absorption, stimulator for growth of some kind of animal cell (as carrier of iron)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>~ 10⁻⁴</td>
<td>Lysis of bacterial cell wall</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>~ 33.0</td>
<td>~ 9.0</td>
</tr>
</tbody>
</table>

Adapted from: Yamauchi, K (1992)

3.0 Dairy Foods And Cardiovascular Health

In the past, much interest has centered on dietary lipids in relation to atherosclerosis and coronary heart disease. Among the several factors that seem to be correlated with high incidence of this condition, is the elevated cholesterol content of the blood serum. Part of the cholesterol in animal comes from the diet, but a large part is synthesized internally. Dietary long chain fatty acids (> C10) tend to elevate and polyunsaturated fatty acids to lower cholesterol levels. Cow’s milk fat contains 300 - 350 mg cholesterol per 100 g fat and has a low ratio of polyunsaturated to long-chain saturated fatty acids of about 0.1. For these reasons, restriction or exclusion of milk fat from the diet sometimes has been recommended. However, it is important to realize, that people do not consume cholesterol, saturated fat or
individual fatty acids as single entities. Rather, these are consumed as milk fat, which itself is a constituent of various dairy foods. Milk fat and dairy foods contain components that may be cardioprotective, such as sphingolipids and conjugated linolenic acid, which may have antiatherogenic properties independent of serum lipid modulation. Dairy products are the most important contributor of calcium to the diet. Calcium can lower low density lipoprotein (LDL) cholesterol in serum and increase high density lipoprotein (HDL) cholesterol levels, reduce blood pressure and provide protection against obesity. Enzymatic proteolysis of milk proteins produces peptides with antihypertensive properties and fermented milk high in these bioactive peptides lowers both systolic and diastolic blood pressure in hypertensive subjects. Milk products offer significant amounts of folate, vitamin B₆ and vitamin B₁₂. These B vitamins can lower serum homocysteine levels. It is now known that an elevated serum homocysteine level (hyperhomocysteinemia) is a strong independent risk factor for coronary artery disease. Homocysteine promotes endothelial dysfunction and damage, an initial step in the development of atherosclerosis, followed by a number of events that leads to platelet activation and thrombus formation.

4.0 Immunomodulatory Properties Of Milk

The immune system plays a central role in host protection against bacterial, viral, parasitic and fungal infections, and also cancers. Deficiencies in any aspect of the immune system can predispose an individual to a greater risk of infection and may enhance the severity of a disease. Several studies have shown that milk contains a range of immunological factors that help the newborn in its defense against infectious diseases until its own immune system becomes fully competent. The lower incidence of infectious illness in breast-fed infants compared with formula-fed infants is well documented. The immune system employs both non-specific and specific immune responses to confer protection against disease. Non-specific components of the host defense include physicochemical barriers (for example skin, mucus, lysozyme, complement, interferons), natural killer cells and phagocytic cells (e.g. neutrophils, monocytes/macrophages). Specific immune responses are mediated by antibodies (e.g. IgA, IgG, IgM, IgD, IgE produced by B-lymphocytes) and T-lymphocytes (e.g helper, suppressor and cytotoxic lymphocytes).

Several in vivo and in vitro studies have shown the presence of factors in bovine milk that are able influence a number of aspects of specific and non-specific host immune response (Table 2). For example, milk proteins and peptides have been shown to modulate a variety of immune functions (antibody responses, T-cell responses, phagocyte functions and natural killer-cell activity) and enhance resistance to disease (bacterial infections, cancers). Components of bovine milk fat (e.g. conjugated linolenic acid) have also been suggested to exhibit potent immunomodulatory properties. A role for milk components in the development/maturation of the immune system of the neonate has also been suggested. The precise mechanism by which milk components impacts on the immune system are not fully understood. Some studies have shown that inclusion of immunomodulatory milk components into dairy products can be used to optimize immune performance in humans (Gill and Rutherford, 1998).
Table 2. Milk components with immunomodulatory properties

<table>
<thead>
<tr>
<th>Whey protein concentrate</th>
<th>Hormones/growth factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Lactalbumin</td>
<td>Casein and casein fractions</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>Whey/casein derived peptides</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Nucleotides</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>Milk fat</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Glycoproteins/glycopeptides</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>Vitamins and minerals</td>
</tr>
<tr>
<td>Complement</td>
<td>Leukocytes</td>
</tr>
<tr>
<td>Cytokines</td>
<td></td>
</tr>
</tbody>
</table>

*Source: Gill and Rutherfurd, (1998)*

5.0 Role of Dairy Foods In Weight Management

Now evidences are there that dairy products may be part of the solution, not part of the problem for obesity. Several studies have demonstrated an inverse effect between dairy foods/calcium intake and body weight. Recent clinical studies have indicated that three servings of dairy foods per day in a reduced calorie diet may help accelerate body weight and, in particular, body fat loss when compare to a calorie-restricted diet low in dairy foods. The addition of calcium via supplements has an intermediary effect. Of importance is the loss of fat around the trunk resulting from higher dairy diets with concomitant reductions in blood pressure, plasma insulin and glucose. Clinical studies also indicate that under weight maintenance conditions, increased dairy product consumption can significantly reduce body fat. Animals and in vitro studies have provided a plausible mechanism on how dairy foods may be exerting their effect on body composition. Low dairy foods/calcium intake results in the flow of calcium into fat cells as a result of increased 1α,25 (OH)2-D3 and other calcium regulating hormones. Higher adipocyte calcium concentrations result in an up-regulation of lipogenic pathways while down regulating lipolytic pathways in the adipocyte. Diets high in dairy products have an opposite effect (DiRienzo, 2003).

6.0 Milk Components And Bone Health

Dairy product consumption has long been associated with good bone health. Calcium is the nutrient in dairy products most often connected to bone because 99% of the body’s calcium resides in the skeleton. However, consumption of many nutrients in dairy products in addition to calcium significantly correlates with bone mineral density of the hip and spine (Table 3). In the past, research has focused upon the calcium content of fluid milk as of critical importance to the growth and maintenance of a strong skeleton. More recently, other milk nutrients, including bioactive peptides, which affect osteoblasts and osteoclasts directly, have been identified. Studies of bone health in children who habitually avoid milk show that they have low calcium intakes and poor skeletons. They tend to be short and overweight, and they fracture more bones at a young age than milk-drinkers. Recent work from a population-based sample of US women also suggests that adults who drank milk daily as children break fewer bones late in life than those who drank milk frequently before puberty (Goulding, 2003).
Table 3. Components of milk with relevance to bone metabolism

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Essential for growth and matrix production; consumption elevates IGF-1 levels which act to increase bone mass and enlarge the circumference of bones</td>
</tr>
<tr>
<td>Minerals (especially Ca, P, K, Mg)</td>
<td>Components of the mineral phase; influence hormones of PTH/vitamin D/Calcitonin cascade; affect the absorption of and excretion of other elements; act as co-factors for enzyme and hormone secretion, e.g. Mg for PTH</td>
</tr>
<tr>
<td>Lactose</td>
<td>The main sugar in milk – contributes energy and is claimed to augment alimentary Ca absorption</td>
</tr>
<tr>
<td>Lipids</td>
<td>Contribute energy/cell wall stability</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Vitamin D has multiple actions on calcium metabolism</td>
</tr>
<tr>
<td></td>
<td>Vitamin K is an important co-factors for osteocalcin</td>
</tr>
<tr>
<td></td>
<td>Vitamin C is essential for bone matrix synthesis</td>
</tr>
<tr>
<td>S-amino acids</td>
<td>Increased endogenous acid load may raise the need for skeletal buffering</td>
</tr>
<tr>
<td>Essential trace elements</td>
<td>May influence mineral stability e.g. Fluoride. Co-factors for enzymes/hormones e.g. Magnesium for PTH, Zinc for growth hormone, Iodine for thyroid function</td>
</tr>
<tr>
<td>Cytokines and bioactive</td>
<td>Some augment osteoblastic activity e.g. lactoferrin; others inhibit osteocalstic activity and bone resorption, e.g. osteoprotegerin, basic milk protein, cystatin C</td>
</tr>
<tr>
<td>fragments in milk can cross</td>
<td>the gut wall and influence bone cell function directly</td>
</tr>
</tbody>
</table>

IGF-1 = insulin-like growth factor-1; PTH = parathyroid hormone
Adapted from: Goulding, (2003)

7.0 Dairy Components In Oral Health

Dental caries (tooth decay) is the localized destruction of tooth tissue initiated by specific dental plaque bacteria that ferment dietary sugar to organic acids. Even though in most developed countries the prevalence of dental caries has decreased through the use of fluoride, the disease remains a major health problem. A substantial volume of literature now exists demonstrating an anticariogenic effect of dairy products (milk, milk concentrates, powders and cheeses) in laboratory animals and human in situ caries models. This anticariogenic effect has been attributed to the multiphosphoseryl-containing sequences of casein and their ability to stabilize calcium phosphate. These sequences can be released enzymically as casein phosphopeptides (CPP), which stabilize amorphous calcium phosphate (ACP) in solution by the formation of CPP-ACP nanocomplexes. The CPP-ACP localize at the tooth surface and prevent demineralization and promote remineralization of enamel subsurface lesions. CPP-ACP is also being used as an ingredient (Recaldent™) in oral care products (Reynods, 2003).
8.0 Dairy Foods And Hypertension

Hypertension, defined as a blood pressure equal to or greater than 140 mm Hg systolic (contracting) and/or 90 mm Hg diastolic (resting). The prevalence of this common chronic disease increases with age and uncontrolled high blood pressure increases the risk for coronary heart disease (CHD), stroke, cardiac failure, and kidney disease. Since the early 1980s, a considerable body of evidence has accumulated from investigations in experimental animals, epidemiological studies, and clinical intervention trials in humans to support a beneficial role for calcium or calcium-rich foods such as milk and other dairy foods in blood pressure control. Apart from calcium, scientific evidence indicates that an adequate intake of potassium and magnesium, the nutrients found simultaneously in meaningful amounts in dairy foods, has a positive effect on blood pressure and helps to reduce the risk of hypertension. Calcium supplements are not advised to reduce risk of hypertension. Rather, food sources of calcium such as dairy foods are recommended because, in addition to calcium, they provide other nutrients such as potassium and magnesium which protects against hypertension. Apart from this, consumption of dairy products also provides a number of hypertensive peptides which may be derived from various milk protein fractions either during digestion or fermentation (Miller et al., 2000).

9.0 Bioactive peptides from milk protein and their physiological effect

Milk proteins contain a great number of encrypted bioactive peptide sequences ranging in size from only two residues through to large oligopeptides with more than 50 residues. These bioactive peptides are released from the native protein either during the process of digestion, or as a result of food processing including bacterial fermentation. The liberated bioactive peptides can then exert their physiological effect either at the gut level, or in target sites through the body after absorption across the gut epithelium. The main physiological effects associated with milk derived peptides are modulation of gastric emptying and gut motility, potential enhancement of cardiovascular function via improved blood flow, immunoregulation and promotion of disease defense. These physiological effects, for the most part, have been determined in vitro using synthetic analogues of the peptide sequences known to occur in milk proteins. In some instances, however, in vivo assays have investigated physiological effects following ingestion of either whole milk proteins (with the anticipation of peptide formation during digestion) or of isolated peptides obtained from chemical hydrolysis, or fermentation with bacterial proteases (Tirelli et al., 1997).

Bioactive peptide sequences can be found in most of the major milk proteins. These include all classes of the major milk proteins, all classes of the caseins, lactoferrin, α-lactalbumin and β-lactoglobulin. The positions for each sequence of bioactive peptide are usually dictated by the presence of peptide bonds that are either totally or partially resistant to proteolysis. A recent database of bioactive peptide sequences uses the known cleavage points of the digestive endopeptidases, pepsin, chymotrypsin, and trypsin to help identify potential bioactive sequence within a protein.
Table 4. Recognition sequence for action of endopeptidase

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Recognition sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin (EC 3.4.23.4)</td>
<td>Phe-X; Leu-X</td>
</tr>
<tr>
<td>Chymotrypsin (EC 3.4.21.1)</td>
<td>Tyr-X; Trp-X; Phe-X; Leu-x</td>
</tr>
<tr>
<td>Trypsin (EC 3.4.21.4)</td>
<td>Lys-X; Arg-X</td>
</tr>
</tbody>
</table>

X- any amino acid residue; Adapted from: Darragh (2002)

Often, regions within a protein may contain overlapping peptides sequences that exert different biological effects, and these regions are termed “strategic zones”. Within a strategic zone, the release of each peptide sequence will depend on proteolytic conditions. For example, digestive proteolysis produces markedly different sequences to those obtained after proteolysis during fermentation with bacteria. The bioactive peptides derived from milk proteins have been grouped in seven categories (Darragh, 2002) and the these major groups of bioactive peptides are

- opioid agonist and antagonistic peptides
- hypotensive peptides
- antithrombotic peptides
- immunomodulatory peptides
- antimicrobial peptides
- cytomodulatory peptides
- mineral-binding peptides.

10.0 Health Benefits Of Oligosaccharides Present In Milk

Human milk contains a large variety of complex oligosaccharides in exceptionally high amounts. For many years these components have only been discussed to play a role in the development of a specific intestinal flora in breast-fed infants. Today, there is striking evidence that oligosaccharides are potent inhibitors of bacterial adhesion to epithelial surfaces, an initial stage of infective processes. Therefore, these oligosaccharides are considered as being soluble analogues of epithelial cell surface receptors. The mechanism for such non-immunological role of oligosaccharides has been suggested; firstly they affect the growth of the intestinal flora and secondly, they prevent the attachment of pathogenic microorganisms to intestinal cells by acting as receptor analogues to mucosal adhesion molecules. In addition, they might function as soluble ligands for selectins influencing inflammatory processes by reducing leucocyte adhesion to endothelial cells (Kunz and Rudolf, 2002).

11.0 Dairy Foods And Colon Cancer

Colorectal (colon and rectum) cancer is the third leading cause of cancer morbidity and mortality for both men and women in the US. Colorectal cancer is thought to be caused by an interaction between environmental factors such as diet and genetic predisposition. Among environmental factors, diet is estimated to be responsible for 30 to 60% of all
cancers. Although some dietary factors are suspected of contributing to specific cancers, others may be protective. It has begun recently been appreciated that several components in dairy foods, specially calcium and vitamin D, bacterial cultures (e.g. *Lactobacillus acidophilus*), a class of fatty acids known as conjugated dienoic derivatives of linoleic acid (CLA), sphingolipids, butyric acid, and milk proteins may protect against colon cancer (Parodi, 1996, 1998, 1999).

Findings from epidemiological, experimental animal, *in vitro*, and clinical studies support the hypothesis that increased calcium and vitamin D intake reduce the hyperproliferation and cancer-promoting effects associated with a Western-style diet. In individuals at risk for colon cancer, hyperproliferation of colon epithelium is reduced towards normal by increased dietary calcium. Based on the evidence to date, the meeting recommended (for USA) intakes for calcium (1200 mg/day for adults over 50 years) and vitamin D (10 to 15 µg/day for adults over 50 years), appears to be a prudent measure to reduce the risk of colon cancer (Miller *et al.*, 2000). There is also suggestive evidence that intake of culture-containing dairy foods such as yogurt may protect against colon cancer, although more research is needed to confirm this finding. The potential anti-carcinogenic role of CLA, sphingolipids, and other components in dairy foods also needs to be authenticated. Apart from the above components, butyric acid, a four carbon short-chain fatty acid in milkfat, may protect against colon cancer, at least according to *in vitro* and experimental animal studies. In a variety of cancer cell lines including in colon, butyrate inhibits their proliferation and induces differentiation and programmed cell death (i.e. apoptosis). At the molecular level, butyrate is associated with down-regulation or inactivation of the expression of cancer genes. Butyrate also may inhibit tumor invasiveness and metastasis (Smith and German, 1995; Parodi, 1997). Milk proteins, especially those associated with whey, are most likely another group of milk components with anti-carcinogenic properties. Whey proteins are rich in sulphur amino acids and may therefore retard the development of colon tumors and tumor precursors through provision of biological available methionine and cysteine. Both amino acids have a positive influence on a cellular methylation status and therefore a stabilizing effect on DNA. In addition, serum albumin, α-lactalbumin and lactoferrin are rich sources of the dipeptide glutamylcystein which is an excellent source of dietary cysteine for cellular synthesis of glutathione. This in turn, is an important molecule in defense mechanisms that protect against cancer (Rogelj, 2000).

For these reason, all individuals, and particularly those at high risk of colon cancer, should consume the recommended number of servings from the milk and other food group each day. Four servings a day of foods from the milk group can provide about 1200 mg calcium, the amount currently recommended for adults over 50 years of age. Although most research on dairy foods and cancer prevention has focused on colon cancer, accumulating scientific evidence indicates that intake of dairy foods such as milk may also reduce the risk of breast cancer (Miller *et al.*, 2000).

### 12.0 Conclusion

An adequate intake of milk and milk products as part of a healthy, balanced diet, is needed throughout the lifespan to promote health. Milk and milk products contain high calcium content, substantial amounts of other essential nutrients and health-promoting
components. Because of their unique nutritional package, dairy foods contribute significantly to the nutritional adequacy of the diets of children and adults. Research has established the benefit of dairy foods for reducing the risk of chronic diseases such as osteoporosis, hypertension, and possibly some cancers. Results from human clinical trials suggest that dairy foods, with their unique nutritional profile, may be more effective than a single nutrient (i.e calcium, potassium, magnesium, vitamin D) for reducing the risk of hypertension and colon cancer.

Unfortunately, the consumption of dairy foods in India is grossly inadequate, which is evident from per capita consumption of milk. Although educational efforts are important for all age groups, children in particular, deserve special attention. Increasing the consumption of dairy food to recommended levels in children and adolescents along with physical activity, appear to be the most effective strategies for reducing the risk of osteoporosis later in life. It affords the best chance to influence peak bone mass and to establish eating habits that will continue throughout life. We will need the concerted efforts of governmental agencies, industry sponsors, individual health professionals and the media, to educate consumers about the important health benefits of milk and milk products and to address personal and social barriers to their consumption.

13.0 Suggested Readings


1.0 Introduction

India has emerged as the largest milk producer in the world with its annual milk productions approximately 85 mMT, surpassing USA annual milk production. This milk is produced in India by more than 100 million milch animals, whereas in the USA only 9.2 million animals produce it. Average milk production in India is 795 liters/animal/lactation compared to 7002, 5417 and 5348 lit/animal/lactation in USA, UK and New Zealand respectively with the World average at 2021 liters.

Livestock in India currently accounts for more than half (53%) of buffaloes and 15% of cattle globally. The total breedable bovine population is 111 millions and total bovine in milk are 59 million. Though India has made great strides to emerge as the largest milk producer in the world, the dairy production on a commercial scale is still not profitable here. Apart from low genetic potential of the majority of our very large bovine population, one of the major reasons for unprofitable dairying is the inadequate feed supply, which leads to a very high feed cost per kg of milk produced. The main fodder resources available for animal feeding in the country are side road grasses, wasteland grazing, agricultural crop residues, edible weeds from cropped lands, leafy fodder from trees (top feeds) and a very low proportion of cultivated fodders. Only 4.4 per cent of the total cultivable area is under permanent pastures and grazing lands. Only 10 percent of the feed is available as compound feed manufactured by the feed industry in the country, which is mostly of inferior quality and used by a small proportion of our farmers.

In the last couple of decades several nutritional technologies have been developed in the Dairy Cattle Nutrition Division of NDRI, Karnal. While some of these technologies have already made impact on the feed industry, others too have a potential for field transfer, provided there is sufficient support from the extension agencies. In India, crop residues happen to be the major feed resource for our large bovine population, which unfortunately are of very poor quality. A lot of effort has been made to develop technologies to improve their nutritional quality. Simultaneously, attention has also been paid to develop technologies, which can boost up milk production from animals having higher milk producing potential.
2.0 Scientific feeding practices

Indian animals may be classified into three groups as per body weight, milk yield & fat percentage

<table>
<thead>
<tr>
<th>Table -1: Categories of animals according to body weight and milk yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cow</strong></td>
</tr>
<tr>
<td><strong>B.Wt. (Kg)</strong></td>
</tr>
<tr>
<td>High Yielder</td>
</tr>
<tr>
<td>Medium Yielder</td>
</tr>
<tr>
<td>Low Yielder</td>
</tr>
</tbody>
</table>

To meet the nutrient requirement of lactating animal, they have to be fed scientifically. To meet the requirement One kg concentrate mixture is to be fed per 2.5 kg milk production with an additional 0.5 kg for maintenance in case of high yielding cows. In case of buffaloes concentrates are be fed @ 1kg/2kg milk production and additional 1 kg for maintenance purpose. Dry matter capacity of animal is to be limited to 2.5 to 3.0 percent of body weight. In roughage feeding, dry matter should be given 50:50 through green fodder and dry fodder in high yielding dairy animals, where as in low and medium yielding animals ratio of green and dry fodder may be 30:70.

<table>
<thead>
<tr>
<th>Table-2: Nutrient requirements for high yielding animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cow</strong></td>
</tr>
<tr>
<td><strong>DMI (Kg)</strong></td>
</tr>
<tr>
<td>Maintenance</td>
</tr>
<tr>
<td>Production</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td><strong>Buffalo</strong></td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>15.0</td>
</tr>
</tbody>
</table>

These nutrient requirements can be supplied to cows by feeding of following given concentrate 6 kg, green fodder (maize)- 4 Kg and dry fodder (straw)- 3.6 Kg. In case of buffalo these nutrients can be supplied by feeding concentrate 6.3 kg green fodder (Maize) 4.8 Kg and dry fodder (straw) 3.9 Kg.
Table 3: Feed formula for concentrate mixture

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Parts</th>
<th>% CP</th>
<th>% TDN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize/Barely/Oats/Bajra/Jawar</td>
<td>42.0</td>
<td>4.20</td>
<td>33.7</td>
</tr>
<tr>
<td>Soya bean Cake/GNC (Expeller)</td>
<td>20.0</td>
<td>8.40</td>
<td>15.6</td>
</tr>
<tr>
<td>Mustard Oil Cake (Expeller)</td>
<td>12.0</td>
<td>4.45</td>
<td>9.4</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>8.0</td>
<td>1.20</td>
<td>5.2</td>
</tr>
<tr>
<td>Deoiled Rice Bran</td>
<td>10.0</td>
<td>1.50</td>
<td>6.1</td>
</tr>
<tr>
<td>Molasses</td>
<td>3.25</td>
<td>0.13</td>
<td>2.2</td>
</tr>
<tr>
<td>Urea</td>
<td>0.75</td>
<td>2.20</td>
<td>-</td>
</tr>
<tr>
<td>Nutrijoule</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mineral Mixture</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Common Salt</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>22.00</td>
<td>74.00</td>
</tr>
</tbody>
</table>

3.0 Technologies To Improving The Utilization Of Crop Residue

Since crop residues constitute almost 80% of the roughage source for bovine feeding in India, even a slight improvement in their nutritional quality can increase productivity from our animals and make a large difference in total milk production pool. Crop residues like straws and stovers are characterized by high content of fiber, including lignin and silica, with negligible amount of CP (2-3 %) and lower TDN (40%) content. In addition to that, these crop residues also have lower digestibility and palatability. Several technologies are available today to improve the utilization of crop residues within the ruminant system.

3.1 Urea-Ammonia Treatment Of Straws

This technology is based on the principle that alkali treatment like urea-ammonia (ammonium hydroxide) treatment results in the breaking of lingo-cellulosic bonds and makes more cellulose and hemi-cellulose available for rumen fermentation. In the process, it not only increases the CP content of the straw from 3 to 8%, but even the TDN content is raised by 10-15% units after the treatment. The straw also becomes soft and is more palatable.

Four kg of fertilizer grade urea is dissolved in 30-40 kg water and the urea solution is sprinkled on a layer of 100 kg of wheat or paddy straw. Urea solution should be thoroughly mixed with the straw. Likewise 20-30 quintals of straw can be treated layer by layer in a dome shape and has to be first covered with untreated straw and then with stitched gunny bags or polyethylene sheets or plastered with mud such as “Dhar”. A reaction period of 3 weeks in winter and 10 days in summer is sufficient for the treatment, during which urea is first converted to ammonia and as ammonium hydroxide it breaks the lingo-cellulosic bonds.

Then feeding the treated straw up to 4-5 kg along with 1.5-2 kg of concentrate can support a growth rate of 500 kg/day. Similarly, 6-8 kg of the treated straw along with 1.0-1.5 kg of concentrate can support a milk yield of 5-6 liters in cows and buffaloes. The technologies can be applied at the farmer’s field and is useful, only where the farmers are short of green fodder. It is effective in areas where green fodder is not at all available.
3.2 Total Mixed Rations (TMR)

This new feeding technology involves reducing the particle size of various feed ingredients that include roughage (straws and greens) and the concentrate portion, and mixing them in proper quantities and proportions. The ingredients are sufficiently blended to prevent separation and sorting so as to avoid the selective eating of specific ingredient in the mixture by the animal is avoided. The combined feed is further enriched with necessary mineral and vitamin supplements and feed additives, so that the final mixture is a complete balanced ration for a specific category of dairy stock.

The technology of TMR is actually a more refined version of the existing technique of “Sani” making followed by the dairy farmers of northern India, which may or may not be a balanced ration. The experiments conducted at NDRI have shown that feeding of TMR increases intake of feed, as it becomes more palatable. There is also less wastage of feed and is labour saving too. Because of the balanced nature of the feed, the performance of the animals in terms of growth and milk production is better and so is the feed conversion efficiency.

Thus, in areas where even “Sani” is not practiced, the farmers may be educated with regard to the beneficial uses of this technology as well as TMR. In such areas, hand or power-driven chaff cutter may also be introduced for reducing the particle size of the straw.

3.3 Technology of complete feed block manufacture

There are two aspects of this technology. Firstly, the straw being bulky in nature, its transportation from surplus areas to deficit areas is a cumbersome exercise. To overcome this problem, newer technologies of densification, block making and bricketting have been developed. Through densification, the stake volume of straw can be reduced five times. This technology has a great potential to deliver feeds to areas hit by floods and draught, where the very lives of the animals becomes a priority. In such areas, even an advance action can be taken to transport the feed blocks and store the same at a proper storage place to serve as a feed/fodder bank in the area.

The second aspect of this technology is that the straw can be further fortified with other nutrients which it lacks, by adding concentrate ingredients and other feed additives, including urea, minerals, vitamins, molasses and even enzymes. Some green fodder in the form of subabool leaf meal or lucerne leaf meal can also be incorporated, to make it a complete feed block, so as to provide all the nutrients in a balanced form for the animals. Incorporation of some strategic feed supplements like maize grain, bypass protein (as formaldehyde treated mustard/groundnut/rapeseed cake), and bypass fat, can further boost up milk production in animals with higher potential for milk production.

The complete feed blocks are now commercially available, manufactured by NDDB (Anand), Milkfed (Punjab) and Poshak Feeds (Karnal). Poshak Feeds is manufacturing blocks for different levels of milk production. The firm is also manufacturing the block-making machine, so that it is now easier to set up more such units in the country. The response from farmers who are using feed blocks to their animals is quite encouraging.
3.4 Urea-Molasses-Mineral Block-Lick (UMMB Lick)

This is one of the nutritional technologies developed under NDRI/NDDB collaborative research project and has more potential to be used under distress conditions of flood and drought. As the name suggests, the lick basically consist of urea, molasses and minerals along with a binder such as bentonite. The earlier version of the lick prepared by “hot process” was soft and liable to get melted at higher temperature, thus sticking to the animals faces, attracting flies. Subsequently, the improved version of the lick prepared by “cold process” has been developed at NDRI, in which some addition of bypass protein as cakes is also there, to make it tougher enough to withstand the effect of high temperature. The animal is able to lick about 400-500 g of the lick/day.

The trials conducted at NDRI on the improved version of the lick has shown that it improves straw intake and straw digestibility and can also supply nutrients over and above the maintenance, to support some growth as well. Feeding of UMMB lick also results in significant reduction in ruminal methane production, which has two advantages viz lesser wastage of feed energy as methane, which is otherwise lost in the atmosphere, causing environmental pollution. NDDB (Anand) has been making UMMB licks on commercial basis.

4.0 Nutritional Technologies For Increasing Animal Productivity

4.1 Bypass Protein Technology

About two decades ago there was a notion that bypass protein technology may not be suitable under Indian conditions, since it was thought that the technology is effective only on high yielding animals. However, over two decades of research work, conducted in the Dairy Cattle Nutrition of NDRI (Karnal) has proved that this technology is very much relevant to Indian situation and has given significant results in increasing growth rate (20-30%) and milk production (10-15%) in cattle, buffaloes and goats. The research studies have further proved that the feeding of bypass protein has also positive effects on both male and female reproduction of dairy animals.

Bypass protein can be present in natural form in some feeds, like maize gluten meal, cotton seed cake, fishmeal and subabool leaf meal. However, bypass protein can also be prepared by the formaldehyde treatment of highly degradable oil cakes such as like groundnut cake, mustard cake,soybean meal and rapeseed cake. The bypass protein by this method can be manufactured in the cattle feed factory, spraying (1% of CP) formaldehyde at the time of mixing. NDDB has set up two factories in Gujarat, exclusively for the manufacture of bypass protein and the response of the farmers who are using this technology are overwhelmingly encouraging.

Studies have also proved that bypass protein feed when fed to medium producing animals result in significant increase in growth rate and milk production. Feeding of formaldehyde treated cake has no adverse effect on the health of animals. No degenerative changes in the organs was found in slaughtered goats fed on formaldehyde treated mustard cake, where as degenerative changes in the organs of the animals fed untreated mustard cake was observed. In fact, the glucosinolate in mustard cake effect was neutralized by the treatment as it was shown that perhaps it slows down the conversion of the toxin present in
cake (glucosinolate) to more toxic form (thiocynate) in rumen. Feeding of formaldehyde treated cake also did not have any adverse effect on the quality of milk. Thus, the technology of the bypass protein feeding is a potentially effective and economically beneficial nutritional technology, which can go a long way in increasing the milk yield of dairy animals and their growth rate, at a reasonable cost.

4.2 Bypass Fat Technology For High Producing Animals:

This is one technology, which is exclusively meant for high producing animals. The diets available in India do not possess sufficient energy level and so feeding of such diets can hardly meet the energy requirement of high yielding animals. Bypass fat is the technology to increase the energy density of the diets without having any adverse effect on fibre fermentation in rumen. This is achieved by protecting the dietary fat from ruminal hydrolysis and biohydrogenation. Thus, the extra fat incorporated in the diet does not affect the growth of cellulolytic organisms and the fibre fermentation in rumen.

The main technique used for the protection of fat is converting fatty acids into calcium salt of fatty acids (CSFA) by fusion method or by double decomposition process, which gives the fat a protection against ruminal hydrolysis and biohydrogenation.

Trials at NDRI and other research institutions have proved that feeding of bypass fat results in significant increase in milk production. At present efforts are going on at several places including NDRI, for the scaling up of the technology of bypass fat. Meanwhile, some firms are already selling bypass fat manufactured in India, in collaboration with some foreign firms, using an imported technology.

5.0 Other Beneficial Technologies

5.1 Chemical Preservation Of High Moisture Green Fodders

The technology of silage making, which is popular among farmers in Europe and America, has never found takers in this country. The reason is that in this system of traditional preservation of green fodders, the DM less is upto 10-15%. There could also be spoilage of the fodder by microbial contamination in the silo pit.

The technology of silage making has now been slightly modified, and is now called chemical preservation of fodder. This is achieved by not allowing the fodder (maize, sorghum, oats) to mature by harvesting at a younger stage, when moisture is 80% (DM 20% only). After chaffing, 1% urea and 0.5% common salt are added/dusted as additives. The fodder is packed in underground pit, making it as much air tight as possible. The pit is then covered properly and sealed with polythene sheets. After two months, the preserved fodder is ready for feeding to animals.

Anaerobically fermented fodder like the one described above, has less chances of spoilage as the preservatives like urea and common salt keep the contaminants at bay. There is also less DM loss. Studies conducted at NDRI have shown that the chemically preserved fodder is more palatable and digestible and much superior compared to the silage made by conventional method.
5.2 Feeding of mineral mixture to dairy animals

Major feeds available for ruminant feeding viz. crop residues, agro-industrial by-products and non-conventional feeds are deficient in mineral content. Also, the intensive crop production for the last four decades has caused an imbalance of minerals in the soils, which not only affects the growth of plants, but also causes mineral deficiency in animals. The lower intake of minerals by the animals can lead to their suboptimal growth, production and reproduction.

For a long time, the problem of repeat breeding in dairy animals was related to hormonal imbalance, and the animal physiologists were trying to solve this problem through hormone therapy. However, the research conducted by DCN Division at NDRI and also at other centers, identified mineral deficiency as the major cause of repeat breeding in dairy animal under the rural set up. Several surveys conducted recently in different regions in the country, including in villages around Karnal, has clearly brought out that the farmers are not aware of the importance of feeding mineral mixture to their animals. Subsequently, the on-farm trials conducted in rural areas for mineral supplementation to animals around many research centers, including NDRI, have yielded positive results in term of improvements in reproductive efficiency of animals and in their better productive performance. NDRI has also developed the technology of chelated minerals, which result in better absorbability of these minerals, which can be used more effectively in smaller quantities.

Thus, it is high time that the extension agencies in the country educate the farmers about the benefits of feeding mineral mixture to their livestock. It is equally important to tell the farmers that they should insist on purchasing mineral mixture prepared as per BIS standard. In the market, many sub-standard brands of mineral mixtures are available and the farmers should resist from purchasing such sub-standard mineral mixtures.

6.0 Conclusion

Dairy production in India on commercial scale can be profitable if scientific feeding and adequate supply of protein and energy in the diet of animals. Crop residue may be utilized better by adopting the technologies developed at NDRI for further milk production. In high yielding dairy animals bypass protein and fat ratio help to enhance the milk production.

7.0 Suggested Reading

A NEW PROSPECT FOR DAIRY BIOTECHNOLOGY -
DESIGNER MILK

Dr. Latha Sabikhi
Dairy Technology Division
NDRI, Karnal

1.0 Introduction

The authentication of the links between diet and chronic diseases has prompted the search for new clues into the relationship between food and the onset (or prevention) of disease. Advances in biotechnology and genetic engineering have hinted at possibilities that were till now, merely theoretical in the field of dairying. It is now proven that a novel bracket of value-added products can be harvested from milk and milk products. While until recently, emphasis has been on breeding large animals to produce more milk, the attention is now tuned to adding more value to milk and studying its health implications. By combining the two approaches of nutritional and genetic interventions, researchers are now hoping to develop 'designer milk' engineered to consumer preferences or rich in specific milk components that have implications in health as well as processing.

To realise the full potential of these advantages, it would be desirable to have the opportunity to alter milk composition in several ways. For diet and human health measures, the actions that would be beneficial are: a) a greater proportion of unsaturated fatty acids in milk fat, b) reduced lactose content in milk in order to cater to persons suffering from lactose intolerance and c) removal of β-lactoglobulin (β-lg) from milk. From technological point of view, there exist vast opportunities in: a) alteration of primary structure of casein to improve technological properties of milk, b) production of high protein milk, c) engineering milk meant for cheese manufacturing that leads to accelerated curd clotting time, d) increased yield and/or more protein recovery, e) milk containing nutraceuticals and f) replacement for infant formula.

2.0 Modifications in Protein

One of the major products of the mammary glands being protein, exciting research and technology opportunities extend the frontiers for better protein supplementation. Improved amino acid profile by the addition of L-taurine, L-leucine and L-phenylalanine offers additional nutritional benefits.

Transgenic cows secreting elevated levels of β- (8-20%) and κ-caseins (two-fold) have been produced by genetic engineering. β-casein, which is the most abundant milk protein, is involved in binding calcium phosphate and thus controlling milk calcium levels. Higher κ-casein content in milk is linked to smaller micelles, better heat stability, and improved cheese-making properties. In the transgenic animals engineered, the total milk protein increased by 13–20% and total milk casein by 17–35% compared to non-transgenic
control cows. This has obviously, a positive influence on the cheese yield and also the casein and milk protein concentrate industry.

Caseins, being easily digestible are quite sensitive to plasmin, a serine protease occurring naturally in milk and also plasminogen. Thus, β-casein the most abundant casein in ruminant milk undergoes limited proteolysis by plasmin. This can be disadvantageous as casein proteolysis decreases the curd yield in cheese and can induce organoleptic defects and gelation of UHT milk. Milk enriched with specific inhibitor of either plasmin or plasminogen activator would therefore be alternative for the process industry.

Industrial interest has focused on the production of high value, low volume therapeutic proteins in the milk of domestic animals. In this context, several human proteins have already been expressed with success. GTC Biotherapeutics (Framingham, MA) uses both goats and cows to produce more than 60 therapeutic proteins, including plasma proteins, monoclonal antibodies and vaccines. PPL Therapeutics (Edinburgh, UK, and Blacksburg, VA) is working with rabbits and sheep to produce α1-antitrypsin, fibrinogen, and a lipase to treat pancreatic insufficiency in digesting dietary lipids. Products such as insulin and growth hormone have also been obtained from the milk of transgenic cows, sheep, or goats. The major advantage of transgenic technology is that proteins can be produced at a very low cost. Economic comparison of production costs of human tissue plasminogen activator (htPA) through bacterial fermentation, mammalian cell culture and cow transgenic technology estimates the cost/g of htPA to be 20000, 10000 and 10 US dollars respectively.

3.0 Modifications in Fat

The “ideal” milk fat for human health would contain <10% poly unsaturated fatty acids (PUFA), <8% saturated fatty acids (SFA), and >82% monosaturated fatty acids (MUFA). Although it may not be possible to achieve this “ideal” milk fat composition, manipulation of composition of milk fat is possible through altering the feeding practices for dairy cows and also through genetic interventions.

3.1 Decreasing the Level of Saturation in Milk Fat

Feeding of unsaturated fats and oils in the natural or encapsulated form results in the rise in the degree of unsaturation of the serum lipids, tissue fat and milk fat although it may lower the milk fat. As the melting point of milk fat containing unsaturated fatty acids (USFA) is more, the spreadability of butter made from this milk is improved. An Australian study involving the feeding of a special blend of canola and soybean meal in the protected form resulted in doubling in butter's spreadability. When taken out of a fridge at 5 degrees C, the butter was nearly as spreadable as margarine, without losing its special eating qualities. Clinical trials revealed that consumption of dairy products made from this milk led to decrease in LDL levels in the blood of the consumers. Studies at the University of California (Davis) are focused on the desaturase gene to produce milk with decreased levels of SFA. Efforts are underway to determine if genetic differences among breeds and individual animals are translated into ratios of SFA and USFA.
3.2 Increasing CLA Levels in Milk Fat

Dairy products are rich in conjugated linoleic acid (CLA), a product synthesized in the rumen during the biohydrogenation of linoleic acid. Reports suggest that diets rich in linoleic acid lead to increasing the CLA levels in milk fat two-fold. Incorporating CLA along with soy oil in the diet of cows increases CLA levels, simultaneously decreasing the SFA in milk fat. Furthermore, milk from a grass-fed cow can have five times as much CLA as milk from a grain-fed animal.

CLAs reportedly suppress carcinogens, inhibiting proliferation of leukaemia and cancers of the colon, prostate, ovarian, and breast. They are the only natural fatty acids accepted by the National Academy of Sciences of USA as exhibiting consistent antitumor properties at levels as low as 0.25–1.0 per cent of total fats. The other reported beneficial health effects of CLA as supported by biomedical studies with animal models are antiatherogenic effect, altered nutrient partitioning and lipid metabolism, antidiabetic action (type II diabetes), immunity enhancement and improved bone mineralization.

Genetic variation is also a major source of differences in the milk fat content of CLA. The 9,11 isomer of CLA in milk fat is synthesised involving a mammary enzyme, delta-9 desaturase by the cow and not rumen bacteria as had earlier been reported. The over-expression of this gene may lead to milk with increased CLA levels.

3.3 Omega Fatty Acids

Scientific research indicates that PUFA intake should not be greater than 4% of the caloric total, in approximate proportions of 2% ω-3 linolenic acid and 2% ω-6 linoleic acid. Milk from pastured cows contains an ideal ratio of essential fatty acids (EFAs). Too much ω-6 in the diet creates an imbalance that can disrupt the production of prostaglandins leading to increased tendency to form blood clots, inflammation, high blood pressure, irritation of the digestive tract, depressed immune function, sterility, cell proliferation, cancer and weight gain. On the other hand, deficiency in ω-3 is associated with asthma, heart disease and learning deficiencies. There are reports that roughly equal amounts of these two fats in the diet will result in lower risk of cancer, cardiovascular disease, autoimmune disorders, allergies, obesity, diabetes, dementia, and some mental disorders.

3.4 Reducing Fat Content in Milk

In contrast to altering the fat composition, modifying the cow’s genetic makeup to enable it to produce milk with two per cent fat would reduce the cost of feed per kg milk by 22 per cent. In changing the fat composition, targeting enzymes that influence the synthesis of fat is important. For example, reduction of acetyl CoA carboxylase that regulates the rate of fat synthesis within the mammary gland would lead to a drastic reduction in the fat content of milk and reduce the energy required by the animal to produce milk.
3.5 Type of Fatty Acids Vs. Product Quality

The type of fatty acids present in milk fat can influence the flavour and physical properties of dairy products. Feeding high oleic sunflower seeds and regular sunflower seeds to cows resulted in butter that was softer, more unsaturated and exhibited acceptable flavour, manufacturing, and storage characteristics. Extruded soybean and sunflower diets yielded a Cheddar cheese that had higher concentrations of USFA while maintaining flavour, manufacturing, and storage characteristics similar to that of control cheese. It is also beneficial from a safety point of view, as the accumulation of fatty acids, namely C12, C14, C18:1 and C18:2 enhanced the safety of cheeses against *Listeria monocytogenes* and *Salmonella typhimurium*.

4.0 Modifications in Lactose

Lactose, the milk sugar has to be hydrolysed by lactase or β-galactosidase (β-gal) into the monosaccharides glucose and galactose before it can be transported to the blood stream. The level of β-gal declines early in life and is virtually absent in adulthood in many human beings. When such individuals ingest milk or milk products, the lactose remains undigested and mal-absorbed in the gut, where it causes retention of water by its osmotic action. This water retention coupled with the bacterial production of large volumes of carbon dioxide leads to intestinal upsets and dehydration. As milk is a major component in the human diet, lactose intolerance limits the use of a valuable nutritional source for many people. In addition, since milk can provide much of the required calcium for maintaining bone health, lactose intolerance can also be associated with osteopaenia in later life - an issue of increasing importance in old persons.

4.1 Pre-Harvest Methods of Lactose Reduction

The preharvest methodologies of reducing lactose involve either the removal of α-LA and gene 'knock-out' methodologies or by introducing the lactase enzyme into milk via mammary gland specific expression. However, these methods reduce the overall sugar content of the milk, resulting in highly viscous milk. Studies on mice have revealed that reduction of lactose via α-LA deletion was inappropriate because it impaired milk volume regulation. The milk of such mice was highly viscous with very high protein (88%) and fat (60%), no α-LA and no lactose. An alternative to produce low-lactose milk is over-expression of β-gal in milk. However, the monosachharides produced within the formed milk increases the osmotic pressure within the alveolar lumen, thereby drawing more water and resulting in further dilution of other milk components. An in vivo technique for low-lactose milk production generated transgenic mice that selectively produced a biologically active β-gal in their milk. In contrast to the previous findings these experiments led to reduction in the lactose content while retaining most of the monosaccharide content of the milk.
5.0 Humanisation of Bovine Milk

It is said that mother's breast milk is the ultimate designer food for babies. However, due to varying reasons, a number of infants are fed formulas based on bovine milk. The composition of these formulas could be greatly improved to suit the needs of the infant by incorporation ingredients that resemble those of human milk, thereby 'humanising' the bovine milk.

Lactoferrin (LF), the iron-binding protein has antimicrobial properties and may also mediate some effects of inflammation and have a role in regulating various components of the immune system. Its level in human milk is about one g/l (in human colostrum about seven g/l). As the levels of LF in cow milk is only about one tenth of that in human milk, this has caught the attention of those involved in designing human milk replacement formulas. Pharming, NV (Leiden, The Netherlands) developed the first transgenic bull in the late 1980s and a line of transgenic cows to produce several proteins including human LF (hLF).

Human milk contains 0.4 g/l of lysozyme (LZ), an enzyme that provides antibacterial activity in human milk. Active human lysozyme (hLZ) has been produced in the milk of transgenic mice at the concentrations of 0.78 g/l. On the processing front, the expression of LZ in milk results in the reduction of rennet clotting time and greater gel strength in the clot. A double transgenic cow that co-expresses both hLF and hLZ in milk may also reduce the incidence of intra-mammary infection or mastitis.

Cow milk allergenicity in children is often caused by the presence of β-lg, which is absent in human milk. Elimination of this protein by knocking out β-lg gene from cow’s milk is unlikely to have any detrimental effects, on either cow or human formula, and might actually overcome many of the major allergy problems associated with cow’s milk.

6.0 Additional Advantages

The use of molecular biology to reduce the presence of pathogenic organisms in milk is a potentially advantageous prospect. Thus, one can foresee antibodies against salmonella, lysteria or other pathogens that will produce safer milk products. Research is also underway to manufacture milk through transgenesis for treatment of diseases such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis. In an interesting combination of sericulture and dairying, goats that produce spider silk in milk have been engineered. When spider genes were introduced into the cells of lactating goats, they secreted silk in tiny strands along with their milk. These polymer strands could be woven into threads after extracting them from the milk and used for applications such as military uniforms, medical microsutures, and tennis racket strings.

7.0 The Future

Mice that produce milk with 33% more total solids (40-50% TS) and 17% less lactose than normal control mice have been generated by transgene. As the increase in the total solids is associated with a decrease in total milk volume, the same amounts of total milk fat and protein are being produced in a lesser total milk volume. If this technology could be propagated in dairy animals, milk that contains 6.5% protein, 7% fat, 2.5% lactose and 50%
less water is not an improbable accomplishment. The advantages would be a) direct economic benefit in terms of 50% reduction in the cost of shipping milk, b) less stress on the cow and on her udder, since the cow would be producing one half her normal volume of milk, c) skim milk with twice protein content and half the lactose content of normal milk, d) easier to produce low lactose or lactose-free dairy products e) better product yields due to concentration f) reduction in total whey output because of low milk volume and lactose content and g) decrease in mastitis as less lactose available for organisms.

The future of biotechnologically derived foods is at crossroads even after two decades of positive results. Hi-tech milk processing may be more acceptable to consumers than transgenesis for altering milk composition. Controversy will inevitably tint all biotechnological manipulations aimed at increasing milk production or altering milk composition because the consequences of such interventions trouble the feelings and instincts of human beings. Numerous ethical, legal and social aspects of biotechnological research need to be addressed before we would see designer transgenic herds similar to the organic herds that have now come up. Ultimate acceptability will depend on the four key factors of animal welfare, demonstrable safety of the product, enhanced health properties of the product and increased profitability as compared with conventional practices.

8.0 Suggested Readings

www.extension.iastate.edu
www.nationaldairycouncil.org
www.publicscan.fi.
1.0 Introduction

Food fortification is thought to be a highly effective solution and among the most cost effective public health interventions currently available. It may be defined as the addition of one or more essential nutrients to food whether or not it is normally contained in the food, for the purpose of preventing/correcting a demonstrated deficiency of one/more nutrients in the population or specific population groups (Codex Alimentarius Commission, 1994). It is practiced in those areas where the problems of malnutrition are prevalent.

According to FAO/WHO guidelines (1995) essential nutrients may be added (i) to replace losses that occur during manufacture, storage and handling of food (restoration). For example, the removal of cream from milk takes almost all the natural vitamins A and D and therefore skimmed milk may be fortified with the same vitamins at levels as fluid whole milk. (ii) To ensure nutritional equivalence in imitation or substitute foods. (iii) To compensate for naturally occurring variations in nutrient levels. For instance, milk and butter are subjected to seasonal variations in vitamins A & D contents. Some dairy products are fortified with the vitamins A & D in order to maintain constant vitamin levels. (iv) To provide levels higher than those normally found in a food. For example, margarine is fortified with vitamins A & D (in western countries) to render it nutritionally equivalent to butter, and (v) to provide a balanced intake of micronutrient in special case (dietetic foods) for example infant formulas, special food for athletics, medical food etc.

2.0 General Criteria for Fortification

- Lower available dietary nutrient
- Larger consumption of fortified food product.
- Addition of nutrient should not create an imbalance of essential nutrients.
- Higher biological availability and stability under processing and storage conditions
- There should be reasonable insurance against excessive intake to a level of toxicity.

(Food and Nutrition Board, 1973)

3.0 Milk and Milk Products as a suitable Vehicle for Fortification

Milk in its natural form is almost unique as a balanced source of man’s dietary need (Table 1). The various steps in processing and storage have a measurable impact on some specific nutrients. Milk also provides a convenient and useful vehicle for addition of certain nutrients to man’s diet and has following benefits:
  - Easier quality control measure implementation
• Wider consumption by all age groups
• Cost is affordable by target population.
• Higher stability and bioavailability of the added micronutrients
• Addition of fortificants usually caused minimum change in colour, taste and appearance.

4.0 Nutrients Generally Added to Milk

Liquid milk fortification with vitamins A and D is mandated in several countries. β-carotene is added as a colour-enhancing agent to some milk products such as butter. Dried milk is often fortified with vitamins A and D, calcium, and iron. Milk based infant formula and weaning foods are fortified with a range of vitamins, minerals, and other nutrients such as polyunsaturated fatty acids. Powdered milk used for complementary feeding in Chile is fortified with vitamin C, iron, copper and zinc.

4.1 Fortification of Milk & Milk Products with Vitamins

Under ambient conditions the water soluble vitamin C and vitamins of the B-complex group such as thiamin, riboflavin, vitamin B₆, niacin, pantothenic acid, folic acid, biotin and vitamin B₁₂ are powdered and thus relatively easy to work with when producing most dairy products. The fat soluble vitamins which include vitamin A, D, E and K, however, exist either as an oil or as crystals, which may cause processing difficulties during the production of certain types of dairy products (Mortensen and Gotfredson, 1996).

One of the problem encountered with the vitamins, is their limited stability in presence of heat, humidity and oxygen. Among the water soluble vitamins, vitamin C, folic acid, vitamin B₆ and vitamin B₁₂ are the less stable. While in the case of fat soluble vitamins vitamin A, D and E are least stable.

In order to improve the stability of these vitamins, a number of different coating technologies have been developed. One of the most important methods to protect the fat soluble vitamins is microencapsulation, which results in a highly sophisticated powder, where the vitamin is kept protected from degradation by the coating material used for the encapsulation. During microencapsulation, the fat soluble vitamins are brought from the form of oil or a crystal – which in some processes would be difficult to handle – to the form of a free flowing powder much easier to handle and mix with other dry ingredients (Mortensen and Gotfredson, 1996).

When two or more vitamins are added to a food product at the same manufacturing stage, this is commonly done in the form of premix or as blend. Premix is a homogenous mixture of desired vitamins in a dry powder from, whereas a blend is the same for the fat soluble vitamins, but in an oily form. A premix can consist of both water soluble and fat soluble vitamins and carotenoids, in which case the fat soluble vitamins have to be microencapsulated.

4.2 Fortification of Milk and Milk Products with Iron, Calcium and other Minerals

Selection of an appropriate mineral fortificant (iron, calcium etc) is based on its organoleptic considerations, bioavailability, cost and safety. The colour of iron compounds is often a critical factor when fortifying milk and milk products. The use of more soluble iron
compounds often leads to the development of off-colours and off-flavours due to reactions with other components of the food material. Infant cereals have been found to turn grey or green on addition of ferrous sulphate. Off-flavours can be the result of lipid oxidation catalysed by iron. The iron compounds themselves may contribute to a metallic flavour. Some of these undesirable interactions with the food matrix can be avoided by coating the fortificant with hydrogenated oils or ethyl cellulose (Jackson and Lee, 1991).

Bioavailability of iron compounds is normally stated relative to a ferrous sulphate standard. The highly water-soluble iron compounds have superior bioavailability (Richardson, 1990). Bioavailability of the insoluble or very poorly soluble iron compounds can be improved by reducing particle size. Unfortunately this is accompanied by increased reactivity in deteriorative processes. The problem of low bioavailability of some of the less reactive forms of iron is often circumvented by the use of absorption enhancers like, ascorbic acid, sodium acid sulphate and orthophosphoric acid, added along with the fortificant.

The other important mineral for the fortification of milk and milk products, which has been studied, is calcium. Several commercial calcium salts are available for calcium fortification, which include carbonate, phosphate, citrate, lactate and gluconate. In general, organic acid salts of calcium are more bioavailable than inorganic salts (Labin-Godscher and Edelstein, 1996). The pH of the milk should be taken care of during Ca fortification. Manufacturers of calcium fortified milk products should consider adding, magnesium, riboflavin and perhaps vitamin D as well, in amounts that would normally be obtained in a serving of vitamin D fortified milk (Weaver, 1998).

Milk and milk products can also be fortified with a range of other mineral salts such as Mg, P, Zn, Cu and Mn. Prudent selection of mineral compounds is based largely on consideration of mineral reactivity and solubility of the salt. To overcome problems of flavour, texture and colour deterioration due to addition of minerals, some companies have engineered new fortificant preparations, which generally involve the use of stabilisers and emulsifiers to maintain the mineral in solution (FAO, 1995).

5.0 Technology for Fortification:

5.1 Liquid Milk

The technology of milk fortification is relatively simple and no additional equipments are needed or can be practiced with minor modifications in the existing plant. Mineral/vitamin fortification can be practiced at several stages in the production. But liquid milk is usually fortified prior to pasteurization or ultra-heat treatment. Homogenization is essential for oily preparations of vitamins. Usually two methods of additions are practiced i.e. batch process for small operations and metered additions for continuous process. A metered injection of the vitamin preparation upstream to the homogenizer has been the standard set up in continuous operation plants (Cornell University, 1994). Oily preparations are diluted with 10 parts of warm oil (45 – 50°C), usually butter oil and homogenized with a suitable quantity of skim milk or it can be mixed with appropriate quantity of milk and cream and finally homogenized. In the case of water soluble or water dispersible micronutrients, a premix can be made by diluting the nutrients to 20 times their weight with milk at 45°C, followed by stirring and thorough mixing (USAID, 2001).
A simple procedure for fortification of skim milk with vitamins A without using homogenizer was developed by Bector and Rani (1998). This process is basically a batch process and is suitable for small plants of low capital cost.

Many iron compounds have been assessed in the fortification of pasteurised whole milk. The best fortification procedure was judged to be the addition of ferric ammonium citrate followed by pasteurisation at 81 °C. In this way fortified milk containing 30 ppm iron was found to be acceptable after 7 days storage. Levels of vitamin E, vitamin A and carotene were not affected by the presence of iron. At pasteurisation temperatures below 79 °C off-flavours developed due to lipolytic rancidity (Edmondson et al, 1971). De-aeration of the milk prior to the addition of iron compounds was also found to reduce flavour problems. In the production of iron fortified evaporated milk, ferric orthophosphate was shown to be useful (FAO, 1995).

Calcium fortificant preparations including stabilizers and emulsifiers have been used for fortification of milk and milk-based beverages. It maintains calcium in suspension so as to improve mouth feel and appearance of products (FAO, 1995). In Germany a milk-based fruit beverage has been marketed which is fortified with calcium, phosphorous as well as vitamins A, E, B and C.

5.2 Dried Milk

Here particle size of the fortificant as well as density of the fortificant has to be taken care as large and heavier size particles will lead to separation. In order to achieve stability of vitamins, the safest way to fortify dried milk is to blend dry forms of premix with the dried milk powder, thereby protecting the effect of microencapsulation. However, this requires an effective mixing system. If blends are used, they are added directly to milk, provided homogenization is done before spray drying. If vitamins are added before spray drying, overage addition (Table 2) will be necessary in order to compensate the losses (Mortensen and Gottfredsen, 1996).

Iron fortification of powdered non-fat dry milk, ferrous sulphate at a level of 10 ppm was found to be stable for a period of 12 months. Ferric ammonium citrate and ferric chloride at a level of 20 ppm iron in the reconstituted product gave acceptable results (FAO, 1995).

5.3 Infant Formulas

The mineral content of cow milk, from which many formulas are produced, is highly variable. Production methods have been adapted to control this source of variability. Operations have been included which remove most of the minerals, but at the same time some vitamins and other components of the milk are lost: technologies used include ion exchange, ultra filtration, electrodialysis, reverse osmosis and gel filtration. Mineral compounds are then added at the required levels. There must be careful selection of mineral compounds added to the formulas, as cereal products are highly susceptible to lipid oxidation during storage. The use of ferrous fumarate and ferrous succinate is recommended for fortification of infant cereals as they gave rise to no objectionable flavours/odours or colours on storage. Ferrous sulphate coated with hydrogenated fats, mono- or di-glycerides and ethyl cellulose caused discoulouration on reconstitution with hot milk and hot water.
Although some allowance is made for the natural vitamin content of the ingredients used, most of the vitamins are added to the formula. The Codex Alimentarius Commission (FAO/WHO, 1994) has published an advisory list of mineral salts and vitamin compounds which can be added to formulas. Predetermined excesses of vitamins have to be added to allow for processing and storage losses. UHT processing followed by aseptic packaging has been preferred to in-can sterilisation since less nutrient losses occur in the former case. Losses have been noted particularly for vitamin C, thiamin, folic acid and vitamin $B_6$.

Iron absorption from formulas has been reported to be 5-10% compared to 50% for human milk. It has been suggested that bovine milk proteins or elevated calcium and phosphorus levels account for this difference. Zinc levels in formulas are also higher than in human milk to make up for reduced bioavailability.

5.6 **Ice-cream**

The unit operations used in the manufacture of ice-cream are not highly destructive to vitamins. Vitamins are added in the dry form to the mix. Since whipping and consequent operation of the mix is carried out around freezing temperature, oxidative losses of vitamins are minimized. The greatest processing losses, which occur during manufacture of fortified ice-cream, is during pasteurization of ice cream mix. Calcium enriched ice-cream is also available in USA and is marketed under the name of TruCal.

5.7 **Fermented Milk Products**

In the production of yoghurt, the low pH renders it unsuitable as a carrier of vitamins such as vitamin A. Water soluble vitamins are best used in a encapsulated form, protected for odour and flavour considerations. Some vitamin losses can occur through metabolism by microorganisms during fermentation (O'Brien and Roberton, 1993). The sensory quality of iron fortified yoghurt was acceptable to when tested by a consumer panel. No significant difference in the appearance, mouthfeel, flavour, or overall quality was observed between iron fortified and unfortified yoghurts (Hekman and McMahon, 1997). In Germany, enrichment of cheese with iodine through the use of iodised salt has been approved.

6.0 **Considerations while Fortification of Milk & Milk Products**

1. Bioavailability of commercial preparations
3. Nutrient-matrix reaction
4. Shelf-life & packaging Process considerations:
5. Cost factor:
6. Safety factor

7.0 **Conclusion**

Fortification should not alter the organoleptic properties (taste, smell, colour, consistency) and shelf life (conditions related to storage, transport) of the product. Often there is a delicate balance between bioavailability and other properties of fortified food. Milk and milk products provide a convenient and useful vehicle for fortification with
micronutrients. The risks associated with fortification are minimal except if good manufacturing practices are not followed and only isolated incidents of this type have ever been reported. Improved understanding of interactions between food ingredients and health and ingenuity of food technologists in food formulation and fabrication will contribute to the advances in food fortification.

8.0 Suggested Reading


HEALTH BENEFITS OF SPECIFIC PHYTOCHEMICALS

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1.0 Introduction

Our food is not only a purveyor of nutrients but also source of a large number of non-nutrients, which are protective against different diseases. Humans are continuously exposed to a vast number of toxicants at very minute levels, but integration of these results in the production of degenerative diseases. A myriad of substances in vegetables and fruits have been shown or postulated to have detoxification properties.

Epidemiological and laboratory findings have provided convincing evidence that increased consumption of fruit and vegetables can significantly reduce disease risk. Interestingly, a large number of chemical compounds found in these foods called phytochemicals, (essential and non-essential) may modify several cellular processes.

2.0 Characterization of foods

A partial list of foods that have been supposed to provide benefits by altering one or more physiological processes is presented in Table 1. Nuts, whole grain, fruit, and vegetables contain a variety of compounds associated with reduced risk of heart disease, cancer, diabetes, hypertension and a variety of other medical conditions. Although substantial evidence exists that foods such as grains, garlic and soybeans can decrease the risk of both heart disease and cancer, evidence for other foods is equivocal. Widely diverse components in whole grains that may provide protection by influencing the environment of the gastrointestinal tract include dietary fibre and other indigestible components; several types of antioxidants, such as trace minerals and phenolic compounds; and several phytoestrogens that may alter hormonal homeostasis.

Table 1 Partial list of functional foods and their physiological effects

<table>
<thead>
<tr>
<th>Food</th>
<th>Physiologic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple, barley, blackberry, blueberry, carrot, eggplant, oats, garlic, ginger, ginseng, mushroom, onion, soybean, tea</td>
<td>Lipid lowering</td>
</tr>
<tr>
<td>Lemon, apple, cranberry, garlic, beet, cucumber, squash, soybean, cabbage, Brussels sprouts, cauliflower, kale,</td>
<td>Enhanced drug detoxification</td>
</tr>
<tr>
<td>Ginseng, licorice, oats, parsley, turmeric</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Cranberry, garlic, onion, green tea</td>
<td>Anti-microbial</td>
</tr>
<tr>
<td>Anise, fennel, soybean, cabbage</td>
<td>Anti-estronegenic</td>
</tr>
<tr>
<td>Orange, green tea, garlic, turmeric</td>
<td>Anti-proliferative</td>
</tr>
</tbody>
</table>
Cabbage, onions, garlic, celery, cucumber, endive, parsley, radish and legumes are among the nongrains that have been reported to have some health benefits. The benefits of functional foods are not limited only to natural foods, since they can result from the generation of novel foods as well. For example, low fat foods may provide some benefits in reducing the risk of heart disease. It is suggested that vigorous lowering of cholesterol by using low-fat foods and lipid-active drugs, controlling hypertension, and abstaining from smoking stabilizes plaque and markedly reduces coronary events and angina pectoris.

Many types of compounds found in foods may be responsible for the health benefits attributed to functional foods. Allyl compounds, such as those found in garlic and related foods, have been used in various parts of the world not only for aroma and flavor but also as antimicrobials, insect repellants, and modifiers for the risks of cancer and heart disease. Other compounds that may contribute to observed protection include carotenoids, found in vegetables and fruits; flavonoids, found in fruit; vegetables, nuts and grains; and indoles and isothiocyanates, both found in cruciferous vegetables.

An enormous number of carotenoids are found throughout the plant kingdom. These compounds, many of which do not possess vitamin A activity, can influence numerous biological functions by serving as antioxidants, modulators of cell growth regulations, regulators of gene expression, and immuno-regulators. Indole-3-carbinol is one of the several carotenoids found in cabbage, broccoli, Brussels sprouts and other Brassica vegetables reported to exhibit anticarcinogenic properties in experimental animals. In addition, recent studies have suggested indole-3-carbinol to be safe, well tolerated, and efficacious treatments for recurrent respiratory papillomatosis.

More than 100 sulfur-containing glycosides known collectively as glucosinolates occur naturally in cruciferous vegetables. On hydrolysis, they yield thiocyanate, nitrite and isothiocyanates. Isothiocyanates influence a variety of biological reactions.

More than 4000 flavonoids, polyphenolic compounds found ubiquitously in foods of plant origin, have been described and categorized into flavonols, flavones, catechins, flavonones and isoflavonoids. Flavonoids potentially have a variety of biological effects, including serving as antioxidants, influencing drug detoxification mechanisms, and altering cell proliferation.

### 3.0 Health benefits of selected plant constituents in foods

The three major classes of plant chemicals identified are terpenoids, phenolic metabolites, and alkaloids and other nitrogen-containing plant constituents. The terpenoids include monoterpenoids, iridoids, sesquiterpenoids, sesquiterpene lactones, diterpenoids, triterpenoid saponins, steroid saponins, cardenolides and bufadienolides, phytosterols, cucurbitacins, norliferpenoids, other triterpenoids and carotenoids. The phenolic metabolites include: anthocyanins, anthichlors, benzofurans, chromones, coumarins, minorflavonoids, flavonones and flavonols, isoflavonoids, lignans, phenols and phenolic acids, phenolic ketones, phenylpropanoids, quinonoids, stillbenoids, tannins and xanthones. The alkaloids include: amarylidaceae, betalain, diterpenoid, indole, isoquinoline, lycopodium, monoterpen, indole, isoquinoline, lycopodium, monoterpenoid, sesquiterpene, peptide, pyrroline and piperidine, pyrrolizidine, quinoline, quinolizidine, steroidal and tropane compounds. Other nitrogen-containing constituents include, non-protein amino acids, amines, cyanogenic glycosides, glucosinolates and purines and pyrimidines.
3.1 Polyphenols

The largest group of phytochemicals comprises of the phenolic chemicals. 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, gallloyl and hexahydroxydiphenoyl esters and derivatives, or phlorotannins. Polyphenols historically have been considered as anti-nutrients by nutritionists, because some, eg. tannins have such adverse effects as decreasing the activities 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 compound.

3.2 Terpenoids (terpenes)

The terpenes, also known as isoprenoids, are the largest class of phytonutrients in green foods, soy plants and grains. The importance of terpenes in plants relates to their necessity to fix carbon through photosynthetic reactions using photosensitizing pigments. Animals have evolved to utilize these compounds for hormonal and growth regulatory functions (vitamin A) and, as it is now being understood, the presence of these molecules in animal tissue also provides a measure of protection from certain diseases, especially those related to chronic damage and growth dysregulation.

Terpenes have a unique antioxidant activity in their interaction with free radicals. Terpenes react with free radicals by portioning themselves into fatty membranes by virtue their long carbon side chain. Generally, the impact of a diet of fruits, vegetables and grains on reduction of cancer risk in part may be explained by the actions of terpenes in vivo. Perhaps the most studied of the terpene antioxidants are the tocotrienols and tocopherols.

3.2.1 Tocotrienols and tocopherols

Tocotrienols and tocopherols are terpenes that occur naturally in grains. It is perhaps impossible to eliminate the effects of tocopherol as vitamin E and its effects as a non – specific isoprenoid. The tocotrienols (α, β and γ) and RRR - δ - tocopherol were effective apoptotic inducers for human breast cancer cells, whereas the tocopherols and the acetate derivative of RRR- α -tocopherol ( RRR – α -tocopheryl acetate) were ineffective. Gamma - tocotrienol, a mixed isoprenoid, suppress the growth of diverse tumor cell lines via initiation of apoptosis and concomitant arrest of cells in the G1 phase of the cell cycle.

3.2.2 Carotenoids

The carotenoid terpenes have been extensively studied as antioxidants. These compounds are highly pigmented, being yellow, orange and red, are present in fruits and vegetables, and when consumed by birds are incorporated into the yolk of eggs. Carotenoids comprise two types of molecules, carotenes and xanthophylls.
Carotenes are tissue specific in their biological activity. \( \beta \)-Carotene, \( \alpha \) – carotene and \( \varepsilon \) - carotene have vitamin A activity. The carotenes, including \( \gamma \)-carotene, lycopene and lutien, protect against uterine, prostate, breast, colorectal and lung cancers. They may also protect against risk of digestive tract cancer. To be effective, lycopene must be absorbed and distributed to the tissue. There are also strong correlations of lutien, cryptoxanthin, \( \alpha \)-carotene and \( \beta \) - carotene, but not of lycopene. The xanthophylls type of carotenoids offer protection to other antioxidants, and they may exhibit tissue specific protection. Zeaxanthin, cryptoxanthin and astazanthin are members of the xanthophyll group.

### 3.2.3 Limonoids

Limonoids are terpenes present in citrus fruit. Limonoids appear to provide chemotherapeutic activity by inhibiting Phase I enzymes and inducing Phase II detoxification enzymes in the liver. D- Limonene, the commonest monocyclic monoterpenpene, found within orange peel oil, inhibits pancreatic carcinogenesis induced in the hamster by N- nitrosobis (2-oxopropyl) amine and gastric carcinogenesis induced in Wistar rats by N-methyl-N’-nitro-N-nitrosoguanidine. Limonoids may also provide protection to lung tissue.

### 3.3 Phytosterols

Phytosterols are another important terpene subclass. Two sterol molecules that are synthesized by plants are \( \beta \) - sitosterol and its glycoside. In animals, these two molecules exhibit anti-inflammatory, anti-neoplastic, anti-pyretic and immune-modulating activity. A proprietary mixture of \( \beta \) - 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.

### 3.3.1 Phenolic constitutions

The most important ones are the phenolic acids (including hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins) and flavonoids, the latter being the most studied group. Phenols protect plants from oxidative damage. They have also been studied extensively as antioxidant protectants for humans.

The beneficial role for wholegrain intake in reducing the risk of coronary heart disease, diabetes, hypertension and some types of cancer is well known. But long-term clinical trials with specific foods have not been done; therefore, this evidence is not available.
3.3.2 Flavonoids

The flavonoid subclasses of phenols include the minor flavonoids (flavonones and dihydroflavonols), flavones and flavonols. Among the biological activities of flavonoids are actions against free radicals, free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, microbes, ulcers, viruses and tumors and hepatotoxins. Mechanisms by which flavonoids have been proposed as chemical protectants involve-modulatory effects on a variety of metabolic and signaling enzymes. Flavonoids have been shown to block the angiotensin-converting enzyme that raises blood pressure; they inhibit cyclo-oxygenase, which forms prostaglandins; and they block enzymes that produce estrogen. The implications of these in vitro inhibitory actions are that certain flavonoids could prevent platelet aggregation, reducing heart disease and thrombosis, and inhibit estrogen to receptors in several tissues, thus decreasing the risk of estrogen-related cancers.

The flavonol kaempferol, which is widely found in the diet, has anti-inflammatory and antibacterial activities and is directly mutagenic. Quercetin inhibits a number of enzymes, inhibits smooth muscle contraction and proliferation of rat lymphocytes. Although it is anti-inflammatory, antibacterial, antiviral and anti-hepatotoxic, it exhibits mutagenic activity and allergenic properties.

3.3.3 Catechins and gallic acids

Major sources of catechins are grapes, berries, cocoa and green tea. Tea contains considerable amounts of gallic acid esters, such as epicatechin, epicatechin gallate and epigallocatechin gallate. Numerous studies have suggested that these components provide protective benefits by their free radical scavenging ability and their inhibition of eicosanoid synthesis and platelet aggregation.

In wines, catechins and procyanidins are involved in the astringency sensation. Catechin is one of the major phenolic in grapes and red wines, and it is considered to be responsible for part of the protective effect of red wine against atherosclerotic cardiovascular disease.

3.3.4 Isoflavonoids

Isoflavonoids are another subclass of the phenolic phytonutrients. Soybeans are an unusually concentrated source of isoflavones, including genistein and daidzein, and soy is the major source of dietary isoflavones. The isoflavones of soy have received considerable attention owing to their binding to the estrogen receptor class of compounds, thus representing an activity of a number of phytochemicals termed phytoestrogens.

3.3 Anthocyanidins

Anthocyanidins are water–soluble flavonoids that are aglycones of anthocyanins. The principal naturally occurring anthocyanidins as pelargonidin, cyanidin, paeonidin, delphinidin, petunidin and malvidin. These compounds are among the principal pigments influenced by
pH and metal ion complexes. Like other flavonoids, anthocyanidins are antioxidants in vitro, and might be expected to have antioxidative and anti-mutagenic properties in vivo.

3.4 Alkaloids and other nitrogen-containing metabolites

3.4.1 Glucosinolates

Glucosinolates, which are present in Cruciferous vegetables, are activators of liver detoxification enzymes. Consumption of cruciferous vegetables offers a phytochemical strategy for providing protection against carcinogenesis, mutagenesis and other forms of toxicity of electrophiles and reactive forms of oxygen. The general importance of the condition of plants to their phytochemical content is illustrated by cultivars of certain crucifers, including broccoli and cauliflower; contain 10-100 times more glucoraphanin than do the corresponding mature plants. Crucifer sprouts may protect against the risk of cancer more effectively than the same quantity of mature vegetables of the same variety.

The mechanism of the protective effects is thought to involve the modulation of carcinogen metabolism by the induction of Phase 2 detoxification enzymes and inhibition of Phase 1 carcinogen-activating enzymes, thereby possibly influencing several processes related to chemical carcinogenesis, eg. the metabolism of DNA binding and mutagenic activity of promutagens.

3.5 Fibre

Whereas dietary meat and fat intake have a positive relation to the incidence of colon cancer, dietary fibre has been associated with alterations of the colonic environment that protects against colorectal diseases. Among the theories on colonic carcinogenesis are those that involve increased concentrations of bile acids and their metabolites, alterations in colonic pH, low Ca\(^{2+}\), elevated NH\(_3\) and long-chain fatty acid concentrations, and alterations in bacterial profiles. Fibre may also provide protection by increasing faecal bulk, which dilutes the increased colonic bile acid concentrations that occur with a high-fat diet. Short-chain fatty acids, including butyric acid, and dietary sugar beet fiber also suppress cholesterol synthesis in a rat liver and intestine model.

4.0 Health benefits of Ethnobotanicals

Several tests for medicinal efficacy of phytochemicals in ethnobotanicals from various indigenous cultures have been reported. Traditional procedures used to prepare the plant preparations may enhance the chemotherapeutic value of the plant derivatives, while at the same time reduce their potential toxicity. Kenyan medicinal plants were extracted and tested for in vitro anti-plasmodial active against chloroquine-sensitive and chloroquine-resistant strains of Plasmodium falciparum. Out of 16 extracts, 12 were active against the sensitive strain. Some plant species from Tanzania and Uganda that are used by traditional healers for the treatment of human African trypanosomiasis (sleeping sickness) have been evaluated. Their in vitro activity against Trypanosoma brucei rhodesiense confirmed the potential of ethnobotanically-selected plants as remedies against sleeping sickness.
4.1 Analgesic and anti-inflammatory effects

The analgesic properties of phytochemical constituents isolated from a methanolic extract of *Sebastiania schottina* roots were evaluated using mice, through intraperitoneal route in an acetic acid-induced abdominal constriction model. The compounds isolated were moretenone, glutinol, b-sitosterol and stigmasterol. Glutinol and moretenone exhibited marked analgesic action, being 16-to 26-fold higher in efficacy than aspirin or paracetamol. The authors suggested that the analgesic compounds in Schottina justified, at least partially, the popular use of this plant for the treatment of urinary problems.

The flavone titonine (7,4’-dimethoxy-3’hydroxy-flavone), isolated from the leaves of *Virola michelli* Heckel (Myristicaceae), was methylated and acetylated and the native compound and the methylated and acetylated compounds were evaluated for anti-inflammatory activity.

A hexane extract of *Eryngium foetidum L* (Apiaceae), a Caribbean endemic plant used in folk medicine for treatment of several anti-inflammatory disorders, has been found to contain a number of terpenoid compounds.

Other studies have reported the anti-inflammatory activity of *Lobelia laxiflora* against carrageen and cobra venom-induced acute inflammation in mice.

Species of the Spanish endemic *Teucrium buxifolium*, traditionally used for treatment of rheumatic and other inflammatory disorders, contained potent anti-inflammatory agents against experimentally induced arthristis and carrageenan paw edema. Teucrium buxifolium species also displayed significant anti-ulcer and cytoprotective activity.

4.2 Antiviral effects

A water extract of the Bulgarian medicinal plant, *Geranium sanuineum L* (Geraniaceae), significantly inhibited the replication of herpes simplex virus Type 1 and Type 2 as shown by the reduction of virus – induced cytopathogenic effect and protection of cells. In preliminary experiments, the extract delayed the development of herpetic vesicles following infection with HSV1 in albino guinea pigs. No mechanism of action has been reported, however the inhibitory effect on virus replication has been reported to be related to the content of polyphenol compounds (flavonoids, catechins, a polyphenolic acid an condensed tannins).

4.3 Cancer

4.3.1 Anti-mutagenic testing

Ten phytochemicals from seeds of the *Casimroa edulis* Llave et Lex (Rutaceae) tree, which grows in Mexico and Central America have been identified. This tree produces edible fruits known as zapote blanco. The ethyl acetate extract from these seeds inhibited mutagenicity induced by 7, 12-dimethylbenz [1] anthracene in *Salmonella typhimurium* strain TM677. The extract completely inhibited DMBA-induced pre-neoplastic lesions in *vitro* in mouse mammary gland organ culture.
5.0 Conclusion

Development of the scientific underpinning of functional foods and their bioactive components as health promoters is emerging as a new frontier for nutritionists and other health professionals. Greater understanding of how diet influences an individual’s genetic potential, overall performance, and susceptibility to disease can have enormous implications for the society. As new discoveries are being made in this area, the new information needs to be transmitted to the consumers so that they can make informed decisions.

6.0 Suggested Reading

1.0 Introduction

In the context of processed and prepared foods, fortification of nutrients has been a long-established practice in the dairy and food industries, the most common example being vitamin and mineral fortification in infant foods and weaning foods. Fortification of ‘Modern’ bread with lysine, an essential amino acid, was once the world’s largest food fortification programme running in India. In recent times the concept of fortification and enrichment of foods which are deficient in certain nutrients or ‘health factors’ has been extended to health foods.

Having little nutritional value, dietary fiber has emerged as a unique health-promoting food ingredient and assumes a special significance with regard to dairy products which, in their pure form, are essentially devoid of fiber. Fortification with dietary fiber would add value to the dairy products not only in terms of variety but also their enhanced healthfulness. On the basis of the several research evidences generated during the last two decades, dietary fiber has covered a long distance in establishing its health-related benefits, particularly with regard to lowering of blood cholesterol level, control of glucose level, and in relieving of constipation. These are mainly the metabolic disorders of body resulting from the fast changing life style and food habits much the same way as has happened in industrialized nations to most of the people doing less physical work and consuming ‘low-residue’ high-energy, protein-rich diets. These disorders can, however, largely be overcome by either consuming the foods naturally high in dietary fiber or the foods fortified with dietary fiber.

2.0 Dairy Products: Highly Nutritious but with a few Limitations

Since time immemorial, dairy products have been an integral part of human diet. Milk is the only food, which has got the power to sustain life in all the stages of development, and is considered an important part of a balanced diet. Besides being a source of first-class protein and energy–rich fat, it contains important elements like calcium, potassium, sodium, magnesium and vitamins, which are vital for an all-round development of the human body. Also, several health attributes are associated with milk or its constituents, such as the role of calcium in controlling hypertension and colonic anticarcinogenicity, protective roles of β-carotene (Ziegler, 1989) and conjugated linoleic acid (Mishra and Rai, 2001) against cancers. Butyric acid, the short chain fatty acid of milk fat has been shown to regulate cell growth and enhance the anti-tumor activities (Pouillart et al., 1992).

Notwithstanding all these and many other virtues of milk, it is implicated in several health related problems. A high risk of developing obesity and coronary heart disease is often associated with high amounts of cholesterol and saturated fat in the diet. Being rich in saturated fat, dairy products also get associated with such risk factors. While falling
consumption of butter in many Western countries during the recent past can be ascribed to the potential role of milk fat, sometimes even milk protein is implicated in certain health problems (Sen, 1986).

Milk and milk products, with no native dietary fiber in them, form an essentially low-residue diet. It seems that many diseases to which milk is associated with, may be attributed to the natural absence of dietary fiber in milk and milk products. Thus the fortification of milk products with dietary fiber as a physiologically functional principle would help enhance the overall health value of these highly nutritious but sometimes ‘suspected’ or ‘risk-raising’ commodities.

3.0 Dietary Fiber in Human Health

3.1 What is Dietary Fiber?

Before moving on to the ways and means of incorporating dietary fiber into dairy products, it would be worthwhile to highlight, in brief, the healthful attributes of fiber. Prior to 1965, fiber was simply referred to as “roughage” and measured as crude fiber. In the 1970s, dietary fiber was defined as “the remnants of edible plant cells including polysaccharides, lignin, and associated substances that are resistant to digestion in the alimentary tract of humans”. Dietary fiber is thus a macro-constituent of food, which includes cellulose, hemicellulose, lignin, gums, modified cellulose, mucilages, oligosaccharides, and pectin and associated minor substances such as waxes, cutin and suberin (DeVries, 2003).

According to the American Association of Cereal Chemists (AACC, 2001) 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 polysaccharides, oligosaccharides, lignin, and ‘associated’ plant substances. Further, dietary fiber exhibits one or more of three major physiological impacts: laxation (fecal bulking and softening, increased frequency and/or regularity), blood cholesterol attenuation, and blood glucose attenuation. Here the ‘analogous’ dietary fiber is that material, which is not necessarily intrinsic to plants as consumed, but exhibits the digestion and fermentation properties of fiber. The AACC definition includes resistant starch, and non-starch polysaccharides in dietary fiber as recommended by AOAC (1995), which also stipulated that the sum of dietary fiber and analytically measured starch values be considered as ‘complex carbohydrates’ (implying that total carbohydrates minus sugar minus ‘available’ oligosaccharides would give the concentration of complex carbohydrates).

3.2 Healthful Attributes of Dietary Fiber

It was in 1974 when Burkitt, Painter and Trowell observed that the rural Africans did not have many of the diseases that plagued the West, and they theorized the positive effects of the high-fiber diet on the gut. Thus, fiber was launched from merely being roughage to a substance with many possible therapeutic and preventive roles in several gastrointestinal disorders including enteritis. Dietary fiber has been found to reduce the risk of
hyperlipidemia, heart disease, diabetes mellitus and obesity in susceptible genotypes (DeVries, 2003).

The best accepted beneficial effect of dietary fiber is relief of constipation. For example, in a study in the UK (Eastwood et al., 1984) involving a random sample of 62 people between 18 and 80 years of age, the correlation between fecal weight and dietary fiber intake was significant. In another study, involving 51 highly motivated volunteers with a wide range of fiber intakes (10-78 g/day), Davies et al. (1986) observed very high correlations between fiber intake and fecal weight ($r = 0.96$), and between fiber intake and stool softness ($r = 0.90$), which strongly suggested that the ease of defecation is dependent on fiber intake. Fiber supplements such as psyllium-seed fiber and methylcellulose, or food components such as wheat bran are known to be used in the treatment of chronic constipation.

Several epidemiological studies have indicated a strong link between a high-fiber diet and the prevention of coronary heart disease. Viscous fibers such as locust bean gum, konjac mannan, psyllium and legume fibers (Jenkins, Wolever, and Rao, 1993), and also pectin, guar gum and high-fiber food fraction such as oat bran and oatmeal (Bartnikowska, 1999), all providing 12 to 30g fiber daily, have been shown to reduce total and LDL-cholesterol levels by 10 to 20 per cent with a less fall in HDL-cholesterol levels.

Further, fiber-rich meals help in weight management. A study on more than 74,000 female nurses in the United States (Liu et al., 2003) showed that those with the greatest increase in intake of dietary fiber gained an average of 1.52 kg less than those with the smallest increase in intake of dietary fiber. High-fiber diets may also provide benefits to diabetic patients by lowering blood glucose concentration, reducing postprandial insulin levels and antidiabetic drug requirements and decrease blood lipid concentration.

Fiber has been associated with the prevention of cancer. A study done by Bingham (2003) on 519,978 individuals aged 25-70 years showed that the dietary fiber in food was inversely related to the incidence of large bowel cancer. The researchers suggested that in populations with a low average intake of dietary fiber, an approximate doubling of total fiber intake from foods could reduce the risk of colorectal cancer by 40 per cent.

On the basis of the several health promoting roles of dietary fiber, WHO recommends an intake 27-40 g dietary fiber per day (Cho, Sullivan and Rickard, 1999) and Indian Council of Medical Research (ICMR) and National Institute of Nutrition (NIN, Hyderabad) recommend 40 g dietary fiber per day for Indians.

4.0 Commercial Fiber Preparations

Several fiber preparations, both soluble and insoluble types, are offered to the food industry for commercial applications in North America and Europe, and a range of food products are fortified with such ingredients. A few of these fiber products are also available in India, but their local production is negligible and the Indian food industry is yet to make any appreciable use of these food ingredients although beginning seems to have been made by the baking industry where certain brands of bread are fortified to raise their fiber content.

Among soluble dietary fiber, several non-digestible oligosaccharides are available in international markets under various trade-names (Roberfroid, 1999). These include natural fructooligosaccharides e.g. inulin (Raftiline®) and its hydrolysis product oligofructose (Raftilose®), synthetic fructooligosaccharide like galactooligosaccharide (Oligomate®),
neosugar (Neo-sugar®, Actilight®), transgalactooligosaccharides (Cup-oligo®), isomaltooligosaccharides (Isomalto®) and palatinose condensates which includes polydextrose (Polydextrose®), pyrodextrins, sololigosaccharides (Soya-oligo®) and xylooligosaccharides (Xylooligo®).

5.0 Fiber Incorporation into Dairy Products

As mentioned earlier, milk and milk products with little or no dietary fiber content constitute a low-residue diet i.e., if consumed without appreciable quantities of complex carbohydrates or foods rich in fiber, it could lead to a less bulky stool. Addition of some dietary fiber in dairy foods would result into healthful products. Such compositional intervention is particularly desirable in respect of fat-rich dairy products. Fiber-fortified dairy products do have greatly enhanced health value. The dietary fiber fortification in milk and milk products could be achieved in two major ways: First, incorporation of fiber-rich natural foods into milk products, and second, blending of commercially available purified fibers or fiber preparations into dairy products.

5.1 Fortification of Dairy Products with Natural Fiber-rich Foods

Incorporation of certain fruits, vegetables, whole grains and legumes has been an important approach in product diversification. Fiber-rich food ingredients providepectin, hemicellulose, cellulose and lignin in the dairy foods, besides, of course, contributing valuable micronutrients. There are a few dairy products conventionally manufactured for a long time, which contain certain non-dairy ingredients contributing varying amounts of fiber. These include fruit-flavoured yoghurt, ice cream and ‘milk-shakes’, of which fruit-and-nut ice cream is most popular. New dairy foods developed recently include vegetable yoghurts like sweet potato yoghurt (Collins et al., 1991) and yam yoghurt, vegetable ice cream e.g. spinach flavoured one, ice cream made with beets, celery and carrots (Elliott, 1986), oatmeal and prune ice cream (Dryer, 1992), and certain desserts.

There are quite a few traditional milk sweets that contain substantial quantities of fiber e.g., Gajar-pak (carrot halwa), Ghiya-ka-halwa (bottle-gourd halwa) (Aneja, et al., 2002), Doda barfi, (Jha, 2003) and Kaju-burfi (Rao, Reddy and Jayaraman, 1993). Traditionally made cereals-based milk desserts like kheer (Jha, 2000) and dalia-dessert (Murli Kumar, 2004) are some dairy food sources of dietary fiber in Indian diets.

In the United States and Europe, yoghurts carrying whole cereal grains (e.g. wheat and oat), soy fiber and fruits like cranberries, blackberries, raisins, blueberries, walnuts, hazelnuts, etc. are already to be found on the market under various names such as Yoplait Breakfast Yoghurt, Yoghurt Diet Meal, Fruits of the Forest and so on (Dryer, 1992). These foods may also be the natural sources of some bioactive components like carotenoids, antioxidant vitamin C and E, saponins, sterols, phenols, allium compounds and indoles. Most of them have been largely associated with anti-tumor activity and hypolipidemic properties.

Though the wholesomeness of the natural ingredients rich in dietary fiber cannot be questioned, the presence of certain nutrient complexing compounds like phytins, oxalates and trypsin inhibitor with the macromolecules of fiber are still the concerns of nutritionists and food scientists. Also, total dietary fiber present in natural foods may vary over a wide range (from less than 1.0% in some fruits and vegetables to 25.0% in certain legumes). Therefore,
the per cent RDA value met for dietary fiber by a single serving of the final preparation developed will be limited by the type and the quantity of fruit, vegetable, cereals or legumes that is added to the milk product. This in turn is dependent on the seasonal availability and compositional variability influencing thereby the technological and sensory characteristics of both the ingredients and the final product. Hence, use of standard fiber preparations would be desirable for the purpose of fortification, although it is sometimes claimed that this approach is probably less effective than the use of food products or food fractions as such to serve as a fiber source.

5.2 Fortification of Dairy Products with Commercial Fiber Preparations

While dairy products are innately poor in dietary fiber, they utilize a variety of ingredients that contain this valuable food constituent. Keeping in view the limited amount of dietary fiber supplied per serving by fruits and vegetables in milk foods in conjunction with the altered flavour and textural attributes of products, incorporation of commercially available pure fiber preparations is gaining importance. Although the health attributes of fiber in its native form as in cereal grains, fruits and vegetables may not necessarily be equally present in the pure fiber preparations, the latter substantially retain the healthful virtues which make them valuable from the point of view of fortification.

There are several insoluble preparations like purified cellulose, microcrystalline cellulose, methyl cellulose, lignin, wheat fiber, oat fiber, apple fiber, orange fiber, pea fiber, etc. as also are soluble fiber preparations such as inulin, short chain fructooligosaccharides, β-glucan, psyllium, gums and mucilages which are available commercially. The gums available in the market may be of various types viz., seed gums (locust bean gum, guar gum, psyllium), plant exudates (gum arabic, gum ghatti, gum karaya, and gum tragacanth) and microbiologically produced gums (xanthan gum and gellan gum) to be used as the concentrated source of dietary fiber. Dairy industry is already familiar with some of these fiber preparations as stabilizers, used to control viscosity and ice crystal growth, or imparting a creamy mouth-feel. Various fiber-added dairy food formulations have been discussed by Nayak, Pattnaik and Mohanty (2000).

Soluble fiber such as Inulin and short-chain fructooligosaccharides also serve as prebiotics and enhances human gastrointestinal system and immune system (Ohr, 2004). These have found successful use in yoghurt such as the Naturally Nutritious Yoghurt (Mountain High, USA) providing 3g dietary fiber per 8 ounces (approx. 230g) serving. Baby Yoghurt and Yo-Yo’s (Horizon Organics, USA) is fortified with dietary fiber claimed to enhance calcium absorption in children. In Japan, several companies are using various oligosaccharides to fortify infant milk formulas, baby foods and yoghurt e.g., Yakult Honsha co., Suntory Co. and Morinaga Food Industry. In Belgium, a dietary fiber-fortified fermented milk drink called Fyos containing inulin is very popular.

The Ultra Slim Fast, a beverage from RTS Deans Foods, prepared by the fortification of skim milk with purified cellulose, corn bran, carrageenan and guar gum has been reported to supply 5g dietary fiber per 12-ounce (340g) serving (Dryer, 1992). It consists of a mixture of purified cellulose, corn bran, carrageenan and guar gum. Extrusion cooked wheat bran has also been reported to be suitable for fiber fortification of yoghurt or quarg specialities (John, Zimmer and Tscheuschner, 1990). While the fiber preparations from Roxler International’s Bakeflora line is in use to replace sugar 1:1 to manufacture low-carbohydrate,
low-energy, dietary fiber-enriched formulations, GTC Nutrition’s Natureal GI oat bran concentrate and Matsutani America’s Fibersol-2 resistant maltodextrin are claimed to be ultra-low glycemic fibers with a high potential for food fortification.

6.0 Conclusion

Nutrient-rich and energy-rich milk products are often associated with potential risks of developing certain health problems. Dietary fiber has come to be widely recognized as a health-promoting food constituent, but is absent in conventional dairy products with a few exceptions which contain non-dairy ingredients. Fiber fortification of protein- and/or fat-rich dairy products can potentially reduce the health risks associated with these products. Incorporation of fiber-rich natural food ingredients such as fruits and vegetables wherever possible is apparently the most practical way of enhancing the functionality of dairy food as, in fact, has already been practiced in products such as ice cream, yoghurt and certain traditional milk sweets. However, this approach may not always be feasible and it may often be desirable to use fiber preparations for the purpose of fortification.

The value of dietary fiber as a nutraceutical seems to have been realized by the food industry in the West as evidenced by a large number of commercial fiber preparations capable of serving as a concentrated fiber source now being available and commercially utilized in a variety of foods. The fiber products presently available in India are only a few and are largely imported, but the potential of such food ingredients can be fruitfully exploited for improving the healthfulness of certain dairy products which, being rich source of fat and/or energy, consumers like to have but hesitate on account of health-related prejudices or perceived risks. Thus, incorporation of dietary fiber in the dairy products offers a new avenue to dairy industry looking for product diversification and value-addition, which would benefit both the consumer as well as the supplier of milk.

7.0 Suggested Reading

1.0 Introduction

With a content of 0.7 - 0.9% phosphorus, covalently bound to the casein by a serine ester linkage, casein as a phospho-protein is a member of a relatively rare class of proteins. Moreover, due to high proportions of essential amino acids, casein is nutritionally excellent protein. Its protein efficiency ratio reported is 2.5, which is mostly unaffected by the processing conditions usually employed during the dairy operations. Casein has some rather unique properties and cannot be replaced by other proteins in certain food applications. Edible casein and caseinates are long established dairy by-products finding use in many dairy and food products. World production of casein is around 2.5 lakh tonnes. The biggest importer of casein is the United States of America, where about 1 lakh tonnes of casein is imported, most of which is utilised for the manufacture of imitation cheese.

Acid casein is insoluble in water. Its soluble form caseinates may be prepared from freshly precipitated acid casein curd or from dry acid casein by reaction with dilute solution of alkali (such as sodium, potassium, calcium or ammonium hydroxide). Sodium caseinate is the most commonly used water-soluble form of casein and is used in the food industry. The two main reasons for using sodium caseinate as an ingredient in foods are its functional properties and nutritive value. Sodium caseinate is valued for its ability to emulsify fat in the production of modified dairy products such as coffee whiteners, whipped cream and ice cream. It also possesses very good water binding and whipping properties. Industries of meat processing, baking and modified dairy products are the largest consumer of sodium caseinate. The various food products in which sodium caseinate is used consist of various kinds of sausages, meat-based and milk-based instant breakfasts, modified milk, whipped cream, coffee whiteners, non-dairy creams, desserts, soups, bread, doughs, crackers (biscuits), dietetic products and various protein-enriched products. Other casein products, used in a descending order in the food industry are calcium caseinate, potassium caseinate, other caseinates, and, finally, pure casein.

Other soluble forms of casein are produced using phosphates, carbonates, and other salts as the solubilizers. Magnesium caseinate is prepared from casein and a magnesium base or basic salt such as magnesium oxide, magnesium hydroxide, carbonate or phosphate by ion exchange. Compounds of casein with aluminium may be prepared for medicinal use or for use as an emulsifier in meat products. Heavy metal derivatives of casein, which have been used principally for therapeutic purposes, include those containing silver, mercury, iron and bismuth. Iron and copper caseinates have also been prepared by ion exchange for use in infant and dietetic products.

One another form of casein that is commonly used in the food industry is the hydrolysate (obtained by acid hydrolysis), which has a meat-like flavor. Casein hydrolysates are, therefore, used to accentuate the meat flavor in heat-treated canned and
dried meat products such as soups. The hydrolyzates are also used as ingredients in crackers, snack foods, and other food products. Today, casein hydrolysates have assumed a new dimension in food industry. They find use in wide ranges of soups, gravies, sauces, drinks, vegetable and fruit juices, flavourings and nutritional, dietetic and formulated foods.

2.0 Bakery Products

Casein and casein derivatives are mainly used in bakery products to enhance flavour and other sensory properties and also for nutritional fortification of the wheat flour. The limiting amino acid (lysine) in most cereal proteins can be very well be complemented with dairy proteins. Casein/caseinates can be added to breakfast cereals, milk biscuits and protein-enriched bread. The PER of wheat flour is only 1.1, compared with 2.5 for casein. By supplementing the wheat flour with casein, it is possible to increase considerably the PER of the mixture. For instance, for a 50:50 mixture of casein and wheat protein, the PER can be raised to 2.2 - 2.3. One of the most important functional characteristics of casein products in bakery products is its water binding capacity.

Acid and rennet casein, sodium caseinate, calcium caseinate can be used in bread making and are added at a level of 15-20% of the wheat flour. A satisfactory loaf volume can be obtained by the use of casein products. The physical structure 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. The structure stabilizes during baking. Bread, with milk proteins added in one form or another, shows a good crumb structure, bread yield, flavour and keeping quality. In the manufacturing of high protein biscuits, milk proteins play an important role as they increase the nutritive value and also the texture.

Milk proteins are often incorporated into the base flour for pasta manufacture for the purpose of enhancing nutritional quality and to improve texture. Products fortified by addition of sodium or calcium caseinate prior to extrusion include macaroni and pasta.

3.0 Modified Dairy Products

The use of sodium caseinate in the dairy industry and in the manufacture of modified milk products has increased all around the world. The addition of 1% sodium caseinate into UHT low-fat milk fortifies this dietetic drink with protein. The addition of sodium caseinate to cultured milk products simultaneously increases the nutritive value and improves the technological quality by products.

Milk Protein products are widely used to supplement the protein content and, therefore, enhance sensory characteristics of conventionally processed dairy products and are also used in the production of a range of imitation dairy products. Imitation cheeses are made from vegetable fat, caseins, salts and water and are used in pizza, lasagne and sauces and on burgers, grilled sandwiches, macaroni etc., at a significant cost-saving compared to the use of natural cheese. The functional properties of caseins that favour their use in imitation cheese include fat and water binding, texture enhancing, melting properties, stringiness and shredding ability. Sodium caseinate is used in powdered coffee creamers, which also contain vegetable fat, a carbohydrate source and added emulsifier and stabilizers. These creamers are cheaper, have a
longer shelf life and are more convenient to use (e.g. they require no refrigeration) than fresh coffee creams. In these products, sodium caseinate acts as an emulsifier/fat encapsulator and whitener, imparts body and flavour and promotes resistance to feathering.

Sodium caseinate is used to increase gel firmness and reduce syneresis in yoghurts, and is added to milk shakes for its emulsifying and foaming properties. Sodium caseinate is also used as an emulsifying and fat encapsulating agent in the manufacture of high-fat powders for use as shortenings in baking or cooking. Dry whipping fats or whipping creams contain casein products.

A procedure for the manufacture of a soluble casein concentrate suitable as an ingredient in baby foods and dietetic foods was developed in the former USSR. Precipitated casein is dissolved by adding 2.8% sodium citrate, 3.2% calcium citrate, and 5% sodium bicarbonate to it and the product is dried and ground.

4.0 Beverages

Casein products are used as stabilizers or for their whipping and foaming properties in drinking chocolate, fizzy drinks and fruit beverages. There is also a large market for sodium caseinate as an emulsifier in cream liqueurs and to a lesser extent in wine aperitifs. Cream liqueurs typically contain 16% (w/w) milk fat, 3.3% sodium caseinate, 19% added sugar and 14% ethanol. Trisodium citrate is also added to prevent calcium-induced age gelation. Casein products have also been used in the wine and beer industries as fining agent, to decrease colour and astringency and to aid in clarification.

5.0 Confectionery

Caseins are used in toffee, caramel, fudge and other confections as they form a firm, resilient, chewy matrix on heating and they contribute water binding and aid emulsification. Casein hydrolysates are used as foaming agents in place of egg albumen in marshmallow and nougat as they confer stability to high cooking temperatures and good flavour and browning properties.

6.0 Meat Products

The possibility of the production of simulated meat by using artificial protein fibers based on casein has been investigated for several years. This field is protected by a variety of patents. The basic principle described in one of them consists of the extrusion of a sodium caseinate solution through a die with small openings into an acidic medium. Care must be taken that the continuity of casein fibers is maintained at all times. The patent covers the production facility scheme and 26 products based on sodium caseinate. Using characteristic flavors, coloring agents, and other ingredients, it is possible to produce simulated beef, chicken meat, pork, bacon, ham, and even fish, all of which have the taste and texture of the products being simulated.

The use of caseinates in meat products is traditional and has been used for 25 years as a potential functional protein ingredient in meat industry and it has gone far since then. Today the best-documented non-meat ingredient in meat products is caseinates round the globe. Besides nutritional value, they are mainly used due to their excellent water binding
and emulsifying properties and, therefore, the major application field is that of the rather comminuted meat products. Amongst many other functional properties, the bland flavour and neutral colour of caseinates deserve a special mention. The caseinates get their functionality from their unique molecular characteristics. They have a random coil structure with a low percentage of helix. They show no heat gelation or denaturation and have a high viscosity in solution. The ability of caseinates to bind moisture through H-bonding and entrapment thereby enhancing the yield of end products, has been used beneficially in various meat and sausage preparations. In addition, their salt tolerance and high protein content attract many meat traders.

The functional behaviour of milk proteins in comminuted meat products have been studied by Hung and Zayas (1992). Milk proteins have been utilised as fillers, binders and extenders in cooked comminuted meat products to reduce cook shrink and formulation cost, as well as to improve emulsifying capacity, emulsion stability, water binding, potential nutritive value and slicing characteristics. The dairy proteins can also improve or alter the consumer acceptance (flavour, mouth-feel, colour, appearance etc.) of the finished product. These proteins significantly increase the gel strength of meat proteins and it has been shown that there is a synergistic effect between milk proteins and salt soluble meat proteins, through covalent cross-linkages. The functionally designed dairy ingredients, especially milk protein products, have held their position in this competitive sector because they exhibit good functionality features and affecting the final product in a desirable way. Choice of dairy by-products in meat industry is often more guided by economic criteria. Surplus caseins have been used for substituting more expensive meat and egg proteins in Western countries. Even in this area, competition from vegetable proteins is increasing. Milk proteins, however, are preferred ingredients for their functional supremacy, their good flavour, colour and nutritional profile, because of which they are able to keep their foothold with vegetable protein products.

In comminuted meat products, a considerable amount of free fat is released during the manufacture of these products, which must be stabilised. Caseinates, when used in the desired way, not only stabilise the fat, but also impart water binding and consistency. They do retain a part of the soluble fraction of meat proteins in their native form, which otherwise prone to denaturation at the interface, enabling it available for gel formation upon heating (Schut and Brouwer, 1971) possibly through protein-protein interactions of meat and milk analogues. Consequently caseinates, a milk protein derivative, is a widely accepted functional protein giving the meat product a better stability. This kind of protein also has many hydrophobic groups. This could be one of the several reasons why they are perfect emulsifiers, and are active at the fat-water interface to prevent fat separation. Besides they are perfectly water soluble, a truly desirable characteristic in several restructured meat products. It further supplements and complements the native meat proteins and does not doubt the naturality of products when utilised in functional proportions, i.e. less than 3 percent (Visser, 1984).

Though caseinates may be employed in dry or pre-solubilised form at the beginning of the comminuting process, optimum stability results are obtained when they are processed in the form of previously prepared caseinate/fat/water emulsion (Schut and Brouwer, 1975; Visser, 1984). Sodium caseinate is the most versatile of all milk proteins and disperses well in water or melted fats. Upon addition of warm water during processing, sodium caseinate dispersion hydrate and the resulting colloidal solution forms a base for subsequent emulsions.
The technologically more difficult and cheaper type of fat, such as of beef and sheep may be perfectly used by pre-emulsifying with caseinates. The rationale of using sodium caseinate in emulsion technology is based on its manifold chemical reactivity. The reactivity of caseinates lies in the unique distribution of the electrical charges on the polymeric molecule, its hydrogen bonding ability and its richness of hydrophilic as well as lipophilic bonding sites. The more complex colloidal chemical performance of caseinates can be accentuated by its reactivity with lecithin and carrageenan.

Addition of caseinate stabilizes the meat emulsion as required in the sausage mix. It thickens the gravy during frying and prevents it running out, but excess incorporation of caseinate may result in drying up of the sausages. Further addition of water-absorbent materials becomes essential when sodium caseinate concentration in sausages exceeds 5% (Salavatulina et al., 1983). The greater water holding capacity, lower viscosity and lower cooking losses of sausage batters containing 2% sodium caseinate in comparison to all meat control were observed by Hung and Zayas (1992).

The nutritional value of sausage products, in which part of the meat protein replaced with other proteins was studied by Safronova (1983). The nutritional value of proteins in sausages depended directly on the levels of replacement; for sodium caseinate 50% of the meat protein could be replaced without any adverse effect on it. Usage of a very high viscosity sodium caseinate as an effective meat binder has been found to be desirable in some meat products, because of its greater water binding and gelling properties.

The effect of sodium and calcium caseinates on the functional quality of nuggets made from spent hen meat has been studied by Rao et al. (1994a,b). They observed that with 2% incorporation of sodium caseinate or 1% calcium caseinate, the yields were improved, protein content was higher and brightness scores were higher for nuggets containing caseinates as compared to control nuggets. The frying losses were also lesser for caseinate added nuggets. The texture profile analysis revealed higher firmness and springiness for sodium caseinate containing chicken nuggets and higher cohesiveness and gumminess for nuggets having calcium caseinate (Rao et al., 1996).

7.0 Dietary, Pharmaceutical And Medical Application

Since milk protein products are of high nutritional quality, they are used extensively in dietary preparations for people who are ill or convalescing, for malnourished children in developing countries on a therapeutic diet and for people on weight-reducing diets. Caseins are used in special preparations to enhance athletic performance and have been incorporated into formula diets for space feeding.

While casein products are not generally used in infant formulae, they are used extensively in specialized preparations for infants with specific nutritional problems. Caseinates are used in low-lactose formulae for lactose-intolerant infants while various types of caseinates have been used in inant foods with a specific mineral balance, e.g. low-sodium infant formulae for children with specific renal problems. Casein hydrolysates are used in specialized foods for premature infants, in formulae for infant suffering from diarrhea, gastroenteritis, galactosaemia and malabsorption. A special casein hydrolysate, low in phenylalanine, has been prepared for use in formulae for feeding infants with phenylketonuria. Casein products are also added to various foods for children and infants and to drinks as a nutritional supplement.
Diets that are suitable for geriatrics, high-energy supplements, weight-control diets, hypoallergenic infant formulas and therapeutic or enteric diets are some of the areas in which casein hydrolysates are most useful. Casein hydrolysates are boon to people who are suffering from protein allergy or stomach disorders and to those who require easily digestible foods. The production of hydrolysed protein provides an opportunity for the dietary management of patients with various digestive disorders as a result of pancreatic malfunction, pre- and post-operative abdominal surgical patients, patients on geriatric and convalescent feeding and others who for various reasons are not able to ingest a normal diet. Casein hydrolysates also have pharmaceutical applications in intensive care foods, anemia treatment, prevention of blood cholesterol, treatment of dental diseases and in administration of amino acid mixture intravenously.

Specific drugs have been produced from casein; β-casein is used as raw material for production of β-casomorphins, tetra- to heptapeptides which can regulate sleep, hunger or insulin secretion. Sulphonated glycopeptides prepared from casein have been used for the treatment of gastric ulcers. It is claimed that the use of casein in toothpaste prevents dental caries, in cosmetics it conceals facial wrinkles and in special therapeutic creams it heals wounds.

8.0 Suggested Reading

1.0 Introduction

Ghee is the most widely used milk product in the Indian sub-continent and is considered to be the supreme cooking medium. It is consumed in several forms including preparation of sweets. Ghee known as Ghritam, Havish, Sarpish, rogngezand, samn, maslea and Ajya, was produced in ancient India as early as 1500 B.C. The Rig-Veda, which is the oldest collection of Hindu hymns, contains numerous references on Ghee (Achaya, 1997), showing its importance in Indian diet. In the Middle East also similar type of products were being made since equally ancient times (Sserunjogi, et. al. 1998). There exists a separate therapy called, “GOVAIDAK", which uses several types of medicated ghee for the treatment of various diseases, thus elucidating the medicinal properties of ghee (Adhvaryu, 1994). The major and minor constituents of cow and buffalo milk ghee are presented in table -1.

Table 1. The major and Minor constituents of cow and buffalo milk ghee

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Buffalo</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saponifiable constituents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryglycerides*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short chain (%)</td>
<td>45.3</td>
<td>37.6</td>
</tr>
<tr>
<td>Long chain (%)</td>
<td>54.7</td>
<td>62.4</td>
</tr>
<tr>
<td>Trisaturated (%)</td>
<td>40.7</td>
<td>39.0</td>
</tr>
<tr>
<td>High melting (%)</td>
<td>8.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Partial glycerides*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diglycerides (%)</td>
<td>4.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Monoglycerides (%)</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Phospholipids (mg %)</td>
<td>42.5</td>
<td>38.0</td>
</tr>
<tr>
<td><strong>Unsaponifiable constituents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg %)</td>
<td>275.0</td>
<td>330.0</td>
</tr>
<tr>
<td>Lanosterol (mg %)</td>
<td>8.27</td>
<td>9.32</td>
</tr>
<tr>
<td>Lutein (µg/g)</td>
<td>3.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Squalene (µg/g)</td>
<td>62.4</td>
<td>59.2</td>
</tr>
<tr>
<td>Carotene (µg/g)</td>
<td>0.0</td>
<td>7.2</td>
</tr>
<tr>
<td>Vitamin A (µg/g)</td>
<td>9.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Vitamin E (µg/g)</td>
<td>26.4</td>
<td>30.5</td>
</tr>
<tr>
<td>Ubiquinone (µg/g)</td>
<td>6.5</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Flavour components</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total carbonyls (µM/g)</td>
<td>8.64</td>
<td>7.2</td>
</tr>
<tr>
<td>Volatile carbonyls (µM/g)</td>
<td>0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>Head space carbonyls (µM/g)</td>
<td>0.027</td>
<td>0.035</td>
</tr>
</tbody>
</table>
According to PFA rules (1976), ghee is the pure clarified fat derived solely from milk or from desi (cooking) butter or from cream to which no colouring matter is added. In India, mainly cow and buffalo milk is used for ghee production. Growing consumer consciousness over the ill effects of food consumption has led to development of specialty food products. Now-a-days people do not want only fat, protein and carbohydrates but prefer to have some components in food which would increase consumer longevity. Producing and marketing nutraceuticals and functional foods has become a big business. “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” (DeFelice, 1995).

Consumption of fat rich products is decreasing steadily worldwide, mainly due to various simplistic nutrition messages showing association of fat intake with some diseases like cardiovascular diseases and cancer. Contrarily, consumption of ghee, a product with almost 100% fat, has beneficial effects on health as revealed by many workers. As such milk fat contributes unique characteristics to the appearance, texture, flavour and satiety of dairy foods and is a source of energy, essential fatty acids, fat soluble vitamins, and several other potential health promoting components. Also ghee finds a valuable place in treatment of various diseases in Indian medicine.

2.0 Ghee As A Food

Fats in general are the storehouse of energy in body and form integral parts of all body cells. The fat layer beneath the skin helps in maintaining the body temperature. Delicate internal organs and some bony projections are protected against chance injury by thick cushioning of fatty tissues. Apart from these, consumption of ghee as a food provides certain health benefits as it contains anticarcinogens, antiatherogens and vitamins.

In Indian diet ghee is considered as a superior fat over other fats and is preferred for cooking and other food preparations. The health benefits from ghee can be fundamentally categorized as, those that are obtained from consuming ghee as a food and those that are obtained by using ghee as a medicine.

Ghee is a valuable source of fat soluble vitamins A, D, E and K. They perform various physiological functions in the body. Their levels in ghee vary with species, storage conditions and manufacturing method. Loss of these nutrients can be effectively controlled by certain environmental factors during storage and the intensity of heat treatment that accelerates the overall process of oxidation. Dietary modifications can also increase the vitamin A and E content in ghee as they are of mainly dietary origin. However average ghee consumption by Indians provides only vitamin E in sufficient quantity and rest of the fat soluble vitamins are mainly obtained from the fat sources other than milk fat.

Ghee is observed to improve the growth rate and digestibility upon consumption. It is a rapid source of energy as compare to other vegetable oils. When fat is consumed in the form of ghee, the lower chain fatty acids are quickly absorbed and metabolized. Studies have suggested that peak absorption of ghee occurs rapidly than other vegetable fats. Ghee also improves digestibility of other food components. Kehar et al. (1956) reported improvement of digestibility of protein by 36% and biological value by 62% when cow milk ghee was added to a diet sub optimal for vitamin A. Milk fat in general was found to enhance digestion efficiency. Chronic sufferers from digestive disorders could eat meals baked or cooked in a certain amount of butter fat with out pain, but not other fats. Mineral absorption from diet
increases with ghee consumption. Studies have shown that cow milk ghee increases the retention of calcium up to 45% and phosphorus up to 57%.

3.0 Ghee As A Medicine

Ghee has been ascribed a very important place as a medicine in Indian medicine, Ayurveda. It is used in various disorders both externally as well as internally. Ayurveda has identified ghee as a ‘Madhura Rasa’ that can be used from birth. In some parts of India a mixture of ghee and honey is given to newborn babies (Pandya, 1996). Classical texts of Ayurveda have classified medicinal properties of different types of ghee based on species, manufacturing method and storage period.

3.1 Based on Species

Although for majority of the medicinal uses cow milk ghee is preferred, Ayurvedic texts have described eight-mammalian ghee useful for medication purposes viz. cow, buffalo, goat, sheep, camel, elephant, mare and human.

- **Cow milk ghee** is good for eyes, heavy in digestion and strength giving. It increases virility, and appetite. It also increases the intelligence capabilities and radiance.
- **Buffalo milk ghee** is heavy in digestion and proves remedial in haemoptysis.
- **Goat milk ghee** is appetizing, and light in digestion. It is also eye invigorating and strength increasing.
- **Camel milk ghee** is anti toxic, appetizing and pungent in digestion. It is helpful in treatment of oedema, worms, cutaneous infections, abdominal glands and ascites.
- **Ewe milk ghee** is light in digestion and beneficial in rigour and phthisis.
- **Mare milk ghee** is light in digestion, anueretic and astringent in taste.
- **Elephant ghee** is astringent in taste, and brings about a suppression of stool and urine. It is helpful in treatment of poisoning, worms, and cutaneous affections.
- **Human milk ghee** is light in digestion, antitoxic, appetizing and helpful in treatment of eye diseases. (Bhisagratna, 1963)

3.2 Based on Method of Production

The major difference between different methods is the fermentation process. In some method ghee is prepared from fermented milk or cream whereas some methods prefers ghee production from fresh milk. This difference in methods of production may change the levels of micronutrients in ghee. Fermentation may cause change in the activity of a particular component in food system that may lead to change in health benefits of ghee prepared by different methods. According to Osada et al., (1994) activity of sphingomyelin obtained from yoghurt, a fermented milk product, is 14 times better than shpingomyelin from other sources. It induces secretion of a hormone interferon-β 14 times more. This hormone plays a vital role in secretion of antiviral proteins and therefore, sphingomyelin from fermented milk play a vital role in antiviral therapy. Similarly ghee prepared from fermented and unfermented milk
may act differently as far as health is concerned. Ayurveda have differentiated medicinal properties of ghee made from fresh milk and fermented milk.

- **Sweet milk ghee** is cool, prevents diarrhea and is beneficial in eye diseases and eliminates blood impurities.
- **Fermented milk ghee** is an appetizer, beneficial to eyes, provides strength, virility and eliminates some fevers. (Adhvaryu.1994)

Although in ayurvedic texts nothing is mentioned about fermentation type i.e. controlled or uncontrolled. Also for most of the medicines cow milk ghee is preferred so, this classification might have been made essentially for cow milk ghee.

### 3.3 Based on Storage Period

Old ghee is considered immensely superior for external applications in aryurvedic treatments; it has been used in the treatment of variety of skin diseases. However, for general dietary purposes fresh ghee is recommended.

The various classes of ghee depending upon their storage period are:

- **Puran ghee** is one year old ghee and used in treatment of coma, U.T.I, ear problems, eye diseases and in healing of wounds.
- **Kumbha ghee** is 11 to 100 years old Ghee and used in treatment of fever, cough, and epileptic fits and skin diseases.
- **Maha ghee** is the ghee stored for more than 100 years and generally used for wound healing and massage. (Pandya, 1996)

### 4.0 Medicated Ghee

There are about 55-60 medicated ghee reported in ayurvedic literature and they are used for the treatment of various diseases. Medicated ghee is always prepared with selective fortification with herbs, so as to acquire all the required fat-soluble therapeutical components of the herbs (Saxena and Daswani, 1996). Different medicated ghee with their main application is listed in the Table (2).

**Table 2. Different medicated ghee used in ayurvedic treatments**

<table>
<thead>
<tr>
<th>Medicated ghee(ghrit)</th>
<th>Treatment</th>
<th>Medicated ghee(ghrit)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arjuna ghrit</td>
<td>Heart diseases</td>
<td>Dhanyak ghrit</td>
<td>U.T.I.</td>
</tr>
<tr>
<td>Anantadhyal ghrit</td>
<td>Syphilis</td>
<td>Dhanvantar ghrit</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Amruta ghrit</td>
<td>Leprosy</td>
<td>Narach ghrit</td>
<td>Ascites</td>
</tr>
<tr>
<td>Amrutadi ghrit</td>
<td>Leprosy</td>
<td>Patoladhy ghrit</td>
<td>Eye diseases</td>
</tr>
<tr>
<td>Amrutprash ghrit</td>
<td>Anti-ageing</td>
<td>Palanbhedi ghrit</td>
<td>Piles</td>
</tr>
<tr>
<td>Asta mangal ghrit</td>
<td>Child diseases</td>
<td>Panchcoal ghrit</td>
<td>G.I. disorders</td>
</tr>
<tr>
<td>Ashok ghrit</td>
<td>Leucorrhoea</td>
<td>Panchgavya ghrit</td>
<td>Hysteria</td>
</tr>
<tr>
<td>Ashwagandha ghrit</td>
<td>G.I. disorders</td>
<td>Panchtikta ghrit</td>
<td>Psoriasis</td>
</tr>
<tr>
<td>Kalyan ghrit</td>
<td>Madness</td>
<td>Phal ghrit</td>
<td>Female disorders</td>
</tr>
</tbody>
</table>
### 4.1 Method for Preparation of Medicated Ghee

Classical texts of Ayurveda have described different treatment for the manufacturing of different medicated ghee, depending up on the herbs used and their physical form i.e powder, paste, or liquid. Prasher (1999) has reviewed different methods of preparations and suitability of different ghee with specific process. However, a generalized method of preparation is described in detail in *Sharangdhara Samhita*, a classical Ayurvedic text. The whole process is summarized in the below given chart.

<table>
<thead>
<tr>
<th>HERBS 1 PART</th>
<th>GHEE 4 PARTS</th>
<th>LIQUID 16 PARTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BOILING</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WATER EVAPORATION</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REQUIRED CONSISTENCY</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FILTERATION</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COOLING &amp; STORAGE</td>
</tr>
</tbody>
</table>

In this process medicated ghee is prepared by mixing one part of herbs with 4 parts of ghee and 16 parts of liquid (water, milk or extract of herb), and boiled till all water is evaporated from the mixture. Once the boiling is completed, ghee is clarified, cooled to room temperature, and stored in appropriate containers.
4.2 Methods of Application

Medicated ghee is used for various external and internal applications.

- **External applications**
  * Netratarpan: submerging eye in medicated ghee.
  * Massage
  * Applied in the form of paste.

- **Internal applications**
  * Used in panchkarma (an ayurvedic treatment)
  * Oral ingestion.

4.2.1 External applications

For external applications aged ghee, which is stored for more than a year is generally used, where as in Netratarpan (eye submerging) fresh ghee is recommended. In this treatment a layer of blackgram flour is made around eyes and medicated ghee is filled into it and allowed for 25-30 minutes, it improves eyesight and used in treatment of various eye diseases. Some types of medicated ghee are also applied in the form of paste to cure various skin diseases. **Shatdhaut ghrit** – a medicated ghee made by washing ghee 100 times with water is used in treatment of swelling, pimples and relieves burns and related pains.

4.2.2 Internal applications

- **Panchakarma** is done to remove toxic materials from the body. Medicated ghee is used in following pachkarma treatments.
  - Forced vomiting
  - Medical enema
  - Nasal administration of drugs.

These procedures are always preceded with some specialized treatments intended to prepare the body for panchkarma treatment, they are known as “poorvakarma”. Ghee is used in one of them, oleation, in which ghee is given to the patient in increasing quantities up to 300gm/day for a specific period or till the patient shows characteristics required for a particular Panchkarma process (Dave et al., 1991)

Forced vomiting is intended to clean the upper gut by oral ingestion of emetic drugs. It is very helpful in the case of food poisoning or other type of poisoning. For the treatment of food poisoning, 150-200 gm of ghee is mixed with hot milk and then given to the patient. This causes a severe spell of vomiting and removes out the poison from the body (Pandya, 1996). It is also used in the treatment of bronchial asthma.

Medical enema is helpful in treatment of G.I disorders. It is a process in which medicines are directly introduced to the rectum. The effectiveness of this method is more, as therapeutical components directly enters the blood without passing through liver.

Nasal administration is essential for ayurvedic treatment of almost all ailments above the neck. It is of three types purgative, nourishing and palliative. Appropriate type is selected according to diseases and its acuteness (Dave et al., 1991).

Oral Ingestion of medicated ghee is being used in treatment of many diseases in Ayurveda and is also found handy in treating diseases like asthma, ulcer, cardiac and skin diseases (Table-3).
Table 3. Some medicated ghee and herb(s) used in treatment of diseases by oral ingestion method.

<table>
<thead>
<tr>
<th>Medicated ghee(ghrit)</th>
<th>Herb(s) used</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arjuna ghrit</td>
<td>Terminalia arjuna</td>
<td>Heart diseases</td>
</tr>
<tr>
<td>Vasa ghrit</td>
<td>Adhatoda vasica</td>
<td>Asthma</td>
</tr>
<tr>
<td>Yastimadhu ghrit</td>
<td>Glycrrhizza glabra</td>
<td>Ulcers</td>
</tr>
<tr>
<td>Panchtikta ghrit</td>
<td>Tinospora cordifolia, Azadirchta indica, Salanum xanthocarpum</td>
<td>Skin diseases</td>
</tr>
</tbody>
</table>


HPTLC studies have shown the presence of vasicinone, an antiasthamine agent in vasa (ghrit) ghee prepared with herb Adhatoda vasica for treating asthma. Clinical studies showed marked improvement in 92.59% cases in 21 days. Also had an additional benefit in reducing serum cholesterol level by 30.16% (Prasher, 1999). Antiatherogenic effect of ghee is well established. Along with antioxidant effects of herb due to phenolic compounds and flavonoids, medicated ghee is used in treatment of heart diseases (Arjuna ghrit). In most of treatments, it is not well established whether ghee or components of ghee have the disease curing ability or the herb extracts. One such study by Joshi et al., (1998) revealed that effect of herbs and herb extract was high when used along with ghee as compared to its usage in powder or tablet form.

This could put us in a situation to explore the components of ghee responsible for such results. Pharmaco clinical studies showed that Panchtikta ghee (ghrit) prepared with different methods has different effect on various therapeutic aspects (Barvaliya, 2001). A thorough study on the components and properties of ghee and effect of different processing conditions used in medication is on the anvil. This could lead us to diversify the usage of ghee in a well-organized commercial way.

5.0 CONCLUSION

Value addition has been the main feature in modern food technology. Evolution of food products not just as a nutrient source but also which benefits an individual from dietary risks is underway and many such products under functional foods, health foods, medical foods do exist in market. Various types of special ghee like ginger ghee and garlic ghee are available in the market for specific uses. Of course, they are priced very high (up to Rs.1000-1200 /kg). Medicated ghee is also available, but process specifications are not well defined. Every pharmaceutical firm has its own different process treatment parameters for manufacturing such products. Very few studies have been conducted in ascertaining the exact components and their concentrations in these kinds of ghee, which are responsible for beneficial effects. It has been already discussed that processing parameters do affect the functional quality of the product. But the exact nature is yet to be revealed. Many of the properties like anticarcinogenic, antiatherogenic etc. are surely exhibited by the components of ghee but their mechanisms need thorough research. In many formulations, it is stated that
herbs that are used have more influence when formulated along with ghee. The reasons are to be explored and established.

6.0  Suggested Readings

1.0 Introduction

The word ‘Geriatric’ has been derived from Greek language, which literally means ‘Gerios’ - old age, and ‘Eatron’ – Medicine. “Geriatrics” is the branch of general medicine concerned with the clinical preventive, medical & social aspects of illness in the elderly. In the backdrop of reduction in human mortality and longer life expectancy there has been a paradigm shift in attention towards nutritional requirement of this segment of population. The global population of persons beyond 60 years stood at 600 million at the end of previous century and is estimated to be staggering 1.2 billion by the year 2025.

The benefit of good nutrition to health is considered as important to elderly as it is to younger people. Good nutrition helps maintain functional status and prevents the onset of disability in the elderly. Conversely nutritional deficiency have been associated with numerous health problems in the elderly including osteoporosis, lung disease, cardiac disease, diabetes, frailty, hip fracture, hypotension, pressure sores, pedal edema, infections and adverse drug reactions.

There are challenges involved in developing nutrition for the elderly because of a number of facts as follows:

- The elderly are a heterogenic group.
- Some of the elderly are malnourished, in a risk of malnutrition or obese, exercise little, have many diseases and a lot of medication, and have a decreased function ability while others are active, energetic and healthy.
- Ageing effects on gastrointestinal function–Sensory perception (food choice), food digestibility, gut function.
- Smaller need of energy due to decreased physical activity and slower basic metabolism (2% per 10 years) –How to assure the uptake of adequate nutrients (such as vitamin D)
  –How to get enough, but not too much, energy?
- Interactions between medication, diseases and nutrition
- Dietary status and general capability are interrelated
- Social background, such as living alone influences eating habits

This area of food for elderly is also bestowed with the opportunity for product development and marketing. Food marketers recognize the enormous spending power of the elderly people especially in developed countries who are ready to spend in a frantic effort to fight the relentless march of time.
2.0 Factors Influencing Nutritional Status

2.1 Physiological Changes

While there are many physiological changes associated with aging, some affect nutrition status more than others.

- Decreased total body protein
- Decrease in total body water. The older adult is more prone to dehydration in hot weather or if he/she develops a urinary tract infection.
- Loss of bone density especially with small framed, thin women—Osteoporosis can develop along with the increased risk of broken bones and hospitalization. Osteoporosis is not just found in women, men who are alcoholics or on long-term steroid use are also at risk.
- Increase in body fat with redistribution of fat stores—More fat accumulates around the middle of the body. Fat in this area is more metabolically active and may increase risk of having elevated blood cholesterol levels.

Impact of physiological changes ion nutritional status is enlisted in Table 1.

Table 1. Impact of Physiologic Changes on Nutritional Status

<table>
<thead>
<tr>
<th>Fewer taste buds and decreased numbers of nerve endings</th>
<th>Reduced threshold for taste and smell, loss of palatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased levels of gastric HCL, IF, and pepsin (20%)</td>
<td>Decreased bioavailability of proteins, vitamins and minerals</td>
</tr>
<tr>
<td>Decreased liver size and blood flow</td>
<td>Reduced albumin production and drug clearance</td>
</tr>
<tr>
<td>Bacterial overgrowth in proximal intestine (in those with atrophic gastritis)</td>
<td>Bacterial folate production counteracts physiologic malabsorption</td>
</tr>
<tr>
<td>Changes in body composition (reduced muscle mass, increased fat)</td>
<td>May be preventable with significant exercise</td>
</tr>
</tbody>
</table>

2.2 Systemic Changes

With the advancement of age, physiological changes are also coupled with a number of systemic changes as follows:

- Enamel thins on teeth.
- Teeth become more sensitive to hot and cold. Some older adults lose their teeth. Dentures can be ill fitting secondary to weight changes and shrinking gums. There is decreased saliva production.
- Food must be in solution for taste buds to work.
- More intense seasonings are needed in order to taste.
- Esophageal motility decreases, and swallowing problems increase.
- Gastric acids are reduced. Emptying of the stomach is slower.
- Peristalsis is slower as well. There can be delayed fat absorption.
- Liver size decreases along with decreased protein synthesis.
- Fewer pancreatic enzymes can mean malabsorption including higher fecal fat losses.
- Changes in bowel function are of great concern to the elderly.
- Constipation is a major complaint. This can be caused by poor intake, laxative abuse, medications, low residue diets, dehydration, fear of pain, and loss of gastro-colic reflex.

3.0 Dietary Guidelines For Aged People

In context of diet, nutrients can be broadly categorizes in to two classes: Macro Nutrients (Carbohydrate, Protein, Fat, and Fiber) and Micro Nutrients (Vitamins, Minerals and Trace Elements). In following section the significance of these nutrients is discussed in perspective of geriatric nutrition.

3.1 Carbohydrates

These should make up 55-60% of the diet. The emphasis should be on complex carbohydrates for these will help meet fiber, vitamin and mineral needs.

Energy needs may be less, but the requirements for other nutrient remain the same in elderly people.

3.2 Protein

Protein sources should make up 15-20% of diet. The elderly tend to reduce the amount of meat and dairy in the diet secondary to chewing and digestive problems. Besides, taste changes make meat very bland tasting. They will need to be encouraged to keep an adequate amount of protein sources in their diet.

3.3 Fat

Fat should be in 10-30% range. Frail elderly require more fat. They will need small, frequent, kcal dense meals. Many complain of early satiety. Supplements may be needed or tube feedings. For older adults who are overweight, a low fat diet can be used for weight reduction in addition to heart disease prevention.

3.4 Fiber

Both soluble and insoluble fibers have well documented positive health benefits of particular importance to aging persons. Insoluble fiber found in whole grains, corn and wheat brans and fruit skins alleviates some digestive disorders and may help prevent colon cancer. Soluble fiber found in fruits, vegetables, dried beans, nuts and rice, barley and oat brans can lower cholesterol and help manage blood glucose. Natural fibers such as inulin and oligofructose, offer a number of health benefits, which may be targeted at reducing of disease and strengthening the body in later years. Consumption of food containing these non-fermentable fibers may help address problem associated with the aged such as chronic constipation, decreased resistance to intestinal infection and an increased incidence of colon
cancer. Clinical studies have shown that these ingredients help increase the levels of bifidobacteria in the colon. Unfortunately, the elderly limit the amount of fruits and vegetables in their diet because of chewing or digestive problems. Good choices to help increase fiber are whole grains, cereals, dry beans, fruits, and vegetables. Recommendations are for 20-35 g of fiber per day. Persons changing to a high fiber diet need to do so gradually to avoid gas formation and diarrhea. They also need adequate water in their diet.

3.5 Vitamins and Minerals

3.5.1 Vitamin B6

The deficiencies can cause people to feel tired or depressed. The immune response can also be impaired. Vitamin B6 is found in a wide variety of foods including meat, fish, vegetables, and fruits.

3.5.2 Folic Acid

Folate, a water-soluble vitamin, includes naturally occurring food folate and synthetic folic acid in supplements and fortified foods. Inadequate folate status is associated with an increased risk for chronic diseases that may have a negative impact on the health of the aging population. Inadequate folate status may result in hyperhomocysteinemia, a significant risk factor for atherosclerotic vascular disease, changes in DNA that may result in pro-carcinogenic effects and increased risk for cognitive dysfunction. Folate status may be negatively influenced by inadequate intake, genetic polymorphisms and interactions with various drugs. In the US, folic acid is now added to enriched grain products and continues to be included in the majority of ready-to-eat breakfast cereals whereas no such fortifications permissible in Europe. Folic acid (not food folate) intake in excess of the Tolerable Upper Intake Level may mask the diagnosis of a vitamin B12 deficiency, which is more prevalent in the elderly than younger individuals. When folic acid supplements are recommended, a multivitamin that includes vitamin B12 should also be advised. To safely and effectively increase folate intake in the elderly, naturally occurring folate-rich food sources should be promoted. Folate-rich foods include orange juice, dark green leafy vegetables, asparagus, strawberries and legumes. These foods are also excellent sources of other health-promoting nutrients associated with chronic disease risk reduction.

The potential for folic-acid masking of vitamin B12 deficiency is considered to be greatest in the elderly, in whom the risk of vitamin B12 deficiency (usually in the absence of overt clinical signs) is most prevalent. Some authors have, thus, recommended that elderly subjects taking folic acid supplements should also take 400-1000 µg/day vitamin B12. Intake of supplemental folic acid should not exceed 1,000 micrograms (mcg) per day to prevent folic acid from masking symptoms of vitamin B12 deficiency.

3.5.3 Vitamin C

Vitamin C is water soluble, heat labile and rapidly destroyed during overcooking. Deficiency is likely to occur in an individual whose intake of citrus fruit and juices and vegetables is low or on those who rely for their vitamin C intake on vegetables cooked by institutions e.g. hospitals and social services departments providing meals on wheels. Requirements after surgery and during acute infection may exceed the recommended level.

3.5.4 Vitamin D

The elderly have increased needs if they have limited exposure to sunlight. Darker skin requires longer exposure to achieve benefit. Conversion to the final active hormone by the
kidney decreases with age. Total needs are elevated to help reduce bone loss. Lack of Vitamin D causes ostomalacia. Symptoms include bone pain, muscle weakness, an increased susceptibility to fractured neck of femur, and a characteristic waddling gait. Food sources include fortified milk, fish oils, and liver.

3.6 Minerals

3.6.2 Calcium

Much attention has been paid to calcium intake, especially among women. From antacids to juice, calcium is being added to a wide variety of products. There are increased losses in the elderly, primarily women. There is less absorption secondary to decreased solubility from reduced gastric acids. The aging population needs additional calcium to prevent the development of the bone thinning disease osteoporosis. Dairy products are the primary natural source of calcium in the diet. Dark green leafy vegetables like broccoli and some canned fish with sardines also represent good sources.

3.6.2 Zinc

A deficiency can cause dermatitis and loss of taste. The immune function is also compromised. Delayed wound healing can be a problem in the hospitalized elderly. Increasing intake of eggs, seafood, and whole grains can prevent a deficiency. Supplements are sometimes necessary for patients with surgical wounds or skin ulcers.

Table 2 Recommended dietary intakes of nutrients for elderly people

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (Kcal)</td>
<td>1800</td>
<td>1400</td>
</tr>
<tr>
<td>Protein (gm)</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>Fat (gm)</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>400</td>
<td>4000</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin A (I.U.)</td>
<td>2400</td>
<td>2400</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>12</td>
<td>0.9</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

4.0 Design Consideration In Product Development

Keeping the heterogeneity of the target population in terms of needs and goals, nutritional requirement of elderly people may be set with different objectives. The ultimate goal should always be to set requirements that should prevent malnutrition, maintain sound health, ensure easy uptake of food and should be economically affordable. Accordingly food industry should be poised to meet the challenging task of formulating and manufacturing ‘geriatric foods’ with due regard and consideration for potential handicaps associated with this age segment as enlisted in Table 4.
Table 3 Design considerations in Product development

<table>
<thead>
<tr>
<th>Handicap</th>
<th>How to Overcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor Vision</td>
<td>Colorful presentation</td>
</tr>
<tr>
<td>Poor sense</td>
<td>Amplification of flavor</td>
</tr>
<tr>
<td>Less taste buds</td>
<td>Mixture of KCl + NaCl use</td>
</tr>
<tr>
<td>Loss of teeth</td>
<td>Soft texture food</td>
</tr>
<tr>
<td>Less secretion of digestive juice</td>
<td>Higher moisture and predigested food</td>
</tr>
<tr>
<td>Constipation</td>
<td>High fiber food</td>
</tr>
<tr>
<td>Changes in colon microflora</td>
<td>Dairy products</td>
</tr>
<tr>
<td>Reduced fat absorption &amp; resynthesis</td>
<td>Low fat products</td>
</tr>
<tr>
<td>Increased need of vitamin</td>
<td>Fortified with micro-nutrients</td>
</tr>
<tr>
<td>Increased susceptibility to infections</td>
<td>Zn supplemented food</td>
</tr>
</tbody>
</table>

5.0 Requirement for special diet

5.1 Low-Fat-Foods
Adsorption of fat appears to be affected in older people significantly in the intestine. This is phenomenon related to impairment of fat resynthesis within the intestinal mucosa. A large number of food products with low fat content such as salad dressing, egg substitutes and margarines are available in Western markets. In such foods, 30-50% of the food component is replaced by thickening agents like vegetable gums and CMC.

5.2 Low Sodium Diet
Sodium Chloride is an essential nutrient required in the daily diet. In various clinical conditions like nephritis, cardiac disease, hypertension, fluid retention or edema in body results due to an increase in concentration of sodium in blood. Hence, the salt or sodium content in diet is restricted. Commercially, several products from which the usual added salt has been omitted are available in developed countries e.g. Low-sodium baking powders and spray-dries sodium milk powders.

5.3 Fiber Rich Foods
Dietary foods are the skeletal remains of cell walls that resist digestion by secretion of the human alimentary tract, but these also include phytate, lectins, non-polymeric phenols which exist in intimate association with them. Fibers used in formulated dairy products are Wheat bran, Sugar beet pectin, Lactitol etc.

5.4 High Protein Foods
These foods contain protein levels ranging from 20-30% and contain protein rich materials such as whole or non-fat dry milk, protein isolates from peanut or soybean. Whey proteins and have been associated with following health benefits:
- Immunological benefits- Whey protein helps modulate glutathione (GSH), a tripeptide, by underlying some of the immunological and antioxidant properties of this ingredient.
- Antioxidant Support- Certain enzymes found within whey, such as lactoferrin, have been shown in animal models to help oxidative stress, through its iron-binding and antibacterial properties.
- Anticarcinogenic Activities- Whey protein concentrate might deplete tumor cells by regulating GSH, rendering these cancerous cells more vulnerable to chemotherapy.
- Weight control- Glycomacropeptide, a component within whey, powerfully stimulates the production within the body of Cholecystokinin (CCK), an appetite suppressing hormone.
- Tissue recovery-healing following metabolic stress say following surgery.

6.0 Product Update

There is a growing realization among the commercial food manufactures especially of developed nations that over-50 crowd can constitute a prime and potential segment of their consumers. Hence their attitude is translating in to a desire for good health, quality of life and age-fighting foods that don’t compromise on great taste. Development and marketing interest in healthy food including nutraceuticals, functional foods and supplements has been viewed as cross generational. As people age there are physiological and cognitive changes that affect their sensory perception, preference, and consumption. Product developers and marketers need to understand the aging consumer. The older age group opportunities for health and nutrition products can be placed in to four broad categories or benefit platform-prevention, wellness, cosmetics (appearance) and performance. Most of such products are based on health promoting food ingredients such as amino acids, proteins, botanical products, antioxidants (Vitamins C & E), calcium, folate, fiber, enzyme, probiotics etc. Some of the commercial brands of such foods available in the market of USA are enlist in Table 7.

Table 5. Commercial brands of Geriatric Foods available in USA.

<table>
<thead>
<tr>
<th>Ingredient/Food/Attribute</th>
<th>Brand name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey Protein Isolate</td>
<td>PowerPro</td>
<td>Land O’ Lakes</td>
</tr>
<tr>
<td>Fiber</td>
<td>Benefiber</td>
<td>Novartis Nutrition</td>
</tr>
<tr>
<td></td>
<td>Ensemble</td>
<td>Kellogg</td>
</tr>
<tr>
<td></td>
<td>Ensure</td>
<td>Abbott Laboratories</td>
</tr>
<tr>
<td>Grape Seed</td>
<td>Nutraceutical Extract</td>
<td>Folexco</td>
</tr>
<tr>
<td>Soya protein Concentrate</td>
<td>Soyarich</td>
<td>Central Soya Co</td>
</tr>
<tr>
<td>Flavour</td>
<td>Masking agents</td>
<td>Prosweer</td>
</tr>
<tr>
<td>Nutraceutical shakes and</td>
<td>Boost, Boost Breeze</td>
<td>Mead Johnson Nutritionals</td>
</tr>
<tr>
<td>bar</td>
<td>Snapple-A-Day</td>
<td>Cadbury Schweppes</td>
</tr>
<tr>
<td></td>
<td>BeginIt</td>
<td>Coca-Cola Company</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Slim-fast</td>
<td>Uniliver</td>
</tr>
</tbody>
</table>
7.0 Conclusion

Good nutrition is as important of elderly people as to any other age group. Food has emotional, cultural and health values. Research has supported that poor nutritional status often precedes acute illness in the elderly and paradoxically there is little research data to support nutritional requirements of this age group. Establishment of such requirements is further complicated by heterogeneity prevailing among elderly on account of status of age, diet and health, preference, culture, ethnicity and social background. Keeping these factors in view, it is challenging but imperative to understand general concepts of geriatric nutrition and the parameter of nutritional assessment of this age group. For food scientists and technologists geriatric nutrition offers not only challenges but some rewards as well. Being fastest growing segment worldwide, nutrition for elderly also ushers in the era of development of geriatric foods with objectives of prevention, wellness, appearance and performance for commercial purposes.

8.0 Suggested Reading

Nutritional needs for older adults. Detroit medical center.com
Soy and bone health. 2002. Talksoy.com
1.0 Introduction

Cardiovascular disease (CVD) is the leading cause of deaths in the most affluent societies all over the world. According to WHO estimates, 16.7 million people around the globe die of cardiovascular diseases each year (WHO World Health Report, 2003) and nearly 25 million deaths are estimated worldwide by the year 2020. CVD is now more prevalent in India and China than in other economically developed countries of the world added together. In India, specifically, every ninth individual can be confidently suspected of having CVD (Krishnaswami, 2002). The two main forms of CVD are Coronary Heart Disease (CHD) and stroke. About half of all deaths from CVD are from CHD and about a quarter are from stroke. The causative factor in both cases is blockage in the artery. CHD is a condition in which the main coronary arteries supplying blood to the heart are no more capable of supplying sufficient oxygenated blood to the heart muscle. This reduced flow is caused due to accumulation of plaques, mainly in the intima of arteries, a disease called “Atherosclerosis” (Lovegrove and Jackson, 2000).

Formulating food products with combination of nutrients may provide health benefits. Recent research indicates that foods rich in omega-3 and omega-6 fatty acids, antioxidant vitamins and fibers may be beneficial for cardio-vascular health. Certain dietary supplements claiming to lower down the serum cholesterol levels or helpful in maintaining cardiac health are available in foreign market.

The health care market in India is expected to grow significantly. The health care spending is expected to increase in the next decade (Mckinsey Study 2002). Presently few dietary supplements for diabetic, arthritic, renal patients, sports people are available in India. However, no supplement for CHD patients has yet been introduced.

2.0 Occurrence of CVD

Heart and circulatory disease or cardiovascular disease includes all the diseases of heart and blood vessels. Atherosclerosis and hypertension are the major causative agents for diminishing the blood supply to the arteries. Atherosclerosis is a condition wherein, narrowing down of arteries takes place due to accumulation of lipid, smooth muscle cells and calcium that build up and harden in the arterial walls. This causes plaque formation in the intima of arteries that reduces the supply of oxygenated blood to the heart muscle. The factors responsible for such a condition are:

(i) Not modifiable such as age, sex, and race and family history
(ii) And those that are modifiable include hyperlipidaemia (high levels of fat / lipid), hypertension, obesity, cigarette smoking and lack of exercise.

Of these, physical inactivity, obesity, smoking and diabetes are major risk factors for CHD.
3.0 Role of Food ingredients in CVD

3.1 Type Of Fat

Fat consumed in the diet is the major factor to be modified to prevent occurrence of CVD. Therefore, first line of treatment for individuals with moderately raised cholesterol and/or TAG is to modify their diet by reducing the percentage of dietary energy derived from fat to approximately 30%, of which not more than 10% of energy should come from saturated fat. The saturated fatty acids can be replaced by polyunsaturated fatty acids (PUFAs) of the omega-6 and omega-3 series and monounsaturated fatty acids (MUFAs). Palmitic acid (16:0), the principal saturated fatty acid (SFA) in most diets and myristic acid (14:0) are the most effective at elevating cholesterol whereas, stearic (18:0), lauric (12:0) and medium chain fatty acids (8:0 and 10:0) have little effect on plasma cholesterol level (Mensink, 1993). Recent studies have shown that oleic acid when substituted for saturated fatty acids decreases plasma cholesterol level. A diet rich in monounsaturated fatty acid has been consumed in the Mediterranean region where the concentration of plasma cholesterol and rates of CHD are low (Sardesai, 1998). Therefore, the present recommendation is to decrease saturated fatty acids and increase the intake of monounsaturated acids.

3.1.2 Omega-3 fatty acids

Omega-3 fatty acids are considered essential fatty acids. These can be found in fish and certain plant oils. There are three major types of omega-3 fatty acids that are ingested in foods and used by the body: (a) alpha-linolenic acid (ALA), (b) Eicosapentaenoic acid (EPA), (c) Docosahexaenoic acid (DHA). Once eaten, the body converts ALA to EPA and DHA, which are more readily used by the body. The benefits of the increased intake of n-3 PUFA lie in their ability to reduce thrombosis and decrease plasma TAG levels (Lovegrove and Jackson, 2000). Many of the properties attributed to the omega-3 PUFAs are believed to be due to them replacing arachidonic acid (omega-6) in the membrane phospholipids. The amount of substitution of arachidonic acid with EPA in membrane phospholipids will depend on the ALA competing with linoleic acid (omega-6 fatty acid) for the enzymes involved in desaturation and elongation. Therefore, the ratio of dietary ALA to linoleic acid is important (Allman, 1995).

3.2 Dietary fiber

These are the mixture of many complex organic substances, each having unique physical and chemical properties. Dietary fibers may be classified as water-soluble or gel forming viscous fibers and water insoluble fibers. Soluble fibers are highly fermentable, are associated with carbohydrate and lipid metabolism and have been shown to have hypocholesterolemic properties, while insoluble fibers contribute to fecal bulk and transit times and have little or no effect on cholesterol metabolism. Results of various human studies indicate that a variety of soluble fibers, including guar, psyllium, pectin and oat bran have hypocholesterolemic properties (Stark and Madar, 1994). It has been suggested that physico-chemical changes in the gastrointestinal tract (i.e. increased viscosity) interfere with micelle formation and lipid absorption, thus resulting in reduction of serum cholesterol. Also, certain varieties of dietary fibers can bind bile salts and neutral sterols and thus enhance their
removal from the body. Animal studies have consistently shown that fibers that reduce plasma cholesterol increase neutral sterol or bile acid excretion (Kritchevsky, 1988).

3.2.1 Oat bran

Oat bran in particular has received a great deal of attention as a fiber source with an appreciable level of soluble fiber that has been shown to reduce plasma cholesterol levels under controlled conditions. The ability of oats to reduce plasma cholesterol and in particular, LDL-cholesterol is because of the soluble beta-glucan gum, which is the major hypocholesterolemic component. The FDA oat claim has determined that an effective daily intake of beta-glucan is 3g (Wood and Beer, 1998).

3.2.2 Inulin

Although is a prebiotic, but also functions as a dietary fiber. A prebiotic is a non-digestible food ingredient that beneficially affects the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson and Roberfroid, 1995). It has been hypothesized that there is modulation of hepatic cholesterol synthesis by fermentation products, e.g. propionate and the increased fecal excretion of bile acids has a major impact on hypolipidaemic effect (Levrat et al., 1994). It is suggested that ingestion of 6-12 g of oligosaccharides per day for 2 week-3 months reduce total serum cholesterol by 20-50dl (Tomamatsu, 1994).

3.3 Antioxidants

A complex antioxidant system normally protects mammalian cells and cholesterol from the injurious effects of free radicals. Antioxidants are substances that, when present at much lower concentrations than an oxidizable substrate, significantly delay or prevent its oxidation. Certain essential antioxidants are provided by the diet. These include Vitamin E, beta-carotene and Vitamin C. Increasing the Vitamin E content of the LDL by dietary supplementation inhibits oxidation of LDL to the atherogenic form.

3.3.1 Vitamin E

This vitamin functions as a cellular antioxidant, protecting susceptible cellular components such as unsaturated fatty acids by interrupting free radical reactions that otherwise can cause membrane damage. The major dietary sources of vitamin E are vegetable oils and soft margarines prepared from vegetable oils. Oils such as olive and rapeseed, which are rich in vitamin E and low in PUFA but high in monounsaturates, are functionally more beneficial. The epidemiological and biochemical studies indicate that protection of high-risk groups from CVD could require an intake of 36-100mg/day (Diplock, 1992).

3.3.2 Vitamin A

Of all the carotenoids β-carotene is particularly effective at scavenging peroxyl radicals under physiological conditions and is also a potent scavenger of singlet oxygen (Burton, 1989). β-carotene and lycopene inhibit the oxidation of LDL to its atherogenic form.

3.3.3 Vitamin C

It is a strong, water-soluble antioxidant and is the first line of defense against oxidative stress in plasma. It serves as an intercellular and extra cellular quencher of free radicals, thereby protecting cells and their components. The incidence of CVD has been reported to be inversely related to plasma Vit.C concentrations (Gey et al., 1987).
4.0 Health Foods

With the evolution of novel technologies and scientific developments in the past years, area of health foods has taken a new dimension. An increasing number of potential nutritional products with medical and health benefits, so called “functional foods” have gained an important place in the world market. The global functional foods market is estimated to be around $47.7 billion. Foods can be modified by the addition of phytochemicals, bioactive peptides, omega-3 PUFA and probiotics and/or prebiotics to become functional (Berner and O’Donnell, 1998). Various ways by which foods can be made functional are given in Table 1.

Table 1. How Foods Can Be Made Functional

<table>
<thead>
<tr>
<th>Food Modification</th>
<th>Examples of possible Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addition of phytochemicals</td>
<td>Antioxidant, lower risk of CHD, lower risk of cancer, lower blood pressure</td>
</tr>
<tr>
<td>Addition of bioactive peptides</td>
<td>Enhanced immune function, enhanced bioavailability of minerals, hypotensive</td>
</tr>
<tr>
<td>Addition of dietary fiber</td>
<td>Prevention of constipation, lower risk of colon cancer, lowering of blood cholesterol</td>
</tr>
<tr>
<td>Addition of omega–3 polyunsaturated fatty acids</td>
<td>Lower risk of heart attack, lower risk of some cancers, enhanced immune system</td>
</tr>
<tr>
<td>Addition of probiotics</td>
<td>Improved gastrointestinal function, enhanced immune system, lower risk of colon cancer</td>
</tr>
<tr>
<td>Addition of prebiotics</td>
<td>Improved gastrointestinal function, enhanced immune system, lower risk of colon cancer</td>
</tr>
</tbody>
</table>

Many lines of evidence suggest that adverse dietary habits are a contributory factor in CVD and so the first line of treatment for individuals with moderately raised cholesterol and / or triacylglycerol levels is to modify their diet. A dietary pattern that incorporates food and nutrients shown to be associated with reduced risks of CHD can produce, within a few years, risk reductions of the same size as those typically associated with Statin drug therapy (Lancet, 1994). A greater benefit might be expected from multifactorial dietary intervention than from changing a single nutrient (Mann, 2002). Some of the common food ingredients having functional attributes are listed in table 2.

Table-2. Common Food Ingredients Having Functional Attributes

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Health claim</th>
<th>Product example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsaturated fatty acids</td>
<td>Reduces risk of heart disease</td>
<td>Spreads, cookies</td>
</tr>
<tr>
<td>Soluble fiber from whole oats or psyllium husk</td>
<td>Reduces cholesterol and risk of heart disease</td>
<td>Cereals, cookies</td>
</tr>
<tr>
<td>Soy protein, soy fiber</td>
<td>Reduces cholesterol and risk of heart disease</td>
<td>Drinks, bars</td>
</tr>
<tr>
<td>Folic acid, Vitamin B 6</td>
<td>Decrease homocysteine and risk of cardiovascular disease</td>
<td>Cereals</td>
</tr>
<tr>
<td>Probiotic bacteria</td>
<td>Cholesterol reduction, anticarcinogen, antiinfective</td>
<td>Fermented foods</td>
</tr>
</tbody>
</table>
Vitamin E | Protects against cardiovascular disease | Supplements
---|---|---
Vitamin C | Protects against CVD | Drinks, sweets
Low in sodium | Reduces blood pressure | Drinks, soups
Plant stanols and sterols | Lowers cholesterol and risk of coronary heart disease | Margarine, yogurt, cereal bars
Catechins | Reduce cardiovascular risk | Tea
Conjugated linoleic acid | Reduces body weight, protects against cancer | Supplements (small amounts occur naturally in milk, beef and lamb)
*LactobacillusGG* bacteria | Reduces diarrhea | Yogurt

### 5.0 Commercially Available Health foods

Some of the supplements claiming to improve heart health are generally available in the form of tablets or powders. These contain ingredients like omega-3 fatty acids, fructooligosaccharides, antioxidant vitamins etc. Fenugreek fiber is being marketed by *SMS Biopharma* as a supplement to improve heart health while herbs like *Guggul* are also available in the Indian market. Various functional foods are available in the markets of Western countries which claim to lower down the cholesterol levels. *OmegaTech’s Gold Circle Farms* have created eggs that deliver higher Vit.E and omega-3 contents by feeding chickens a feed containing a variety of healthy ingredients such as flaxseed, menhaden oil, antioxidants and grains. *Good Habits DHA-enriched foods* are producing eggs, beefs and chicken with higher levels of DHA, Vit.E and omega-3 fatty acids than their traditional counterparts. However, in India, no such supplement is as yet available and there is a need for development of such a supplement.

#### Table 2 Commercially available health and nutraceutical food products

<table>
<thead>
<tr>
<th>Product name</th>
<th>Ingredients</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf-stable milk with added omega-3s</td>
<td>Omega-3 fatty acids, milk</td>
<td>Nestle and Parmalat</td>
</tr>
<tr>
<td>The Heart Bar</td>
<td>L-arginine</td>
<td>Cooke Pharma</td>
</tr>
<tr>
<td>NovaDigest</td>
<td>Fructo-oligosaccharides and Fiber</td>
<td>Novartis</td>
</tr>
<tr>
<td>NovaCol</td>
<td>Oat-beta-glucan, soy isoflavone, Vit.E and C</td>
<td>Novartis</td>
</tr>
<tr>
<td>Goodhabits DHA-enriched Foods (eggs, beef and chicken)</td>
<td>Eggs, beef and chicken with up to 20 times normal level of DHA, 7 times more of Vit.E, 6 times the normal omega-3 content</td>
<td>American Nutraceuticals</td>
</tr>
<tr>
<td>Taste of Life Salad dressings</td>
<td>Vitamin E enrichment</td>
<td>Kraft foods</td>
</tr>
</tbody>
</table>

### 6.0 Conclusion

In the recent past the area of special dietary products and their consumption has received considerable attention due to increased consumer awareness towards health improvement. The importance of diet as a therapeutic adjunct in the form of nutraceutical has become the trend of the millennium. The approach of using diet to improve health seems to
be more attractive than drugs that are more aggressive and are associated with unpleasant side effects.

According to recent report of WHO, prevalence of CVD has increased progressively in the past few years. It has been estimated that one-fifth of the deaths in India are due to CHD that is inflicting at much younger age in Indians than in the west. Current projections suggest that in the next 20 years India will have the largest CVD burden in the world. An insight on the occurrence of CHD suggests that cardiac health needs protection. Need to formulate a dietary supplement would be a boon for providing protection against cardio-vascular disease in our country.

7.0 Suggested Readings

1.0 Introduction

Consumer convenience along with safety is the key to value addition in dairy and food processing industries. In this context, technology development for process upgradation of certain traditional dairy products is obviously the topical area of research geared to cater to the need of the industry for avenues to diversify its activity with local and export markets in view. In this context, kheer and dalia are two important cereal-based food products, which are popular throughout the country but have no organized manufacturing and marketing system (Aneja, 1997).

Kheer has been the premier milk delicacy associated with festivities and celebration from time immemorial. Hindu mythology refers to it as the celestial nectar, 'Amrit' or elixir and gives it a place of prominence among foods as the secret of immortality - the life giving food. The two great epics of the Hindu mythology provide evidence of its popularity. Since those times of kings and nobles, kheer has maintained its place of prominence in the Indian diet. No fiesta is considered complete without kheer as dessert. Similarly, dalia as a milk-wheat porridge is quite common in many parts of the country both as a breakfast food as well as health food.

Many traditional foods have remained unchanged with regard to processing or packaging for centuries, due to the fact that they developed in a particular location and are deep rooted in the natural, cultural, religious and socio-economic environment. Some have disappeared without a trace as a result of modern influences, while some have expanded on a global scale, becoming household products. Unfortunately, as is the case with other traditional dairy products, kheer and dalia making have also remained confined to domestic level only. Their poor shelf life coupled with lack of technology for organized large-scale manufacture have been major deterrents in exploitation of its commercial advantage, both in domestic and international markets. Concerted attempts were made at National Dairy Research Institute, Karnal to develop commercial processes for organized manufacture of kheer in the form of a dry mix and also as ready-to-serve product in retort pouches and dalia in the form of ready-to-serve product in retortable cans.

2.0 Instant Kheer Mix – Product Diversification For Dairy Industry

Though popular throughout India, its limited shelf life even under refrigeration imposes severe restrictions on its organized manufacture and marketing. It was envisaged that if a process were developed for kheer in a shelf-stable form, it would offer significant value addition and product diversification for Indian Dairy Industry. In the past two decades, several attempts have been made including the use of preservatives to extend the shelf life of kheer, but these have met with a limited success. Production of kheer in a dry form suitable for ready reconstitution could be conceived to help overcome the problem of shelf life of this popular traditional product.
A process has been developed at NDRI, Karnal to manufacture an instant rice-based kheer mix. It consists of separate drying-cum-instantization of the milk and rice phases of the product. The powdered portion obtained by two-stage spray-bed process, and containing 1.9% moisture, 18.2% fat, 15.3% protein, 2.5% total ash and 62.1% total carbohydrates, is somewhat similar to the sugar-containing partially skimmed milk powder, or the presently marketed dairy whiteners. The rice grains which have been so processed as to make them quick-cooking are carried in a small pouch placed in the metallized polyester/LDPE bag used for packaging of the kheer mix powder. The mix has a shelf life of at least 6 months at 37°C. Reconstitution of kheer mix involves rehydration of instant quick-cooking rice in boiling water for 10 min followed by dispersal of the powdered component into the rice-water mixture. The reconstituted product could be suitably flavoured and enriched with dry fruits etc. This product has been found to have a high acceptance rating in a consumer study (Jha, 2000). Such a dry mix has a great potential to be commercially viable commodity in the Indian dairy products market.

2.1 Functional Properties of Instant Kheer Mix Powder

The physical nature of spray dried powders are vastly different from those obtained by other drying methods such as drum drying, freeze drying etc. In order to fully characterize the new product, systematic studies were conducted to analyze its functional properties (Jha et al., 2002). Some of the important functional properties are presented in Table 1. The powdered milk fraction has a better flowability and higher bulk density as compared to whole milk powder or skim milk powder.
Table 1. Functional properties of instant *kheer* mix powder

<table>
<thead>
<tr>
<th>Property</th>
<th>Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowability (angle of repose, degrees)</td>
<td>40.09 (38.30-42.64)</td>
</tr>
<tr>
<td>Wettability (min)</td>
<td>2.00 (2.00-2.00)</td>
</tr>
<tr>
<td>Dispersibility (%)</td>
<td>75.38 (69.73-86.51)</td>
</tr>
<tr>
<td>Insolubility index (ml)</td>
<td>4.00 (3.00-5.00)</td>
</tr>
<tr>
<td>Loose bulk density (g/cc)</td>
<td>0.69 (0.67-0.73)</td>
</tr>
<tr>
<td>Packed bulk density (g/cc)</td>
<td>0.81 (0.80-0.83)</td>
</tr>
<tr>
<td>Particle density (g/cc)</td>
<td>1.25 (1.25-1.25)</td>
</tr>
<tr>
<td>Occluded air content (cc/100 g)</td>
<td>6.63 (5.90-7.00)</td>
</tr>
<tr>
<td>Interstitial air content (cc/100 g)</td>
<td>45.00 (45.00-45.00)</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>44.80 (41.60-46.40)</td>
</tr>
</tbody>
</table>

2.2 Advantages Of Instant *Kheer* Mix Over Conventional *Kheer*

Product development work on *kheer* mix assumes greater significance in the context of numerous advantages, which it offers over the conventional product. Some of the important advantages of instant *kheer* mix powder are enumerated below:

- Process upgradation for the manufacture of traditional dairy products
- Shelf life of *kheer* enhanced to 6 months as compared to 2-3 days for conventional product.
- Considerable convenience to consumers - no sourcing of raw materials
- *Kheer* making time reduced to about 10 min as compared to nearly 1 hour in conventional process
- Safe and hygienic product in attractive packaging
- Value addition and opportunity for dairy industry for product diversification
- More avenues for tapping export market using indigenous dairy products
- Offers nutritional advantages of rice and milk in packaged form
- Nutritious and delicious food for our Armed Forces, which can be easily transported to difficult areas without any refrigeration
- Easy to be manufactured even by small scale industries and existing dairy plants having spare spray drying facility
- Low cost of manufacture
- Removal of drudgery for housewives and working women during conventional *kheer* making.

3.0 Developments In Instant Vermicelli *Kheer* Mix

In some of the studies conducted at DFRL, Mysore, a *kheer* mix has been developed based on reconditioned vermicelli, milk powder, sugar and flavourants. It is a nutritious, low
fat and energy rich food which provides 386 Kcal/100g. The product has been found to have the required chemical and microbiological stability for 6 months at 37°C (Jayathilakan et al., 2000). The product is highly suitable for all ages, particularly for hospital patients, school children and also useful for disaster relief operations.

4.0 Long-Life Rice Based Kheer

A process has been recently developed for in-package cooking and sterilization of kheer in retort pouches with the objective to enhance its shelf-life at ambient temperature. Sterilization was done in steam-air environment, using a Rotary Pilot Scale Retorting System employing a constant rotation of 2 rpm. Retort temperature and pressure were set at 121.1°C and 15 psi respectively. An overpressure of 30 psi was maintained to prevent bursting of pouches. Concentrated milk, raw rice (washed and soaked at 30°C/30 min.) and sugar were filled in retort pouches (size 200 mm x 170 mm) having a configuration of 12 polyester, 12 al-foil and 350 gauge cast polypropylene. Retort pouches used in the study had a bursting strength of 40 psig for 30 seconds and heat seal strength of 84 N/25 mm width in cross direction and 98 N/25 mm width in machine direction. Retort pouches were fitted with a temperature-measuring probe consisting of a Cu/Cu Ni thermocouple, a packing gland made of polyoxymethylene and two rubber O-rings. Time-temperature data were recorded during heat processing using an Ellab data recorder-cum- F₀ & cook value integrator. Heat penetration indices were determined by constructing a graph on an inverted semi-log paper (Temperature deficit in 0°C versus time in min.). A typical process studied had J = 0.514, fₜₜ = 6.30, U = 15.10 min., fₜₜ/U = 0.417, B = 19.65 min., Total process time = 24.29 min and F₀ value of 14.76. Kheer obtained by this process with a composition of fat 6.21 %, protein 10.75 %, total solids 28.67 %, ash 0.74 % and carbohydrates 53.63 % and the product had a shelf-life of more than 4 months at 37°C. This approach to enhance the shelf life of one of the important traditional dairy desserts could also be applied to other dairy/food products.

5.0 Long-Life Dalia Dessert

The advent of retort processing technology has made the availability of shelf stable Ready-to-Eat (RTE) foods a reality in the Indian market. A variety of lip-smacking Indian dishes such as Dal Makhani, Aluchhole, Chana Masala, Navrattan Korma, Palak Paneer, Sambar Rice etc. are now readily available off the shelf (Rangarao, 2002). With food processors like Tasty Bite Eatables, MTR Foods, ITC Foods, Satnam overseas, ADF Foods and many others dishing out newer and newer products to meet the demands of the Indian palate, the RTE foods business is reported to have reached a turnover of Rs. 60–80 crore during the current year (Menon, 2003; Bhushan, 2003). In line with the current scenario of ready-to-eat products picking up great markets, a commercial process for manufacturing dalia dessert has als been developed. Various wheat varieties (both durum and aestivum) were screened for their physico-chemical properties before converting them into dalia grains. The fully developed product contained 29.60% total solids, 3.56% protein, 0.84% ash and 21.54% carbohydrates including 2.88% amylase. Product in tin free steel cans had a shelf-life of 72 days at 37°C (F₀ = 4.15 and nisin 376 IU/g). Technology can be easily adapted by the user industry more diversified production operations in the increasingly competitive market.
6.0 Commercial Milk-Cereal Formulations

In India, Farex has introduced a wheat apple milk-cereal based weaning food. It has a balanced mix of essential nutrients which contain protein, carbohydrates, vitamins, minerals and iron. It is easy to digest and does not contain any artificial preservatives, colours or flavours. Weaning formulations have also been developed based on sorghum, groundnuts, sesame seeds, chikpeas and skim milk powder and processed by a twin-roller drum dryer. These formulations were found to have compositions and properties comparable to those of cerelac, which is a more popular weaning food (Mahgoub, 1999).

7.0 Challenges Ahead

Technology of making these appetizing delicacies remain confined to domestic level in the absence of any viable alternative for their commercial manufacture. Growth in this area has also been hindered because of the inadequate shelf life of these desserts for commercial marketing. To exploit the full potential of these products in terms of their nutritional qualities and foreign exchange earnings, strong support is needed to develop the processes for improving the shelf life as well as uniformity of product quality and hygiene. Valuations of several traditional dairy products such as ghee, khoa and chhana in terms of their annual output and their monetary contributions to the output of Indian dairy industry have been worked out. However, no such attempt has been made for kheer and dalia. One reason for lack of information is the absence of organized manufacturing and marketing of this product. In our country, large-scale marketing of several brands of ready-to-reconstitute kheer/payasam mixes on regional basis has been started especially in states like Karnataka, Kerala and Tamil Nadu. Most of these products are reconstitutable in milk and are basically a blend of processed rice or semolina, ground sugar, and dry fruits. Of late, such mixes have become very popular as they offer considerable convenience to consumers. Development of suitable processes for commercial manufacture of cereal-milk based convenience foods will hopefully provide a necessary fillip to the contribution of traditional dairy products in National dairy economy.

8.0 Suggested Readings

1.0 Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use. An expert panel commissioned by FAO and WHO defined probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” This is the definition that should be used, and probiotics should not be referred to as biotherapeutic agents.

Indeed, different products containing probiotic bacteria have gained in popularity with consumers. Strains of *Lactobacillus acidophilus* and *Lactobacillus casei* complex are well represented in commercial probiotic products, followed by *Bifidobacterium* spp. (*B. animalis*, *B. bifidum*, *B. breve*, *B. infantis* and *B. longum*), some other lactic acid bacteria (lactococci, leuconostocs, enterococci) and non-lactic acid bacteria (propionibacteria, aerobic bacilli, yeasts). The consumption of these micro-organisms may affect the composition of indigenous microflora and may have several beneficial effects on human health such as the maintenance of a balanced flora, alleviation of lactose intolerance symptoms, resistance to enteric pathogens, immune system modulation, an antihypertensive effect, antioxidative properties as well as certain anti-carcinogenic effects.

2.0 Guidelines For The Evaluation Of Probiotics

In May 2002, a joint working group of the FAO and the WHO drafted new guidelines for the evaluation of probiotics in food. FAO and WHO and the countries they represent requested guidelines and recommendations for the criteria and methodologies required to identify and define probiotics and establish the minimum requirements needed to accurately substantiate health claims. Although the FAO and WHO reports focused on foods, many of the recommendations, including the definition of probiotics, were endorsed at a May 2002 meeting of the International Scientific Association for Probiotics and Prebiotics. Based on these guidelines, several important criteria and standards must be introduced to ensure that consumers know that the products are of suitable quality and reliability. In brief, these guidelines address the following points: isolation of *Bifidobacterium* and *Lactobacillus* from their natural environments, classification into species, examining of their physiological and biochemical properties, survival in unfavourable physiological conditions of gastrointestinal tract, adherence to colon epithelium, antibacterial activity, enhancing immunological system, influence on ecosystem of gastrointestinal tract (microflora, activity of faecal enzymes of microbial origin, amount and proportions of SCFA in colon contents and histological changes of gut epithelium), bioavailability of calcium from diet, as well as ability to utilise oligosaccharides as non-digestible nutritional compounds.
2.1 Strain Identification

The first consideration is to identify and characterize the organism to the genus and species level with internationally accepted methods, such as DND-DNA hybridization and sequencing of DNA encoding 16S rRNA. For strain typing, pulsed-field gel electrophoresis is the gold standard, but PCR methods exploiting species specific primers or randomly amplified polymorphic DNA (RAPD) can also be used. Determination of the presence of extrachromosomal genetic elements such as plasmids can also contribute to strain typing and characterization. Once strains have been identified, their nomenclature must be corroborated by reference to the “Approved Lists of Bacterial Names” or updated lists cited in the International Journal of Systematic Microbiology. This is important, for example, to exclude the term Lactobacillus sporogenes, a species that is not recognized but which is used by a number of companies to describe the organisms contained within their products.

The second consideration for particular strains that are being targeted for probiotic use is to have a clear and consistent strain designation, such as L. casei Shirota strain or L. rhamnosus GG. This will allow physicians and consumers to track publications associated with that strain and to verify that the strain has indeed been shown to have probiotic benefits.

2.2 In Vitro and In Vivo Experiments

In the early 1980s, when a few groups were discovering probiotic strains of lactobacilli and developing the field, in vitro assays provided useful selection systems. Thus, characteristics such as adhesion to cells, production of bacteriocins, acids, and hydrogen peroxide, and the ability to inhibit adhesion of pathogens were deemed to be important to confer probiotic effects. These methods still have their place in characterizing strains, but they are insufficient on their own to define a probiotic organism. Simply put, the expression of such factors in vivo and verification that they comprise key mechanisms of action is needed before they can adequately predict the function of probiotic microorganisms in the human body. In vitro tests, such as bile salts resistance, can correlate with gastric survival in vivo. Of the other tests that require validation of in vivo performance, many still have merit in assessing and characterizing organisms as well as investigating potential mechanisms of action. For example, research that showed that lactobacilli could adhere to intestinal cells and signal mucus production which prevented pathogen adhesion was extremely valuable and provided a new appreciation for function in the gut. Various in vitro tests are given below:

2.3 Survival In Synthetic Stomach Juice

This test is important to judge the survival of bacterial cells while passing through the stomach, as conditions in the stomach are not tolerated by majority of the eubacteria. In the stomach/ small intestine model, strains can be selected for survival and growth under GI tract conditions, whereas the effect of probiotics, prebiotics and dietary intake on composition and various metabolic activities of the intestinal flora is studied in the colon model. The stomach studies are performed with synthetic stomach juice (8.3 g of Proteose Peptone, 3.5 g of glucose, 2.05 g of NaCl, 0.6 g of KH2PO4, 0.11 g of CaCl2, 0.37 g of KCl, 0.05 g of bile, 0.1 g of lysozyme, and 13.3 mg of pepsin dissolved in 1 liter of distilled water), adjusted to
pH 2.5 with 1 M HCl. The juice is heated to 37°C for 30 min and filtered in a sterile manner before use. To test tubes containing 10 ml of juice, 10 µl of *Lactobacillus* culture is added (giving approximately 10^6 CFU ml^-1). Samples are taken at 0, 30, and 180 min, and the survival rate measured by spreading 100 µl of different dilutions on MRS agar plates, which are incubated anaerobically at 37°C for 48 h.

### 2.3.1 Bile Salt Tolerance

The bacteria are examined for their ability to grow in the presence of porcine bile extract. One milliliter of an overnight MRS broth culture is added to 9 ml of MRS broth supplemented with 0.1, 0.3, 0.5, 1.0, or 2.0% porcine bile extract (B8631; Sigma, St. Louis, Mo.), and the bacteria are incubated at 37°C for 24 h. Growth is characterized as no growth, weak growth, or strong growth. Strong growth represents an OD comparable to that of a nonsupplemented culture, while weak growth is at least a doubling of the OD.

### 2.3.2 Assay For Toxicity Of Conjugated Bile Salt.

The strains are tested for the capacity to resist the bactericidal activity of a conjugated bile salt (TDCA). A stationary-phase culture inoculum (1%) is added to MRS broth supplemented with TDCA at a concentration of 0, 1, 3, or 5 mM. At zero time and after 1, 5, and 10 h of anaerobic incubation at 37°C, dilutions of the bacterial suspensions are prepared. Aliquots of the dilutions are smeared onto MRS agar plates, which are then incubated anaerobically at 37°C for 48 h. Population estimates are made from viable counts.

### 2.3.3 Mucus Binding Assay

The bacteria are grown at 37°C in MRS broth for 24 h. This medium is supplemented with 0.1% pig gastric mucin (M1778; Sigma) to test for the induction of binding. Microtiter wells are coated with mucus from pig small intestine. Wells coated with bovine serum albumin are used as a control. The bacterial strains are grown as described above, washed once in PBS supplemented with 0.05% Tween 20, and diluted to an OD600 of 0.5 in the same buffer. One hundred microliters of bacterial suspension is added to each well and incubated overnight at 2°C. The wells are washed with PBS–0.05% Tween 20, and binding is examined with an inverted microscope. The buffer is poured off and, after the wells have dried, the OD405 is measured with an enzyme-linked immunosorbent assay plate reader. All measurements are obtained in triplicate.

### 3.0 Safety

Probiotics are viable organisms, and therefore it is feasible that they could infect the host. Their occurrence as normal commensals of the mammalian microbiota and their established safe use in diverse food and supplement products worldwide support this conclusion. Nevertheless, side effects have been reported, including rare systemic infections. There is a need to be careful when administering live bacteria to immunocompromised subjects and those with intestinal bleeding. Care must also be taken to ensure that excessive immune stimulation is not induced in individuals who are susceptible to the development of arthritis or other complications.

The issue of safety becomes more complicated if one considers organisms such as *Enterococcus* spp. as probiotics. These bacteria are present in relatively high numbers in the intestine and are often included in so-called probiotic cocktails, particularly in animal feed.
However, enterococci have emerged as an important cause of nosocomial infections, and isolates are increasingly vancomycin resistant. The same is true of *Saccharomyces boulardii*, an organism used widely as a probiotic yet one which has been associated with episodes of fungemia.

A case has been made that *Enterococcus* and perhaps also *S. boulardii* not be referred to as probiotic for human use, but the onus should be on the producer that this and any other organism contemplated for human use not be a significant risk. For example, *E. coli* would not be regarded as a prime probiotic candidate because of so many of its strains are pathogenic, yet *E. coli* 83972 has been used quite effectively to prevent bladder infections in spinal cord-injured patients. That protocol was a prospective, nonrandomized, pilot clinical trial on 44 patients with spinal cord injury who had neurogenic bladder and had frequent episodes of symptomatic urinary tract infection. The bladders of the patients were inoculated with *E. coli* 83972, and among the 30 who became colonized with this organism there was a 63-fold reduction in the rate of symptomatic urinary tract infection versus the baseline prestudy period (mean, 0.06 versus 3.77 episodes of symptomatic urinary tract infections/patient-year, \( P < 0.001 \)). In this example, safety did not appear to be an issue, and indeed the benefits outweighed the risks as far as could be determined. A more subtle form of safety may involve minimizing the transfer of drug resistance genes.

In order to establish safety guidelines for probiotic organisms, recognizing that many are Generally Recognized as Safe, the FAO and WHO recommended that probiotic strains be characterized at a minimum with a series of tests, including antibiotic resistance patterns, metabolic activities, toxin production, hemolytic activity, infectivity in immunocompromised animal models, side effects in humans, and adverse incidents in consumers. One possible scheme for testing toxin production has been recommended by the European Union Scientific Committee on Animal Nutrition.

### 3.1 Phase 2 Clinical Studies

Phase 2 clinical studies assess the efficacy of a product against a placebo. The outcome for the individual should be a statistically and biologically significant improvement in condition, symptoms, signs, well-being, or quality of life, reduced risk of disease or longer time period to the next occurrence, or faster recovery from illness. More clinical evidence of this type is needed to gain credibility among the broader medical community. These need to provide a physician with the name of the strain, its product formulation, and the specific use for which it has been shown to be effective. Strains *L. rhamnosus* GG and *L. reuteri* SD2222 have accumulated some good clinical data, but trying to correlate these with the product formulations for sale in the United States is not simple. The GG strain is produced in liquid form in Finland, while in the United States the product is in capsules. The SD2222 studies are even less clear in that the strain number is rarely stated in published studies and is not on the label of the product sold in the United States.

### 3.2 Phase 3 Clinical Studies

Phase 3 studies assess the effectiveness of a product in comparison with a standard therapy for a particular disease. In general, randomized, blinded studies will provide the answer, assuming that sample size has been properly calculated and outcomes realistically predicted. Such studies should include quality-of-life measurement tools and consider risk-benefit ratios. For example, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor
product (statin) may reduce low-density lipoprotein cholesterol by 45% but may also cause rhabdomyolysis, kidney damage, or death (http://www.fda.gov/cder/drug/information/baycol/baycol-qa.htm). On the other hand, if animal studies that show that $10^4$ L. reuteri CRL1098 per day for 7 days prevent hypercholesterolemia and produce a 17% increase in the ratio of high-density lipoprotein to low-density lipoprotein could be repeated in humans, would this provide a clinically significant treatment option without side effects? In other words, phase 3 studies require careful planning and an evaluation of multiple endpoints before probiotics should be discarded from the health care armamentarium. The performance of more phase 3 studies on probiotic strains is required to determine fully their place, if any, in the treatment and prevention of more serious clinical conditions and whether or not this approach can replace or complement pharmaceutical use.

4.0 Survival In Product, Compatibility With Starter And Product Characteristics

Strains claimed to be present in a product should survive in relatively high viable cell numbers, retain metabolic activity and provide desirable organoleptic qualities. For instance, Bifidobacterium bifidum, B. breve, B. infantis and B. longum, which are species isolated from humans, can be used to produce fermented milks. It has been established that B. breve and B. longum could survive up to 15 d at a level higher than $10^6$ cfu.g$^{-1}$ in fermented milk made with mixed cultures of Lactococcus lactis ssp. lactis and Lc. lactis ssp. cremoris. Similarly, Bifidobacterium infantis and Bifidobacterium lactis cell counts remained higher than $10^6$ cfu.g$^{-1}$ after 3 months ripening in Cheddar cheese and goat cheese, respectively. During production of fermented products, bifidobacteria may be simultaneously added to the lactic starters. It is well known that changes in the composition of the starter could disturb the ecological system and lead to changes in the characteristics of the product. For instance, fermented milks containing bifidobacteria contained higher amounts of acetaldehyde, diacetyl and ethanol. From a general point of view, the impact of the addition of bifidobacteria on the kinetics of fermentation of lactic starters and their contribution to the organoleptic qualities of the resulting fermented product is little known in the literature.

5.0 Future Trends

The use of fermented milks as carriers of probiotic microorganisms is growing all over the globe. Yakult is already well established in Japan, South Korea and other countries in the East. Probiotic yoghurt is already commanding a good demand in the western countries and cheddar and other varieties of cheese are coming up fast. Dahi is a popular fermented milk in India and other South Asian countries. Probiotic cultures which are compatible with Dahi starters are being developed and there is a big commercial potential for probiotic dahi in this region. Molecular tools will continue to be used to understand and manipulate lactic acid bacteria with a view to producing vaccines and new and improved probiotic products. The critical step in wider application will be to make products available that are safe and clinically proven in a specific formulation easily accessible to physicians and consumers. Efforts are needed to advance the scientific knowledge of probiotics and determine their mechanisms of action, as well as describe when and why they fail in certain situations. Various processing advances, such as microencapsulation and bacterial coating and addition of prebiotic compounds used as growth factors by probiotic organisms, will provide a means to optimize the delivery and survival of strains at the site of action.
1.0 Introduction

There has been an upsurge of interest in the recent years, in the potential of food to promote health and prevent disease. Incorporation of beneficial bacteria into foods to counteract harmful organisms in the intestinal tract has been the most visible component of this new area. The theoretical basis for selection of probiotic micro-organisms include safety, functional aspects (survival, adherence, colonisation, antimicrobial production, immune stimulation, antigenotoxic activity and prevention of pathogens) and technological details such as growth in milk and other food base, sensory properties, stability, phage resistance and viability. Newer avenues as carriers of probiotic organisms are being sought. Cheese being a 'live' food, is potentially an excellent vehicle for these beneficial microbial cultures. Recognising the beneficial properties of probiotic organisms and challenged with the possibility that these organisms may produce end products that may be different from those produced by the normal starters in cheese, several attempts have been made to manufacture probiotic cheeses. This lecture, besides listing the technological bottlenecks faced while attempting to make probiotic cheese, reviews several studies on the manufacture of such cheeses.

2.0 The Current Market For Probiotic Foods

Consumers in Europe have a good understanding of the beneficial effects associated with daily consumption of dairy foods and the role of probiotics. The European market is set to triple in probiotic sales over the next six years, and is projected to reach $137.9 million in 2010. The U.S. market for probiotics is in its infancy, but with growing consumer awareness and more publicity, the U.S. market is projected to vastly overcome the European market with sales reaching $394 million. Marketing campaigns are insuring that live active cultures in fermented dairy foods carry a positive health image and blend well with the healthful properties of dairy products, such as building strong bones and maintaining a healthy weight. Brand leaders such as Dannon, Stonyfield Farms® and Yoplait are push probiotics in their yogurt formulations. Lifeway Foods has introduced a probiotic cheese spread and Horizon Organic Dairy offers cottage cheese containing *Lactobacillus acidophilus* and *Bifidobacteria bifidus*. In Europe and Japan, probiotics are marketed to enhance infant formulas (Anon., 2004).
3.0 Technological Challenges In Manufacturing Probiotic Cheese

The selection of suitable organisms that survive in the cheese as well as the choice of the appropriate variety of the cheese poses tremendous challenges before the researcher. The major concern in the incorporation of probiotic organisms in cheese is, whether or not their viable cells survive the normal processing and ripening conditions. There are significant differences among species and even strains of bifidobacteria with respect to the acid- and bile-tolerance of some of these organisms. Since most of the bifidobacteria are reported to be pH-sensitive, the choice of a strain that would survive the lowered conditions of pH in the cheese is imperative. Additionally, this strain should also be able to withstand the acidic conditions of the human stomach and tolerate the high bile salts concentration of the upper small intestine.

The relation between the normal lactic cultures that are used in cheesemaking and the probiotic bacteria that would be introduced is another area of technological importance. They should, from the outset, provide no competition to each other and should be able to survive in harmony, so as to produce no undesirable flavour or textural trait in the product. The amount of inoculum and the appropriate time of its addition in milk (or to cheese) are some other parameters to be investigated. Since bifidobacteria being slow growers, some workers suggest that the amount of inoculum must be large, often to the extent of 3 to 5 per cent, whereas others are of the opinion that such large quantities of cultures would lead to biochemical fermentations in the cheese that are atypical of the product.

The oxygen sensitivity of bifidobacteria is a major block in their successful cultivation in milk. This can be overcome by using super-concentrated bifidus cultures. Dairy products that are direct set with these cultures contain almost 4 million live bacteria per ml. The counts of bifidobacteria in cheeses increase during a ripening period, probably owing to the anaerobic conditions produced in the cheese.

4.0 Probiotics in Cheese

Hard cheeses, such as Cheddar, may offer certain advantages over yoghurt-type products in terms of delivery of viable probiotics, such as the reduced acidity of the cheese compared with yoghurt environments and the high fat content and texture of Cheddar cheese, which may offer protection to the microorganisms during passage through the gastrointestinal tract (GIT). Studies have demonstrated that bifidobacteria survived well in Cheddar and Gouda cheeses. One report (Stanton et al. 1998) documents the incorporation of a number of strains of probiotic lactobacilli into Cheddar cheese and assessment of their performance during ripening. These strains had previously been isolated from human upper GIT during surgery. Two L. paracasei strains grew and sustained high viability in cheese while L. salivarius strains did not survive during ripening. Thus, probiotic Cheddar cheeses containing high levels of L. paracasei strains (10^8 cfu/ g cheese) can be manufactured by conventional manufacturing procedures at a relatively low cost. Studies showed that incorporation of these strains had no negative effect on cheese quality, including aroma, flavour and texture. The cheeses also compared very favourably with yoghurt regarding delivery of viable cells to the GIT, despite the apparent age difference of the products. The major achievements and conclusions of this work are as follow.
Cheddar cheeses containing high levels of probiotic strains (L. paracase, Enterococcus faecium etc.) following ripening, were successfully produced using normal manufacturing procedures. The sensory quality of the cheeses was not adversely affected by the addition of probiotic strains and flavour was in fact enhanced by one probiotic strain.

A patent has been filed on the process, which has attracted intense industry interest and commercial trials are being conducted in one case.

In pig feeding trials it was shown that mature Cheddar, containing high levels of probiotic bacteria compares favourably with fresh yogurt as an effective delivery system in colonising the gastrointestinal tract.

A positive immune response (serum IgG) was found in pigs fed the probiotic strains.

Spray dried skim milk powders containing high numbers of probiotic strains, (even after storage at 4°C for 3 months), were successfully produced for use in functional foods including Cheddar cheese, yogurt, ice cream, etc. (Gardiner et al., 1998, 1999a, 1999b, 2000; Stanton et al. 1998).

Taking advantage of the consumer interest in the improved therapeutic and nutritional attributes of such cultures, American and European manufacturers have introduced several varieties of cheese with added bifidobacteria. Cheddar has been most abundant in this category, being marketed as the normal to reduced fat variety. Several reports suggest that bifidobacteria introduced into hard-pressed cheeses remain viable during storage for as long as 3 to 4 months and could be metabolically active.

The method of addition of the starter varies from direct addition in to the vat to addition in the form of lyophilized powder along with the salt (Dinakar and Mistry, 1994). The counts are reported to increase by four to five log cycles. This increase in counts have been attributed to the favourable anaerobic atmosphere developing in cheese during ripening and a simultaneous decrease in other lactic acid bacteria. The probiotic organisms are reported to have no effect on flavour, flavour intensity, texture or appearance of the cheese variety.

A hard-pressed Cheddar-like cheese was produced using micro-filtered milk standardised with cream enriched with native phosphocaseinate retentate and fermented by B. infantis by Daigle et al. in 1999. The bifidobacteria remained viable (above 3X10^9 cfu/g) in cheeses packed in vacuum-sealed bags kept at 4°C for 84 days. No significant difference was observed between cheeses produced with or without bifidobacteria for fat, salt, protein, moisture, ash and pH. No bifidobacteria grew during cheesemaking. After 12 weeks of storage, >56% of the alpha_s1-casein was hydrolysed in cheeses that were produced with bifidobacteria and above 45% of hydrolysis was observed in the control cheese, although there was no significant differences in the electrophoretic SDS-PAGE patterns. Small quantities of acetic acid were detected in bifidus cheeses.

Bifidobacterium sp. strain Bo and L. acidophilus strain Ki have been used by different groups of workers in cheeses. Malcata et al. in 1995 manufactured a 'probiotic' cheese from the milk of a native Portuguese goat breed using these organisms. Gomes et al. (1995) used a starter entirely composed of the same organisms for the manufacture of a Gouda-type cheese. High rates of inoculum (3.5%) were necessary to meet technological requirements. After 9 weeks storage period, the numbers of bifidobacteria and lactobacilli were 6-18 x 10^8 cfu/g and 0.2-5 x 10^7 cfu/g respectively.
In a study by Gobbetti et al. (1998) B. bifidum, B. infantis and B. longum were incorporated into Crescenza cheese individually or as multispecies mixture to a concentration of about $10^6$ cfu/ml cheese milk. When added individually, the cell counts of B. bifidum, B. infantis and B. longum were $\log_{10} 8.05$, $\log_{10} 7.12$ and $\log_{10} 5.23$ cfu/g respectively. Presence of bifidobacteria did not influence the aerobic microflora, the growth of Streptococcus thermophilus used as a starter culture or the composition of the cheese. No difference in primary proteolysis was observed, but higher levels of pH 4.6-soluble nitrogen and more pronounced activities of amino-, imino-, di- and tripeptidase were detected in cheeses with added bifidobacteria. The sensory scores for the control and experimental cheeses were similar.

Sabikhi and Mathur (2002) prepared probiotic Edam cheese incorporating B. bifidum (ATCC 15696). The probiotic cheese had over 7.5 log cfu/g of viable bifidobacteria after three months of ripening. The ingestion of the probiotic Edam cheese resulted in intestinal implantation of bifidobacteria in Albino rats with the concomitant reduction in the faecal coliform count. The anticarcinogenic activity of the bifidobacteria was also illustrated by the reduction in the activity of beta-glucuronidase, the faecal enzyme that converts procarcinogens to potential carcinogens (Sabikhi and Mathur, 2001, 2004).

Supplementation of cream dressing with freeze-dried concentrates is a suitable method of incorporating bifidobacteria in cottage cheese. Incorporation of these organisms may also be a way of making beta-galactosidase available for lactose-intolerant consumers.

Some probiotic cultures, (e.g., L. rhamnosus) produce antibacterial substances that act specifically against undesired micro-organisms such as clostridia. The use of these organisms is a possible replacement for nitrate addition to suppress the growth of gas-formers in cheeses like Edam and Gouda. Thus they promote the natural preservation, with minimised use of chemical preservatives.

Songisepp et al. (2004) attempted making a probiotic cheese based on the Estonian open-texture, smear-ripened, semisoft cheese ‘Pikannte’. The probiotic L. fermentum ME-3 reported having antioxidative properties was added into milk simultaneously with starter cultures (cheese A) and into drained curd (cheese B). The probiotic strain was found to withstand the technological processing of cheese, surviving and sustaining moderate antimicrobial and high antioxidative activity throughout ripening and storage of cheese. The ripened cheese contained approximately $5 \times 10^7$ cfu/g viable ME-3 cells.

5.0 Conclusion

Spurred by the biochemical, microbiological, nutritional and physiological role of live microflora derived from fermented milk products, there is increasing interest in realizing similar potential advantages from cheese varieties also. There is abundant scope in the development of diverse range of cheeses that may afford opportunities as carriers of probiotic organisms in the altered macro-environment of the product. Cheese manufacturers searching for avenues to diversify their enterprises would gain tremendously, in view of the enormous value addition that may be envisaged in making and marketing a probiotic cheese. Although some information is available about the successful incorporation of bifidobacteria in Cheddar cheese, paucity in the knowledge of manifestation of their presumed probiotic action as well as process modifications required to be done in other varieties of cheeses also.
6.0 Suggested Reading


104
1.0 Introduction

An increase in the consumption of several fermented milk products such as yogurt, Dahi (an Indian fermented milk product) acidophilus sour milk, cultured buttermilk, Kumis has been highlighted in the literature because of their palatability, and nutritional and therapeutic attributes. Among all the aforesaid products Lactobacillus acidophilus based milk and related food products occupy an important place, since acidophilus is a friendly bacteria which supports a favorable environment, encourages intestinal microflora balance and promotes the health functioning of the intestinal system. Acidophilus is a beneficial bacteria that synthesize nutrients in the intestinal tract and counteract pathogenic organisms. It may also be used for digestive maintenance and flora restoration after long courses of antibiotic treatment.

The history of L. acidophilus in fermented milk products begin with interest in Lactobacillus spp. initiated by Metchnikoff in the beginning of century. He developed a hypothesis on longevity and his well-known statement that “There are many useful microbes amongst which lactic acid bacteria (LAB) have an honorable place. These hypothesis and statement have stimulated a lot of extensive investigation on a beneficial effect of fermented milk till present and led to development of variants of particularly L. acidophilus-based dairy and non-dairy health foods.

2.0 Characteristics of L. acidophilus:

L. acidophilus is a member of one of the eight main genera of LAB. The genera, Lactococcus, Leuconostoc, Bifidobacterium, Carnobacterium, Enterococcus and Sporolactobacillus can then be divided into species, subspecies, variants and strains. L. acidophilus is best-known species of lactobacillus. (Jones, 1999) It is naturally found in human and animal G.I. tract, mouth and vagina. L. acidophilus is characterized as being a rod shaped bacteria grows in or without the presence of oxygen. L. acidophilus is also characterized as a homofermentative that only produces lactic acid as its sole product. It is being used as a probiotic or living organism, which upon ingestion in certain numbers, exert health, benefits beyond inherent basic nutrition (Wood, 1992).

2.1 Beneficial Effects of Intestinal Flora

The beneficial effects of intestinal flora usually called beneficial flora including lactic acid bacteria in particularly probiotic cultures such as L. acidophilus (Hosono, 2002) The beneficial effects of these cultures are:
• Inhibit the harmful bacterial by production of antimicrobial components like organics acids hydrogen peroxide and bacteriocin in the gastrointestinal (GI) tract.
• Lactic acid bacteria have the ability to scavenge the oxygen radicals because they can produce an enzyme called “superoxide dismutase”.
• So far there is no definite mechanisms explaining how lactic acid bacteria improve the immune effects, however, some researchers presumed that lactic acid bacteria increase the immune system by stimulating the B and T cells of human macrophage.
• Improve the digestion and absorption of food by producing various kinds of enzymes and by lowering the pH of GI tract and speeding up the transport of gastric contents.
• Some species of lactic acid bacteria have been shown to increase vitamin B complex in fermented foods and others could synthesize vitamin K in the colon.

2.2 Harmful Effects of Intestinal Flora

The intestinal flora, which is usually known as “harmful or putrefactive flora”, is able to synthesize and produce harmful substances during the growth in the gastrointestinal tract. The compounds produced are putrefactive products such as NH₃ and H₂S, phenols, amines, carcinogens as well as toxins, and they are responsible to cause the incidence of various diseases like diarrhea, constipation, cancer, hepatic coma, autoimmune disease, hypertension and opportunistic infection. The putrefactive compounds and other metabolic products formed in the presence of various fecal enzymes of intestinal flora as shown in Table-1. These enzymes have an important role in the incidence of diseases. For example, N-nitroreductase and azoreductase could produce nitro compounds from amines; β-glucuronidase involves in the formation of glucoronic acid and β-glucosidase forms diazomethane. All these formed compounds show intense carcinogenity and cause cancer in the intestinal tract and in the kidney.

Table – 1 Metabolic products of Harmful Microflora

<table>
<thead>
<tr>
<th>Fecal Flora Enzyme Activities</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophanase</td>
<td>Indole</td>
</tr>
<tr>
<td>Decarboxylase</td>
<td>Amines</td>
</tr>
<tr>
<td>Deaminase</td>
<td>H₂S</td>
</tr>
<tr>
<td>Urease</td>
<td>NH₃</td>
</tr>
<tr>
<td>Azoreductase</td>
<td>N-nitroso compounds</td>
</tr>
<tr>
<td>N-nitrosation</td>
<td>Nitrite</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>Hormonal substances</td>
</tr>
<tr>
<td>N-nitroreductase</td>
<td>Secondary amines</td>
</tr>
<tr>
<td>Nitrification</td>
<td>Neutral steroids</td>
</tr>
<tr>
<td>N-denalkylation</td>
<td>Acids steroids</td>
</tr>
<tr>
<td>Deconjugation</td>
<td></td>
</tr>
<tr>
<td>Dehydroxylation</td>
<td></td>
</tr>
<tr>
<td>Aromatization</td>
<td></td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td></td>
</tr>
<tr>
<td>β-glucosidase</td>
<td></td>
</tr>
</tbody>
</table>
3.0 Lactobacillus acidophilus as Therapeutics

Acidophilus is often recommended to as a safeguard during antibiotic therapy, which can suppress beneficial bacteria and trigger the growth of yeast infection. Acidophilus may offer general health protection, as well. Several studies suggest that its functions as an immunity enhancer, and may suppress the toxic effects of carcinogens. Specifically, acidophilus products are most commonly used to control following disorders and to maintain appropriate ecosystem.

3.1 Ease Irritable Bowel Syndrome.

Acidophilus may bring relief to many people suffering from irritable bowel syndrome (IBS), a constellation of gastrointestinal symptoms that include abdominal bloating, cramping and diarrhea. A recent study in the American Journal of Gastroenterology found that acidophilus showed a significant benefit in 50% of patients with the primary symptoms of IBS.

3.2 Control Diarrhea.

If diarrhea is due to antibiotic use, acidophilus will help to correct the bacterial imbalances caused by the drug. Some strains of traveler’s diarrhea may be weakened by acidophilus, perhaps because the immune-boosting effects of probiotics help to reduce intestinal inflammation.

3.3 Reduce Flatulence.

As it restores a healthy balance of bacteria in the digestive tract, acidophilus can keep gas-producers in the large intestine from multiplying. Increasing the gut’s level of good bacteria relieves flatulence while lessening gas and bloating.

3.4 Combat Vaginal Yeast and Other Infections Associated with Candida.

Yeast that normally lives in harmony in body, Candida albicans can begin to overgrow following antibiotic therapy. Which may lead to digestive disturbances, fatigue, and allergies, among other symptoms. Because they promote a healthy intestinal environment, acidophilus and other probiotics can help to halt Candida overgrowth.

3.5 Relieve Urinary Tract Infection

Lactobacilli such as acidophilus are the dominant members of healthy bacterial life in the urinary tract. Studies have shown that as “bacteriocins” or antibiotic-like substances, they are powerful enough to suppress Escherichia coli bacteria, the source of many urinary tract infections.
3.6 Battle Bad Breath.

As bacteria in your mouth work to break down food particles, they can multiply and release foul-smelling chemicals. Usually, good oral hygiene is adequate to clear out offensive mouth odors. But when one need extra help, adding acidophilus to the system can encourage more efficient digestion and reduce the number of odor-producing bacteria.

4.0 Selection of Strains of \textit{L. acidophilus} for the Development of Health Foods

Strains of \textit{L. acidophilus} suitable as starters for production of acidophilus fermented milk products and related health foods should be selected according to three main criteria.

- The strain shall belong to the species of Acidophilus.
- The strain shall be able to grow and survive in milk giving a product with acceptable organoleptic properties
- The strain as included in the fermented milk product and related health foods shall be able to pass through the stomach and shall have a favorable influence on the intestine. A fermented milk product shall by definition be fermented by the specified species It is not possible to conclude that a \textit{Lactobacillus}, isolated from the human intestine, belong to \textit{L. acidophilus} only by showing that it is obligate homofermentative and by characterizing it with phenotypic tests The final decision demands further tests including DNA-homology and comparison with the type strain.

A new selected strain shall fulfill the following technological criteria. It shall be able to grow in milk either alone or as a part of a mixed starter. Further the strain shall be able to hydrolyze lactose and ferment it to mainly L + lactic acid. During fermentation the pH shall drop to 4.7 or less in maximum 20 hours (Kurman, 1988). To limit the final acidity of the product, pH during storage shall not decrease to a value lower than 4.0. The proteolytic properties of the strain shall be low and balanced without forming bitter peptides, other off-flavour or result in wheying-off. The growth shall result in high number of surviving \textit{L. acidophilus} preferably considerably more than 100 million per gram of product.

In practice a fermented acidophilus milk has to be produced using the new selected strain, alone or as a part of a mixed starter. The properties of the product have to be examined and compared to existing specifications and the survival of \textit{L. acidophilus} has to be calculated. Considering a mixed starter all the strains of lactic acid bacteria, which are to be used in full-scale manufacturing have to be inoculated and grown together in order to ensure that none of them decrease the survival.

5.0 Development of \textit{L. acidophilus} based products

There are many types of fermented dairy products that use \textit{L. acidophilus} and the most familiar acidophilus sour milk, sweet acidophilus milk and acidophilus yogurt, acidophilus paste, etc(Gandhi,2002) Sweet acidophilus milk is consumed by individuals who suffer from lactose mal-digestion and intolerance, a condition that effect approximately 75% of the worlds population (Wardlow, 1999) Mal-digestion and intolerance occurs when enzymes (lactase) cannot break down lactose or milk sugar in the intestine. Failure to digest lactose results in discomfort, cramps and diarrhea .In addition to this there are cheese,
fermented vegetables, in which probiotic strains of *L.acidophilus* have been incorporated. Acidophilus is also being used in the form of tablets, capsules, and powder.

Acidophilus sour milk is made by inoculating the *L. acidophilus* bacteria in sterilized milk. After inoculation, the milk is incubated for 24 hrs. and yields a type of coagulated milk product that has a low content of lactose *L. acidophilus* is also used in the preparation of acidophilus yogurt. In this case *L. acidophilus* along with other lactic acid bacteria are added to heat-treated milk, followed by incubation to decrease the pH when milk becomes acidic, proteins in the milk break down and coagulate to form a gel. There are several *L.acidophilus* based fermented milk products which have been marketed in various countries as shown in Table-2. New research is underway to test more targeted health benefits associated with taking *L. acidophilus* supplements. Many manufacturers of this supplement link intake of *L. acidophilus* with a decrease in certain diseases like yeast infections, gastrointestinal distress and low immune activity. However, there needs to be more research in this area before their claims are substantiated.

**Table – 2 Therapeutic L. acidophilus Based Fermented Milk Products**

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Microflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidophilus milk</td>
<td>Many countries</td>
<td><em>L. acidophilus</em> Yoghurt culture + <em>L. acidophilus</em></td>
</tr>
<tr>
<td>Aco-Yoghurt</td>
<td>Switzerland</td>
<td></td>
</tr>
<tr>
<td>Cultra-Ab</td>
<td>Denmark</td>
<td><em>L. acidophilus</em>+ β. bifidum</td>
</tr>
<tr>
<td>AB-Yoghurt</td>
<td>Denmark</td>
<td><em>L. acidophilus</em>+ β. bifidum+ Yoghurt culture</td>
</tr>
<tr>
<td>Biogarde</td>
<td>Netherlands</td>
<td><em>S. salivarious</em> sub sp., <em>Thermophilus, B. bifidum</em></td>
</tr>
<tr>
<td>Yakult</td>
<td>Japan</td>
<td>*L. acidophilus, L. casei, β. bifidum &amp; Yoghurt culture</td>
</tr>
<tr>
<td>Miru Miru</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.0 Conclusion

There are enough evidence to support the beneficial effects of *L.acidophilus* based health food products, however some issue like, targeted health benefits, viability and stability of the culture in the standardized products, industrial standardization and consumer awareness are to look into.

7.0 Suggested Readings

Faro Jones (1999); *Lactobacillus acidophilus*; http://dwl.unl.edu
Gandhi, D.N. (2002); Potential application of lactic acid bacteria in the development of fermented milk products and in bioprocessing of whey. Indian Dairyman; 54((12):64-67
Wardlow, G.M (1999); Perspectives in Nutrition,Mebraw- Hill,.Boston
1.0 Introduction

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. The demand for low fat or no fat food products but with a good taste has increased. Fat contributes to flavour, appearance, creaminess, palatability and lubricity of food. Fat also carry lipophillic flavour compounds, act as a pre-cursor for flavour development and stabilize the flavour. 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. 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. However the processors have to take the existing legislative requirements in to account for the use of fat replacers in the formulations.

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. Fat replacers are categorized in two groups, i.e., Fat substitutes and Fat mimetics.

2.1 Fat Substitute (F S)

F S 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. F S resemble fat in physical and thermal properties and often referred to as lipid or fat based fat replacers. However their use in food products require legislative approval.

2.2 Fat Mimetics (F M)

The substances, which can imitate physical or organoleptic properties of fat are called F M or referred to as Texturizing Agents. These substances are often proteins or carbohydrates based fat replacers. F M bind water and denature or caramelize at higher temperatures. Caloric value of these compounds range between 0-4 kcal / g. F M 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 F M require use of emulsifiers for successful incorporation of lipophilic flavour compounds. F M cannot replace fat on gram for gram basis. It is suggested that the following three ingredients system is necessary for a good F M:

- 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.

The differences in the characteristics of fat substitutes and fat mimetics are summarized in the table given below.

Table.1 Comparison between Fat substitute 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 taste</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>

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.

4.0. Available 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. These include; fatty acid based products, protein based products and carbohydrates based products.
4.1. Fatty Acid Based Fat Replacers (Fat Substitutes)

4.1.1 Sucrose Fatty Acid Polyester

This is commonly known as Olestra. It is manufactured from saturated and unsaturated fatty acids of chain length of 12-carbon atoms and higher obtained from edible fats and oils. The process involves hydrolyzation 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 CHDs, 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.

4.1.2. 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. They provide and stabilize aeration, promote and stabilize foam, control syneresis, carry flavours, control rheology and provide lubricity. 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 FS include Sorbitol, Raffinose, Trehalose etc. Sorbestrin or Sorbitol polyester is another such product, which is a mixture of tri, tetra and penta esters or Sorbitol and Sorbitol-anhydrides with fatty acids.

4.1.3. 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(DDM), Esterified propoxylated glycerol(EPG), Trialkoxytricarballylate(TATCA), Trialkoxycitrate(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 alkylmalonic acid, synthesized by reacting a malonyl dihalide with fatty alcohol. It may be used for replacement of oil in food formulations and for frying purpose. EPG be used in frozen desserts, salad dressings, baked goods, spread and for cooking and frying. TATCA, TGE, and TAC are polyacarboxylic acids with two to four carboxylic acid groups esterified with saturated or unsaturated alcohol having straight or branched chains of 8-30 carbon atoms.
4.1.4. 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 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.

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. In some cases gels are formed which give a fatty mouth-feel. The interaction between lipids in food products and carbohydrate based F M is enhanced in the presence of a fatty acid based emulsifier and result into product which has properties more like fat. Carbohydrate based F M 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. Gums, starches, pectin, cellulose and other carbohydrate ingredients perform the functions of fat in foods by binding water. Fat free salad dressings contain xanthan gum and carrageenan as stabilizers. Carbohydrate based F M 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, crosslinking 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. Modified starch is a reduced caloric fat replacer supplying 1-4 Kcal/g energy.
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.

4.2.3.1. 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.

4.2.3.2. 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. Their applications include salad dressing, frozen desserts, sauces and dairy products.

4.2.3.3 Sodium carboxy 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 number of functions in processed foods.

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 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.

4.2.4.1. 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 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.

4.2.4.2. Oattrim

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, confectionery, frozen desserts, cereals baked goods and meat products.

4.2.4.3. 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. 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 FM is 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 FM 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. 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. Microencapsulated Proteins

Simplesse is one example of micro-particulated protein. It is manufactured from WPCs 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. 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. K-Blazer, Ultra-Bake., Ultra-Freeze, Lita are some other protein based FM which are prepared from different protein sources as well as different processes.

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.

6.0 Suggested Readings

1.0 Introduction

With the changing lifestyle in affluent and technologically developed societies diseases like obesity, diabetes and cardiovascular diseases have become major health problems. Developing countries are also no exception in this regard. 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, hypercholesrolemia, 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 low sugar and low fat products are designed so as to meet the dietary requirements of obese, persons at risk of CVDs, diabetics and persons on weight management diets.

A major part of milk produced in our country is being consumed in form of a wide array of dairy delicacies - widely diversified range of sweets and other specialties viz. burfi, peda, kalakand, sandesh, rasogolla, paneer etc. These dairy products are quite high in fat and sugar content, so their Low fat and low sugar versions help in greatly restricting the calorie intake. 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 confirm to legal requirements. Though it is very difficult to deliver all the functionality of the sugar as well as fat in the product particularly in following cases

- where sugar and fat ingredients constitute a large proportion of the original formulation, and
- where processing conditions require more sever heat treatment for example in heat desiccated dairy products. However in products which 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

Sweeteners, which can be used to replace sugars, fall into two basic categories (Nabors, 2002):

- 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. Intense sweeteners are those substances, which on weight basis are substantially sweeter than the common carbohydrate sweeteners such as sucrose.

Intense sweeteners are constituted of compounds that mimic the effects of sugars on the tongue and pass the human intestine unmetabolised without producing any calories. High potential 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. Functional attributes of a sweetening system in a formulation that need to be considered before choosing the ingredients to replace the sugar are shown in the figure.

**Fig. Functional attributes of a sweetening system**

Physicochemical properties of some important sugar replacers including intense sweeteners and bulking agents are discussed as below
2.1 Aspartame

It is a dipeptide ester, 200 times sweeter than sucrose and 1 g of aspartame provides 4 calories. Safety of aspartame has been extensively studied and Acceptable Daily Intake (ADI) for aspartame has been established to be of 40-mg/kg body weight by Joint WHO/FAO Expert Committee on Food Additives (JECFA). No effects have been noted in clinical studies at doses many times higher than those safe for human consumption (Nabors, 2002; Du Bois, 1992). Food products containing it are required to bear an informational statement for people having phenylketonuria (PKU). Stability of aspartame is dependent on various factors like water activity, temperature and Ph of the product.

2.2 Acesulfame-K

It is a potassium salt of Acesulfame, which is 200 times sweeter than sugar, is used as intense sweeteners in foods and cosmetics. It is excreted completely unmetabolized and does not interfere with the normal functions of the human body. It is approved for use in a wide range of products, including tabletop sweeteners desserts, puddings, baked goods, candies and soft drinks with an ADI of 15-mg/kg body weight by USFDA. It dissolves readily in water and is very stable in aqueous solution. It is not affected by pasteurization and can be used in UHT and sterilized soft drinks. There is no evidence that it can react with other food constituents and (Raymon-Lipinski and Klein, 1988). It has good synergy when blended with aspartame and sodium cyclamate (Mahindru, 2000).

2.3 Saccharin

It is commercially available in three forms: acid saccharin, sodium saccharin and calcium saccharin. Sodium saccharin is most commonly used form because of its high solubility and stability. JECFA has established 2.5-mg/kg body weight as ADI for saccharin. Taste profile of saccharine has bitter aftertaste.

2.4 Sucralose

It is 600 times sweeter than sugar available under the brand name “Splenda”. It is a derivative of sucrose with zero calorie content. Sucralose is white, odourless and biodegradable, free flowing powder. It can withstand high temperatures of UHT and pasteurisation processes, and can be used in cooking, baking and extrusion (Nabors, 2002). Sucralose is used in dairy products, confectionery, beverages, desserts, syrups and as a tabletop sweetener.

2.5 Other Sweeteners

Neotame is a derivative of aspartame, and 7000 to 13000 times sweeter than sucrose (Nofre and Tinti, 2000). It provides zero calories. It has a clean, sweet, sugar like taste with no undesirable taste characteristics and can be used in a wide array of beverages and foods alone or blended with other high intensity or carbohydrate sweeteners.

Sugar alcohol they are chemically defined as saccharide derivatives in which ketone or aldehyde group is replaced by hydroxyl group. (Dias, 1999). Polyols are often used in combination with low calorie sweeteners to provide bulk and improve texture and mouthfeel.
Some of the more useful bulking agents under this category are, Maltitol Lactitol, Sorbitol, xylitol.
Polydextrose is a non-sweet, highly soluble, randomly linked, highly branched glucose polymer available under the brand names polydextrose® and Litesse®. It exhibits functional properties suitable as sugar and fat replacer. It has been claimed to improve texture and palatability of low calorie food products like frozen desserts, puddings, baked goods, hard candies and soft chewy candies.

Maltodextrins are concentrated saccharide solutions or dry products derived from corn starch and have low DE (1-19). They are less highly converted and have very bland flavour (Dziezak, 1986). Maltodextrins have been reported to provide stabilizing effect, prevent coarser texture and improved meltdown.

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 the product
- Carrier for fat soluble components of the formulation

Fat replacers are divided into 2 main groups, fat mimetic 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 mouthfeel 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 (trialkoxycarballylate), 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 Aspects Related to The Use of Fat Replacers and Artificial Sweeteners in Milk Sweets

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 (whey protein concentrate) and Simplesse (commercial whey protein based fat replacers) are used for these products to overcome the above mentioned defects as well as increase the yield. Our experience with simplesse and WPC used in khoa shows that these 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 butter fat is replaced by these protein based fat replacers.

Milk sweets contain wide range of sugar levels ranging from 20 to as high as 70 percent. As sugar in sweets help to develop the desired body and texture of the sweets, its replacement with artificial sweeteners will adversely affect these properties. Therefore the addition of bulking agents becomes inevitable along with artificial sweeteners. This replacement directly affects the profitability of the product, owing to the lower yield of the of the product and higher costs of the bulking agents and artificial sweeteners. Most commonly used bulking agents, which are more compatible with sweets like burfi are polyols like sorbitol and lactitol. The following practical difficulties are likely to be encountered in using the bulking agents and artificial sweeteners under the normal technological protocols adopted for burfi.

- The excessive intake of polyols, such as sorbitol has laxative effect. So they need to be used in combination with other bulking agents.
- Burfi should have smooth texture, which is obtained by vigorous whipping and beating. If this practice is adopted in presence of maltodextrin at described temperatures, the product will become unacceptable due to excessive chewiness.
In manufacture of traditional dairy products a high heat treatment is employed during their processing and are generally stored at ambient temperatures. These conditions are unsuitable as far as stability of some of the artificial sweeteners is concerned.

As bulking agents like poyols and artificial sweetener do not participate in Maillard and caramalization reactions, these sugar free products cannot impart the typical brown color and flavor of some of the sweets like milk cake and brown peda.

5.0  Suggested Readings

TECHNOLOGY FOR INDIGENOUS DAIRY PRODUCTS USING ARTIFICIAL SWEETENERS

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1.0 Introduction

It is a recognized fact that a high calorie diet with inadequate physical activity results in overweight or obesity, which may lead to diseases like diabetes, hypertension, atherosclerotic cardiovascular disease, endometrial Cancer and gall stones. Obesity, the accumulation of excessive amount of fat, has become a major health problem. In order to maintain an average weight it is essential to have a right energy balance. In case of overweight or obese individuals a negative energy balance has to be achieved by intake of low energy foods without reducing the essential nutrients such as minerals, trace elements and vitamins, and by increased energy expenditure. However, with the modernization and comfortable lifestyle has resulted into excessive amount of energy intake contributing to the weight gain and ill health. A survey conducted in 1988-1989 in the city of Madras showed that the prevalence of diabetes mellitus in adults was 8.2%, which has increased to 11.6% in 1994-1995 (Ramachandran et al., 1997). According to WHO report there is increase in the number of diabetic people in developing countries as compared to developed countries and India will have largest number & people with diabetes by the year 2025 (Ramachandran, 2000).

The apparent connection between diet and health has increased the awareness for healthier eating. Consumers are showing strong interest in low calorie foods with reduced fat, reduced cholesterol, artificial sweeteners and enhanced fibre content. Low calorie foods can be divided into ingredients such as carbohydrates, proteins and fat, and additives, such as high intensity sweeteners, fat substitutes and bulking materials. Whenever a new food additive or ingredient is developed its safety aspects and risk management of the product are to be governed by the health authorities before giving its approval for use.

2.0 Types of sweeteners

Due to lot of efforts of researchers, now there are non-caloric and high intense sweeteners available, which do not have much food value but are useful as sugar substitutes in various food. Based on calorific value there are three types of sweeteners.

2.1 Caloric Sweeteners

Caloric sweeteners are those substances, which when consumed, not only provide sweet taste but also contribute 4 K calories per gram of substance. It includes sugar, honey, D-glucose, invert sugar, caramel maltodextrin, high fructose corn syrup and dextrose syrup (Babu and Bhat, 1997). Maltodextrin and fructose has been tried in the manufacture of frozen desserts with excellent organoleptic properties. Due to higher sweetening effect (40 times than sucrose) or fructose, it is possible to obtain sufficient sweetness in ice cream with a relatively small addition of fructose and without addition of artificial sweetener. When fructose or maltodextrin is used it is also possible to produce an ice cream with a low fat content (Bhandari, 2001). In several countries, fructose is allowed in ice cream for diabetics
and it has been observed that fructose upto 25 gms can be safely consumed daily be diabetics (Kilara, 1991)

2.2 Low Calorie Sweeteners

Low calorie sweeteners are those substances, which provide energy between 1 to 3 K calories per gam. Relatively they are less sweet than sucrose. Polyols are known to occur naturally in number of fruits, all vegetables, cereals, algae, mushrooms, seaweeds etc. Although they occur in nature, their concentration in plant is too low to enable cost effective commercial extraction. They are industrially obtained under high temperature by catalytic hydrogenation of the relevant saccharides. The calorific value fo all polyols is 2.4 Kcal / gm. Different polyols such as maltitol, lactitol, sorbitol and mannitol are very effectively used alone or in combination with other sweeteners in various food products.

2.3 Non-calorific sweeteners

Artificial or synthetic sweeteners are those intensely sweet substances, which do not normally occur in nature. They are the result of specific chemical reactions and their structures are very diverse (Lawerence, 1993). They are also termed as non-calorie sweeteners or low calorie sweeteners as they contain less or no calorie. Artificial sweeteners are very sweet and devoid of unwanted metabolic characteristics of sucrose. Non-caloric sweeteners include natural and synthetic. At present only a few artificial sweeteners have received government approval for use and these vary, depending on the country. Saccharin, Cyclamate and Aspartame are perhaps the most widely used sweeteners today. New artificial sweeteners with approval in a limited number of countries are acesulphame-K, Alitame and Sucralose. In our country also according to recent notification issued by PFA artificial sweeteners like saccharine, aspartame, sucralose and acesulfame-K are now allowed in the various dairy products like burfi, laddo etc. Previously they were only allowed in the carbonated water and soft drink concentrates.

Intense sweeteners are constituted from compounds that mimic the effect of sugar on the tongue, but do not react with biochemistry of the human anatomy. In other words they pass through the human body unmetabolised i.e. without producing any calories (Mahindru, 2000).

Table 1 Functional and ADI of Some Popular Intense Sweeteners

<table>
<thead>
<tr>
<th>Intense Sweetener</th>
<th>Energy Content</th>
<th>Sweetness in comparison to Sucrose</th>
<th>Acceptable daily intake (ADI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartam</td>
<td>4 Kcal/gm</td>
<td>130-200 times</td>
<td>40 mg / kg body weight</td>
</tr>
<tr>
<td>Acesulfane-K</td>
<td>Nil</td>
<td>200 times</td>
<td>5 mg/kg body weight</td>
</tr>
<tr>
<td>Sucralose</td>
<td>Nil</td>
<td>600 times</td>
<td>-</td>
</tr>
</tbody>
</table>

3.0 Bulking Agents

One of the limitation of these intense sweeteners particularly aspartame is its intense sweetness due to which very meager amount is needed. It contributes very little to increase the total solids of foods in which is added. The bulk that is provided by sugar is not seen in
the products prepared by the artificial sweeteners. The lost volume or bulk is replaced by bulking agents. Bulking agents are the compounds that act in conjunction with water to provide the missing bulk. Bulking agents binds water through molecular attraction, capillary action or by slowing the water molecule movement through gel. There are various bulking agents, which have been tried in various foods such as sorbitol, maltodextrin and polyols etc.

4.0 Low Calorie Traditional Dairy Products

Sugar is an important ingredient in the preparation of enumerable traditional dairy products, such as khoa and chhana based sweets and the various frozen dairy desserts. However, the high content of sugar at times makes it an undesirable item of consumption from health point of view, especially for diabetic and obese individuals. Recent studies revealed that one out of every twelve urban Indian population, above the age of forty, is likely to be diabetic by the year 2025 (Ramachandran, 2000). Keeping in view of this problem some of the traditional dairy products have been prepared by using artificial sweeteners.

Table 2: Average sucrose content in various dairy products

<table>
<thead>
<tr>
<th>Products</th>
<th>Average Sucrose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetened condensed milk</td>
<td>43</td>
</tr>
<tr>
<td>Non fat sweetened condensed milk</td>
<td>45</td>
</tr>
<tr>
<td>Ice cream</td>
<td>15</td>
</tr>
<tr>
<td>Chocolate ice cream</td>
<td>18</td>
</tr>
<tr>
<td>Infant milk foods (% of total carbohydrate)</td>
<td>5</td>
</tr>
<tr>
<td>Sherbet</td>
<td>33</td>
</tr>
<tr>
<td>Frozen Yoghurt</td>
<td>19</td>
</tr>
<tr>
<td>Flavoured sterilized milk</td>
<td>7</td>
</tr>
<tr>
<td>Rasogolla</td>
<td>52</td>
</tr>
<tr>
<td>Sandesh</td>
<td>33</td>
</tr>
<tr>
<td>Burfi</td>
<td>30</td>
</tr>
<tr>
<td>Peda</td>
<td>32</td>
</tr>
<tr>
<td>Kulfi</td>
<td>13</td>
</tr>
<tr>
<td>Misti dahi</td>
<td>13</td>
</tr>
<tr>
<td>Srikhand</td>
<td>41</td>
</tr>
<tr>
<td>Lassi</td>
<td>7</td>
</tr>
</tbody>
</table>

4.1 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.
4.2 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 and Sucralose have been developed. Other traditional dairy products such as burfi, Sandesh and Lassi are being tried to manufacture using different bulking agents and artificial sweeteners.
Process flow chart for the manufacture of artificially sweetened Kulfi
(Pandit Peeyush, 2004)

1. Buffalo Milk
2. Standardization
   - Fat/SNF ratio 0.66%
3. Open pan Concentration to 32% TS
4. Addition of bulking agent
   - i.e. Sorbitol 5.5% and Maltodextrin 4.26%
5. Cooling to room temperature
6. Dissolution of Aspartame
   @ 742 ppm in 5 times water at 35 to 40°C
7. Addition of flavouring
   - i.e groundnut cardamom @ 0.1%
8. Filling in to Moulds
9. Freezing in ice and salt mixture
   - In ratio 4:1 with constant agitation
10. Kulfi
11. Storage in Deep Freezer

5.0 Suggested Reading

1.0 Introduction

Ice cream is a delicious, wholesome, nutritious frozen dairy food. Its past goes back to century, but it’s future seems endless. Ice cream represents one of the most dynamic sector of the dairy industry and over the last two decades there have been considerable projects in developing novelty value added ice creams. Now a days, there is a trend for health foods. Low calorie, reduced calorie and diabetic foods are becoming increasingly popular in all segment of food industry. According to recent survey conducted in India, on routine market research, the consumers have to cut back on the ice cream consumption because of fear of adverse impact on health (Ray, 2000). A market survey in the U.S.A also confirmed a strong interest in low calorie frozen desserts and ice cream (Carson, 1988). Hence, healthier ice cream and frozen desserts for specific group of people will be the focus point of prominence for the Indian ice cream manufacturer in the coming decade. Ice cream and frozen desserts, generally considered rich in calories and indulgent, are now required to be of special category, which can be achieved by reduction of fat and sugar, incorporation of fruit and vegetables pulp and fibre into ice cream to give the benefit of nutraceuticals.

2.0 Low Fat or Reduced Fat Ice Cream

In recent years, demands for low fat and non-fat dairy products has shown considerable increase. But the low fat level in ice cream results into texture and flavor impairment of the product. As a result, product development require use of 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 involve 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. Several products with extender and/or fat replacer properties are now commercially available in the market. These fat replacers create the creamy sensation and improve the meltdown properties. Some of research workers have made efforts in this direction to develop low fat product using fat replacers. Schmidt et.al. (1993) reported that ice cream mixes made with protein based fat mimickers had similar rheological and melting properties as compared to control but higher air incorporation. Whereas use of carbohydrate based fat mimickers changed the rheological properties resulting in higher viscosities, greater deviation from Newtonian flow and less air incorporation.
Prindiville *et al.* (1999) analyzed low fat and non fat chocolate ice cream made with 2.5% milk fat, cocoa butter or one of the two whey protein based fat replacers, Dairy Lo or Simplesse, they reported that Simplesse was more similar to milk fat than Dairy Lo in its effect on brown colour, cocoa flavour, cocoa character and textural stability but was less similar in terms of thickness and mouth coating.

Under the provision of Nutritional Labeling and Education Act (NLEA), frozen desserts can now be labeled “Reduced fat” if the reformulated product contain 25 percent less fat than the original formulation. The term “Lite/Light” can be used if a 50 per cent reduction of calorie is achieved. A product can be labeled “Low fat” if one serving of the food contains no more than 3 grams of fat and food may be labeled as “Fat free” if it contains less than 0.5 grams of fat per serving. Serving sizes are defined and an average serving size for ice cream and frozen desserts is 118.75 ml (Kilara, 98).

### 3.0 Low Calorie Frozen Desserts

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. A number of bulking agent for use in ice cream/frozen desserts e.g., Litesse polydextrose (1 Kcal/g), Lacitol (2 Kcal/g) and Maltodextrin (4 Kcal/g) are available in the market. In the west, ice cream and frozen desserts have been tried using aspartame as a sweetener with a variety of bulking agents based on polydextrose, microcrystalline cellulose etc. (Wolkstein, 1986). A calorie reduced frozen dairy dessert was formulated using aspartame as the sweetener and polydextrose as bulking agent. Panel data showed that microcrystalline cellulose played an important role in the formula. The taste panel data also showed that usage level of aspartame between 0.05-0.1 percent and a maximum of 12 per cent polydextrose produced acceptable product. The total energy reduction was found to be 30-40 per cent (Goff and Jordan, 1984).

Rothwell (1985) developed a formulation for diabetic and dietetic ice cream and one which uses polydextrose include 4% fat, 15% polydextrose, 0.5% microcrystalline, 0.2% sodium citrate, 11.3% SNF, 0.75% stabilizer and emulsifier and 0.75% aspartame. Acceptance of this ice cream was described as ‘good’. Steinsholt and Longava (1985) suggested an acceptable low calorie ice cream product with 50% less energy than standard ice cream. The manufactured ice cream contained 11% SNF, 4% fat, 0.8% stabilizer, 0.05% aspartame and 12% polydextrose. Addition of 1.5% microcrystalline cellulose had no effect on product quality. The hardness of the product was reduced by adding 1% glycerol.

Verma (2002) studied the preparation of low fat/sugar free frozen dessert by using aspartame, acesulfame-k, sucralose and sodium saccharine as artificial sweeteners and Maltodextrin and sorbitol as bulking agent with different level of low fat combinations. The panel report showed that frozen dessert with 5.0% fat, 12.5% MSNF, 9.9% maltodextrine, 9.3% sorbitol, 1.5% WPC and 0.3% stabilizer and emulsifier with 400 ppm aspartame was the best combination. He concluded that artificial sweetener can compensate sweetness but to get desired body and texture, maltodextrin and sorbitol were necessary. No sign of
deterioration in terms of flavour, body and texture, melting quality, and colour and appearances were observed during the storage period of 90 days. In India, PFA permits the use of artificial sweeteners such as aspartame, acesulfame-k and saccharin in soft drink concentrate and carbonated water, but not in ice cream and frozen dessert.

4.0 Low-or Reduced Lactose Ice Cream

A significant number of consumers suffer from some degree of inability to completely digest lactose. These lactose malabsorbers sometimes experience discomfort in the lower bowel when lactose that escape being absorbed in the small intestine is fermented into acid and gas in the colon of the individual. The situation for persons who are highly affected is to consume frozen desserts in which there is low amount of lactose. Such product can be made by either ultrafiltering and/or diafiltering the milk to remove much of the lactose or by prehydrolysing the lactose (Marshal et al., 2003). Marshal has made high quality reduced lactose goat milk ice cream from goat milk concentrated by ultrafiltration. Geilman and Schmidt (1992) developed formulas for ice cream made with ultrafiltered and diafiltered cow’s milk. They found, as has others (Hofi, 1989; Masters and Kosikowski, 1986; Tong et al., 1989) that these products were harder and melted more slowly than those produced with equivalent non milk solids, probably because the ultrafiltered retentate retain virtually all the proteins but loose two-third of the lactose. The traditional mix contained 4 percent protein and 6.6 percent lactose, whereas mixes made with ultrafiltration contain 9 percent protein and 2.2 percent lactose. The type of sugar added in replacement of lactose caused significant differences in hardness of finished products. The removal of lactose by ultrafiltration reduces the concentration of dissolved solids in the product and raises the freezing point. On the contrary, hydrolysis of lactose produces two molecules for every lactose molecule hydrolysed and therefore, lowers the freezing point. Removing 50 percent of lactose by ultrafiltration and then hydrolyzing the remaining portion of lactose can keep the freezing point at the level (Marshal et al., 2003).

5.0 Nutraceuticals and Functional Foods

The international food information council (IFIC) has defined the functional foods as “foods that provide health benefits beyond basic nutrition”. A more concrete definition was provided by the Institute of Medicine of US National Academy of Sciences, which defined functional foods as, “Those foods in which the concentration of one or more ingredients have been manipulated or modified to enhance their contribution to a healthful diet, while a nutraceutical is any substance that may be considered as food or part of food and provide medical and health benefits, including the prevention and treatment of disease.” To provide added nutritional value using our knowledge of nutrition and health relationship the functional ice cream and frozen desserts can be designed in the following ways:

5.1 Pre- and Probiotic

Recent interests in prebiotics and probiotics in foods have caused product developers to attempt to gain market advantage by creating healthful frozen desserts. Many consumers have considered frozen yoghurt, which is fermented by “friendly” bacteria, as more healthful than low fat ice creams. Due to its nutritional value and refreshing taste, yogurt has become
the choice of people interested in health, athletics and weight control. In this respect, frozen yoghurt has become a delicious alternative to ice cream. This product contains the nutritional value of yoghurt and refreshing taste of ice cream. Among the probiotic bacteria are species of Lactobacillus and Bifidobacterium. These bacteria thrive when they are consumed with prebiotic materials such as fructooligosaccharides (FOS). One supplier of FOS claim that not only does its product, NutraFlora®, enhance the growth of probiotic bacteria in the human intestine, but also reduce the beany flavour of soy and the bitter flavour of the aspartame by masking those flavour notes.

Lot of work in this direction has already been done. Ice cream and frozen yoghurt have been developed containing both probiotic and prebiotic carbohydrate (Laroia and Martin 1991; Hekmat anh McMohan 1992; Christiansen et al. 1996; Ravula and Saha 1998; Haynes and Playne 2002). Haynes and Playne also examined the use of resistant starch as a prebiotic ingredient in ice cream. Once frozen, the studies showed that the viability of the probiotic organisms in ice cream changed little over a one year period and so the ice cream provide a stable matrix for the consumption of probiotic strains.

A probiotic Kulfi was developed using probiotic culture Bifidobacterioum and L. acidophilus. Such Kulfi contain high level of probiotic organisms, even after four weeks of frozen storage. This type of Kulfi becomes a good source for delivering these probiotic bacteria to the consumers and has proven health benefits (Rao and Prakash, 2004).

5.2 Addition 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, and helpful in regulation of fat metabolism etc. Ice cream and frozen desserts can be used as a vehicle to deliver bioactive peptides.

5.3 Vitamin and Calcium fortification

Ice cream and frozen desserts can be most readily used for nutrient fortification and inclusion of nutraceuticals and vitamins. 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).

5.4 Fiber enrichment

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 soluble fiber has numerous health benefits, as it is effective in controlling obesity, stroke, diabetes, cancer and gastrointestinal disorders. Fibre is a substance found only in plants, such as fruits, vegetables, and grains. It is well known that fruits and vegetables are rich sources of fibres, minerals and vitamins. So, this could be well achieved by incorporation of fruit and vegetable pulp into ice cream.
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.

The US Food and Drug Administration has authorized the following claims that food manufacturers can place on their labels:

- Diets low in saturated fat and cholesterol and rich in fruits, vegetables and grain products that contain fiber, particularly soluble fiber, may reduce the risk of coronary heart disease.
- Diets low in saturated fat and cholesterol that include 3g of soluble fiber from whole oats per day may reduce the risk of heart disease.
- A low-fat diet rich in fruits, vegetables and whole grain containing fiber (particularly soluble fiber) may lower blood cholesterol levels and reduce risk of heart diseases. This type of diet may also reduce the risk of some types of cancer.
- Diets low in saturated fat and cholesterol that include a daily intake of soluble fiber from whole oats or psyllium seed may reduce the risk of heart disease.

Polydextrose could be the economical source of fibre for incorporation into ice cream. Another fibre source is Nutrina’s Soy Fiber, which contains both soluble and insoluble fiber. Soluble fiber acts as nutrition for the digestive tract while insoluble fiber speeds the passage of foods. This fiber mixes readily with milk or juice and has a neutral taste.

Sensing the need of the health conscious consumers Amul has recently launched a new variety of isabgol-enriched ice cream. Isabgol is the seed derived from Plantago ovata, which is mainly cultivated for the ‘mucilage’ or husk, obtained from the seeds outer layer (coat). Being a ‘true dietary fibre’, the isabgol husk is considered to be a natural laxative that aids easy bowel movement. Besides it is also known to possess serum cholesterol reducing properties (Damodaran, H. 2002).

6.0 Conclusion

Consumers are becoming more and more health conscious. As a result of this, the demand for healthy foods with functional benefits is increasing constantly. Nutraceuticals has opened a new vista for the food scientist to develop range of products with proclaimed health benefits. Ice cream and frozen desserts, which are considered indulgent due to their high fat and calorie content, now need to be reformulated without compromising the taste and texture.

7.0 Suggested Readings


http://www.healthcatalog.com/soy_fiber.htm


SPECIALIZED PROCESSED FOODS FOR METABOLIC DISORDERS

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1.0 Introduction

In recent times, studies have shown that moderating the diet can control several of the metabolic disorders. The processed food industry has also realized this fact and producing a variety of foods with appropriate composition associated with appropriate processing parameters to produce foods suitable for different metabolic disorder conditions. Natural colours in foods have also been linked to good health as detailed in Fig. 1.

Table 1 Natural Colour and Ailments

<table>
<thead>
<tr>
<th>Colour</th>
<th>Ailment</th>
<th>Food</th>
<th>Dosha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violet</td>
<td>Nerve and skin related problems</td>
<td>Brinjel, fruits like jamuns</td>
<td>Pitta</td>
</tr>
<tr>
<td>Indigo</td>
<td>Secretory gland and urinary tract problems</td>
<td>Fruits like mulberries</td>
<td>Pitta</td>
</tr>
<tr>
<td>Blue</td>
<td>Gland and throat problems</td>
<td>Fruits like blueberries</td>
<td>Pitta</td>
</tr>
<tr>
<td>Green</td>
<td>High blood pressure, ulcer and skin problems</td>
<td>Green cabbage, green peas, fruits like green grapes and guavas</td>
<td>Vaata</td>
</tr>
<tr>
<td>Yellow</td>
<td>Liver, constipation and bone-marrow related problems</td>
<td>Yellow coloured pulses, fruits like lemons and pineapples</td>
<td>Kapha</td>
</tr>
<tr>
<td>Orange</td>
<td>Mental problems, asthma, gout, kidney, fever, bleeding</td>
<td>Carrots, fruits like oranges and ripe papayas</td>
<td>Kapha</td>
</tr>
<tr>
<td>Red</td>
<td>Anaemia, circulatory and bone related problems</td>
<td>Beetroots, tomatoes, spinach, fruits like apples and strawberries</td>
<td>Kapha</td>
</tr>
</tbody>
</table>

2.0 Special Dietary Foods

Foods meeting special dietary needs can be classified into two categories.

2.1 Foods supplying a special dietary need resulting from physical, physiological, pathological or other conditions, such as convalescence, allergic response to food, underweight, overweight or need to control sodium intake.

2.2 Foods that meet the normal but distinctive physiological conditions of infants, children, pregnant and lactating women, the aged, and those required to cope with intense physical exertion.

Examples of processed foods belonging category 1 are: high protein foods; low protein foods; low calorie foods; high calorie foods; foods with modified carbohydrates; low fat foods; foods with low levels in certain amino acids; low gluten foods and low sodium foods.
Examples of processed foods belonging to category 2 are Infant; weaning foods; foods for pregnant and lactating women; and geriatric foods.

3.0 Low Calorie Foods:

A medical practitioner recognize over weight as an abnormal physiological condition since it places a considerable strain on the biological system of the organism and often leads to diabetes, heart disease, etc. Weight reduction is considered important in the treatment of renal and bladder diseases. Low calorie foods are essentially of two basic types. In the first sugar is replaced by non-calorie sweeteners, as in jams, canned fruits and confectionery. In the second, bulking agents, which do not supply energy, are used, such as agar, cellulose derivatives, pectins, gums and carrageenans.

In U.S. Food & Drug Regulations, a ‘Low calorie’ food must not have more than 40 calories per serving and no more than 0.4 calories per gram. A food may be called “reduced calorie” if the calories are reduced by at least one-third that of the traditional product. The availability of aspartame with almost no odour, a clean sweet taste and sweetness 180-200 times that of sucrose has opened up exciting opportunities for formulating a wide range of low calorie foods in the Indian context.

Formulated dietary foods that satisfy all the nutritional needs, except energy have been manufactured successfully. In one classic example, intended for consumption as a drink, one packet of 225 g. provides the full daily requirements of all the nutrients for adults, but only 900 calories in place of the usual 2200-2500. Such preparations are easy to make from basic foods such as skim milk powder, casein and soy flour, with the addition of a limited amount of carbohydrate and the full complement of vitamins and minerals. Alternatively, they can be made in tablet or biscuit form, sometimes with added carboxymethylcellulose. Low calorie soups based on dehydrated vegetables or purees and guar gum have also been commercially marketed.

The availability of the bulking agent like Polydextrose (a polymer of dextrose providing only 1 calorie per gram) has contributed to the development of low calorie foods that have palatability and texture without sweetness, like baked goods, salad dressings, frozen dairy desserts, confectioneries etc. Another significant development is the commercial availability of sucrose polyester, a non-calorie fat substitute.

4.0 Foods with Modified Carbohydrates:

These foods are designed for abnormal physiological conditions where utilization of some carbohydrates is impaired. For example, a deficiency of intestinal enzymes such as lactase leads to incomplete absorption and utilization of disaccharides. Many commercial formulations are available with hydrolyzed lactose.

5.0 Low Fat Foods

Several physiological disorders interfere with the normal processes of dietary fat utilization. A low fat diet is recommended in the treatment of the following conditions:

- Fat malabsorption due to inadequate intraluminal digestion or absorption.
- Disorders involving a defect in the lymphatic transport of fat.
- Type 1 hyper – lipoproteinemia caused by abnormal metabolism of fat.

Since fats are concentrated forms of energy, low fat foods are used in weight control programmes. Typical low calorie, low-fat foods include salad dressings, egg substitutes and
margarines. In reduced fat salad dressings, the 30-35% fat component is compensated and modified by incorporation of vegetable gums and carboxy methylcellulose (CMC) or modified starches.

Incorporation of soy flour in frying batters has been shown to prevent excessive fat absorption. For example, in doughnut frying addition of 4% soy flour reduced fat absorption by 60%. The mechanism is not clear, but could be due to a heat denatured protein layer at the doughnut surface acting as a fat barrier. There is thus a distinct possibility of having low fat fried foods such as puris, pakodas, sevs etc. through supplementation of the basic wheat flour or Bengal gram flour with soy flour to the extent of 5 to 20%. In puris for example addition of 5% soy flour leads to 20-35% reduced fat absorption. Similarly many hydrocolloids are gaining attention for their ability to reduce fat uptake during deep fat frying. Use of textured vegetable proteins (TVP) derived from soy flour offer innovative opportunities for designing low fat foods such as hamburgers, cutlets, kababs and so on.

6.0 Low Sodium Foods

Sodium chloride is an essential nutrient in the human diet and occurs naturally in most foods. Sodium chloride is added during cooking as well as in processed foods flavour enhancer, dough conditioner, curing agent preservative and so on. Safe and adequate levels of sodium intake as established by the Food and Nutrition Board of the U.S. (in mgs per day) from 115-350 for children below 6 months, 250-750 for children 6-12 months, and so on upwards to 1100-3300 for adults.

Fluid retention or edema in the body results from an increase in sodium concentration in plasma under surgical clinical conditions, like nephritis, cardiac disease, liver cirrhosis, hypertension and toxaeamias of pregnancy.

The most successful treatment for control of edema and hypertension is restriction of sodium intake through the diet. The salt or sodium content of the diet should be restricted as required by the severity of the disease, using the following guidelines.

- Mild restriction as in mild heart disease, to 2-3g/day b) deleting highly salted foods from the diet and permitting light salting of cooked foods.
- Moderate restriction, in patients with edema to about 1g/day by deleting sodium chloride completely from the diet, and permitting only foods low or moderate in sodium.
- Severe restriction, as in congestive heart failure, severe edema and hypertension, to about 0.5g/day. Only foods low in sodium and specially formulated low sodium foods can meet this requirement.

Use in food manufacture of sodium hydroxide and of salts that would lead to uptake of sodium by the foods, must be eliminated, such as use of sodium salts in blanching and lye-peeling, or the use of brine in preparing fruits and vegetables to prevent discouloration and so on. Ion exchange and electrodialysis offer means of reducing sodium content during processing.

According to the Codex Alimentarius Committee, “Low Sodium Foods” are defined as those containing not more than 120 mg per 100gs of the final product. “Very Low Sodium Foods” are those with not more than 40 mg sodium pr 100g.

Commercially, several products are available in advanced countries from which the usual added salt has been omitted. For example, special low sodium baking powders are used made up of potassium bicarbonate: 39.8g, corn starch.29.0g, tartaric acid: 7.5g and potassium
bitartrate: 56.1g. One and half volumes of this mix is equivalent to one volume of standards baking powder.

Milk contains 70 mg sodium per 100g and so is permitted in limited amounts in the 500 mgs sodium diet but not allowed at all in the 250 mg sodium diet. Such limitation on milk intake severely reduces the nutrient content of the diet and low sodium milks are therefore useful. There consist of spray-dried powders of blends of vegetable oil, casein, lactose, vitamins and minerals. A number of low sodium salt substitutes have been marketed, with potassium chloride as the base and various additives, flavour enhancers and free flowing salts in addition.

7.0 Conclusion

A clear understanding of human nutrition and the nexus between diet and disease has forged a very healthy collaboration of nutritionists and food processors in the developed countries in marketing a whole range of dietary foods for various specific metabolic disorders. The industry in our country has barely scratched the surface of such manufacture. Cost apart, one of the major constraints in the development of such dietary foods is the non-availability of food additives with specific functionalities so very essential in building the necessary consumer appeal into the finished product. We have tended to consider food additives with a jaundiced view for too long to permit any meaningful new product development and it is time that clear guidelines are set forth for the new food processing ministry to act on. It is hoped that at least some of the dietary foods covered in this paper would see the light of the day in our country. It would indeed be most propitious if the special dietary foods are considered neither special nor dietary but as healthy foods with nutritional breadth and balance to meet the needs of the healthy as well as the diseased population.
1.0 Introduction

In modern times packaging has been identified as an integral part of processing in the food industry. Packaging is science, art & technology of protecting products from the adverse effects of the environment. Consumer preferences and new technological innovations have always been the major forces that led change in the food industry. Packaging of products materially contributes to trade promotion and conserves valuable manpower and raw materials that have gone into their making. Worldwide packaging industry is estimated to be worth $300 billion. Projected growth rate of demand and consumption for packaging in India is 10%. In the past century particularly, the increase in population, urbanization, education, life spans and communication drove the food industry to be large scale and health conscious, with safety, attractiveness and convenience of foods being the forerunner of technological transformations.

2.0 Health Beverages: Market And Scenario

The beverage market is a diverse business involving brands and private label, refillable and recyclable one-way packaging, licensers and licensees, turnkey or industry standard packaging, and custom designs for gift-wrapped and commodity favourites. The impact of efficient consumer response (ECR) is increasing and will affect packaging sizes and formats, particularly in respect of secondary and tertiary packaging. Today’s consumers want more than just refreshment from their drinks. They must be functional as well as satisfying. Packaging serves a number of important functions in the beverage industry. It must protect the beverage throughout its lifetime, from the filling of the container to consumer use. Beverage packaging consists of beverage cans, bottles of glass and plastic, pouches and cartons. These come with a different variety of closure systems and pack everything from vodka to water, beer to carbonated soft drinks (CSDs).

Today’s consumers have turned into a demanding bunch. First, they started to demand that their foods provide the convenience that matched their increasingly busy lifestyles. Now they want their beverages to “do something” for them. Beverages must fit with their latest diet, be it low fat, low carbohydrate, low sugar, or something else. They must boost flagging energy, rehydrate exercised bodies, calm and soothe rattled nerves, or provide vitamins and minerals for better health. “Refreshment is the cost of entry, and consumers are looking for added value benefits beyond refreshment”.

The result has been a plethora of products in what is vaguely defined as alternative beverages or enhanced beverages. They are as simple as calcium-enriched orange juice and as complex as an herbalist’s concoction. The near endless variety offers great opportunity for convenience stores because most of these beverages are sold in single-serve packages and are generally regarded as impulse purchases. They are not cheap and can provide a healthy profit margin. Consumption of commercial beverages around the world is growing @ 3% per annum in volume terms. Rates of growth vary depending on the type of beverage and region.
The average global consumer drinks 190 litres a year of commercial beverages but consumers in North America, Australia and Western Europe now consume more than three times that.

2.1 Carbonated Soft Drinks (CSD)

Though carbonated soft drinks cannot be considered as health beverage but because of the higher energy content and addition of certain   It is the largest single packaged beverage segment and is still growing at around 2% per annum. However, packaged water is experiencing strong growth worldwide, as are soft drinks such as juice and energy drinks. Over the last five years, global consumption of packaged water has grown on average around 11% per annum, while energy drinks registered 9% growth. In the US, energy drinks have in fact grown over 40% in 2003 compared to 2002. These statistics appear to reflect a consumer trend to focus on health and nutrition. Spirits continue to decline but in some markets, such as the US, spirits are showing a positive trend, driven by premium brands of vodka, tequila, rum, cocktails and liquors. Beer remains a mature market but it is still growing at around 2% per annum, while wine shows modest growth.

As consumers become more concerned about their health, there are increasing demands for more convenient ways to satisfy their needs. At the same time, the demographic trend in developed countries is towards an ageing population and not only smaller but also single-person households. The over 50s are equally discerning and demanding and the packaging industry faces a big challenge. It needs to match the needs of diverse, individualistic baby boomers with creative packaging that adds value to products, such as new lightweight and easy-to-handle packaging and new technologies for closures. The industry also needs to provide alternatives suitable for smaller households, such as wine in 250ml cans. Purchasing power has driven packaging growth and will continue to influence the development of new technologies and packaging to satisfy particular consumer needs, such as on the go (OTG) consumption. For example, new features are being developed such as sports caps for ease of consumption and smaller pack sizes introduced to serve this mobile consumer.

The category can roughly be divided into new age or functional drinks, energy drinks, sports drinks, and waters. Each has its target audience, with properties designed to meet a need.

2.2 Sports Drinks

A sports drink is a beverage that contains carbohydrates and other supplements to help replenish fluids and nutrients used during vigorous exercise and sporting events. Sports drinks are available in a variety of flavours. Some are pre-made liquids while others are mix-it-yourself powders.. The three types of sports drinks are available in the market and they can be classified as isotonic, hypertonic and hypotonic drinks. They differ in their osmomolality and carbohydrate content.
2.3 Energy Drinks

Energy drinks are the beverages that specifically claim to provide an energy and stimulation boost. These products also have a marketing positioning which stresses energy; many generally include active ingredients such as glucose, caffeine and taurine, as well as other health–oriented ingredients such as ginseng and various vitamins and minerals.

2.4 Functional Drinks

These are marketed broadly as ‘lifestyle beverages’ and ‘healthy beverages’, ranging from herbal adult soft drinks and teas to drinks claimed to relieve stress or boost the immune system.

3.0 Considerations For Selection Of Packaging Material

The packages mainly perform three functions viz. to contain, to protect and to sell the product. It is essential to know the nature and composition of the product, its desired shelf life under specified conditions of storage in terms of light, temperature, humidity, presence of oxygen and the types and causes of deterioration including mechanical stress, which the product may undergo during handling and storage. The packaging material must not impart its own odour to the product. It should be inert to food and non–toxic. The consumer expects that package should not only protect the product but also give information about the contents, storage conditions, methods of use, date of manufacture and expiry, price and nutritional considerations.

3.1 Choice Of An Appropriate Packaging Material

Choice of an appropriate packaging material is governed by several factors such as:

- The specific sensitivities of the contents, e.g. moisture, oxygen, etc.
- Factors affecting the contents viz. temperature, RH, pH, and the reaction mechanism involved.
- Weight and shape of the container.
- Effect on filling and sealing speeds.
- Contamination of food by constituents of the packaging material.
- Storage conditions – How long the product needs to be protected!
- Bio–degradability and recycling potential.
- Many more peculiarities, like product range, market, consumer needs and operating margins, determine the packaging of beverages.

4.0 Packages For Beverages

4.1 Cans

Cans are highly developed to deliver a drink to the consumer efficiently and at a comparative cost. They are distinctive and versatile. The can is a reliable and convenient package for distributing beverages, whether alcoholic (beer) or soft drinks, especially if carbonated, since it perfectly protects the product. Consumers continue to appreciate beverage cans because of their lightweight, ease of chilling and because they keep the contents carbonated. For the filler and the retailer, cans offer easy transportation and storage.
and are excellent for supermarket shelves in multi-pack formats, which are also much appreciated by consumers.

4.2 Glass Bottles

The glass package has a modern profile with distinct advantages, including quality image, low-cost production tooling, brand differentiation through shape, design and texture, product compatibility, impermeability, odour resistance, good transparency, tamper resistance, ease of opening, in-bottle pasteurisation, good internal –pressure resistance, resealability, recyclability, reuse opportunity, sleeving and decorative possibilities, UV protection properties, and good top load strength/rigidity. The general range of colours available in the marketplace today is:

**White flint:** made up of silica (MgO) 72%, from high purity sand; lime (Cao) 12%, from limestone (calcium carbonate); soda (Na$_2$O) 12% from soda ash; alumina (Al$_2$O$_3$), present in some of the other raw materials or from feldspar-type aluminous material; and magnesia (MgO) and potash (K$_2$O).

**Pale green:** slightly less pure materials are used, the iron content rises and a pale green glass is produced. Chromium oxide can be added to produce a slightly denser blue green colour.

**Dark green:** obtained by the addition of chromium oxide and iron oxide.

**Amber:** this colour is normally obtained by melting a composition containing moderate iron oxide under strongly reduced conditions. Carbon usually is added to ensure condition. Amber glass has UV protection properties and could well be suited to usage with light sensitive products.

**Blue:** Blue glass is usually obtained by the addition of cobalt to a low-iron glass. Almost any coloured glass can be produced by either furnace operation or by glass colouring in the conditioning fore hearth.

4.3 Pet Bottles

Plastic materials have experienced widespread growth over the last 50 years, becoming commonplace as alternatives to traditional materials, such as glass and paper, and enabling new applications because of their unique properties. PET bottles were first introduced commercially in the USA around 1976, and in Europe in the latter part of 1979. The material has excellent characteristics—it is clear, unbreakable, does not taint the beverage and has new market potential. PET is such an engineering polymer. It is polymerised from terephthalic acid and ethylene glycol, under vacuum, in the presence of catalysts proprietary to the manufacturers. PET has one of the highest gas barriers of plastics commonly used for packaging. Its use was primarily driven by the soft drinks companies, who wished to increase the volume of product sold from the traditional 1 litre glass bottle and also to find a safer, more convenient package for the consumer. It was initially introduced as a 1.5 litre bottle, which conveniently fitted the majority of refrigerators. Since the product contained carbon dioxide, it was necessary to design a vessel that could withstand pressure. This resulted in a “torpedo” like design with a hemispherical base fitted with a basecup, the
sole purpose of which was to allow the container to be positioned vertically during packing, filling and storage.

4.4 Plastics

Plastic bottles showed strong growth driven by consumers’ preference for convenience and low price of large family-sized packs. Over the years, plastics in both rigid and flexible forms, have been gradually replacing metal and glass as beverage packaging materials. This can be attributed to their versatility, formability, lower manufacturing costs and lightness of weight. There are a number of high-barrier plastics in existence, which must be combined with cheaper plastics as a result of their extremely high cost. Ethylene vinyl alcohol (EVOH) falls within this category and has excellent barrier properties. It is usually combined with a base polymer, such as PE or PP, which provide the main structure of the laminate and are also cheap and easy to process. Other base polymers include PVC and PS, which are chosen for their transparency.

Advancements in processing technology combined with the broad range of plastic materials available on the market today have enabled packaging components to be tailor-made to meet the specific needs of an application. Co-extrusion, a technique used both in rigid and flexible packaging, allows a number of plastic layers to be bonded together, each layer contributing certain properties, the combination of which can provide a perfect packaging solution. These advances in technology give a competitive edge over glass and metal. Plastic materials used for packaging applications must be resistant to the diffusion of gas/vapour in order to keep the product fresh. Diffusion is caused by the difference in concentration of gaseous substances on the outside and inside of the pack. In beverage packaging, the four main types of permeation are:

- Loss of carbon dioxide, causing a drink to lose its fizz.
- Loss of water vapour, which can reduce the fill level.
- Absorption of oxygen, which can cause degradation of the product.
- Loss of aroma, which can affect flavour.

4.5 Drink Cartons

The birth of the beverage carton is closely linked to the development of milk distribution. Like other packages, beverage cartons are sometimes criticised for having an adverse effect on the environment. They are used only once (like all other paper-based packages) and make use of valuable resources, trees that are of great natural importance. However, the beverage carton industry has responded with some strong arguments that should be considered prior to the selection of a packaging system, claiming that the beverage carton is not a part of the problem, but a part of the solution:

- **Cartons are lightweight** – a 1litre carton for juice weighs less than 30g and, consequently, represents less than 3% of the weight of the product it contains.
- **Cartons are made from a renewable raw material** - wood fibre is the basic material in beverage cartons and is usually derived from forested areas.
- **Cartons increase distribution efficiency** - thanks to their low weight and compact shape, beverage cartons offer a huge potential in improved distribution compared with, for instance, glass bottles.
• **Cartons are hygienic** – a beverage carton is made from virgin material and, consequently, has never been used for anything prior to being filled. This is an assurance of food safety that can never be claimed for a returnable system—there is no knowing what a returned bottle may have contained.

• **Cartons are one-way containers** – they do not use resources in return transport, in rinsing or washing. The detergents employed in washing reusable bottles remain a significant environmental hazard.

• **Aseptic cartons do not require refrigeration** – both milk and juice have to be transported or stored in energy demanding refrigerated lorries, cold stores, display cabinets and refrigerators, unless they are packed in aseptic containers.

• **Cartons are easy to dispose of** – once used they can be folded to take up very little space in the dustbin or at the collection site for recycling.

• **Cartons can be recycled** – they satisfy the demands of society for a reduction in the use of raw materials and recycling of materials.

There is an increasing need among on-the-go consumers for “single service” beverage packages that fit conveniently in car drink holders and are easy for kids to use. To meet this growing need, IP developed Micro-Pak, a paper carton for beverages. Micro-Pak has a unique size and shape making it portable, easy to handle, convenient for on-the-go snacking and a perfect fit for car drink holders. It offers longer shelf life for high quality refrigerated products and makes life easier for families on the go. Tru Taste Gold™, IP’s patented Barrier-Pak™ board, enables Micro-Pak to retain the true taste of the beverage and a higher level of vitamin C. It enhances the shelf life of the product and provides superior package integrity. The printability of the package drives brand recognition and shelf appeal, obvious key requirements in today’s marketplace.

To maintain the quality of beverages over extended periods of time and provide superior protection during distribution, even when refrigerated distribution is inconsistent, IP’s beverage packaging scientists developed Tru Taste™ Maxx, a carton that features advanced barrier properties. Unique coatings on the board reduce oxygen loss, which means juice/drink has fresher taste, better colour and increased shelf life. A special side seam provides better protection against leakage. The high strength of the patent-pending barrier paperboard allows the container to resist rough handling during distribution. And for either hot-fill or “broken cold chain” applications, Tru Taste™ Maxx can eliminate the need for foil-lined cartons, providing an economic and environmentally friendly alternative.

### 4.6 Foil Pouches

Flexible packaging is considered by some people to have been the Cindrella of the beverage packaging industry. It possesses many advantages, such as compactness when empty and disposability. It offers the consumer lightness and unbreakability, together with the potential for excellent graphic decoration, often built on a metallized surface. Pouches are the most recent development in drinks packaging; they are usually made from a multi-layer composite of which aluminium foil is the essential barrier layer. These packs, some of which are ‘stand-up’, have enormous visual impact and have been adopted for a wide range of sports and health drinks. These are ideal packs for children, being safe, hygienic and easy to carry. Many of them incorporate tamper evidence features, and closure systems that make consumption ‘on the move’ even easier. Their very low weight-to-contents ratio saves resources. Even though the weight of an empty 200ml pouch is less than that of a sheet of
copier paper, the pack is strong enough to meet tough drop and burst resistance tests as well as providing the essential barrier property for sensitive products. The remarkably light weight of the pack enables enormous transport cost savings to be made. The packaging laminate is delivered in space saving reel form, so saving transport costs.

5.0 Global Beverage Packaging

Changes in market developments are influencing beverage packaging. In particular, a shift has been observed from the use of refillable packs, to increasing use of recyclable containers like metal, plastic and glass. Consumption habits also continue to change. For example, consumers have shown to a large extent that they prefer premium-packaged beer to draught beer. As more consumers buy beer for home consumption, they look for convenient formats. This has led to further opportunities for packaging manufacturers to develop recyclable packaging.

Today’s global beverage packaging arena is a wild, dynamic for the packaging industry. Caught squarely between material suppliers at one-end and product manufacturers at the other, beverage packaging industries are being challenged in every direction. Where there is challenge, there is opportunity. In a still-uncertain economy, beverage-packaging growth appears strong, and forecasts are optimistic. One survey conducted late last year by Food Engineering magazine showed that while packaging capital equipment spending was sluggish as a whole, beverage packaging spending was still robust. Thirty-two percent of survey respondents in the beverage category planned to spend $5 million or more on packaging materials, and 36 percent planned to spend $1 million or more on packaging equipment. By comparison, 39 percent of respondents from other categories expected to spend less than $50,000 on packaging equipment.

The beverage category includes spirits, wine, beer; carbonated and uncarbonated soft drinks, waters and sport drinks; health and nutrition drinks, coffee and tea; and juice drinks. According to a new report by Zenith International, soft drinks are slated to become the largest consumer product in the beverage category. Lighter-weight plastics with more heat resistance and better barrier properties are fuelling the demand, along with new aseptic filling technologies. Convenience-minded consumers have also accepted pouches and boxes as thirst-quenching alternatives.

5.1 Beverage Packaging Innovations

- **Multi-packs**: Consumers might like to get beverages in single-serve sizes, but they prefer to buy in bulk. Multi-packs provide the answer, and retailers like the fact that they can put fewer units on the shelf with higher price points.
- **New shapes**: Manufacturers are differentiating their products by changing the shape of beverage containers—not just to create a more distinguishing profile, but also to improve “grippability” and handling.
- **Regionalization**: Eager to exploit local and national events, beverage marketers and retailers are re-labelling products in smaller, targeted quantities for greater emotional impact. Cross-promoting drinks with sporting events, movies and other attractions promises to be a hot sales strategy for the new millennium.
- **Culturalization**: More than just translating product labels into different languages, marketers are looking to build ethnic brand loyalty through packaging that reflects cultural preferences and lifestyles.
- **Line extensions**: Owners of successful brands are leveraging consumer loyalty by offering new twists on old favourites. Expect to see marketers launch variations on tried-and-true themes with new and enhanced flavours, shocking colours and modified nutritional contents.

- **Convenience**: When Coca-Cola marketers discovered that people who buy 12-packs only chill six cans at a time, they saw an opportunity. Born was the Fridge Pack, an elongated carton that fit nicely on the bottom shelf of the fridge. When the carton’s perforated end is removed, the package becomes a dispenser unit.

- **Non-traditional distribution channels**: It wasn’t too long ago that the idea of selling milk and water in a soda vending machine seemed like a stretch. As marketers seek new venues for their products, expect to see beverages repackaged and re-positioned—in different grocery store aisles and retail outlets as well as at schools, health clubs and sporting arenas.

Every one of these trends presents a new opportunity for packaging printers and converters. Individually, most of them translate to new designs, new graphics and new job orders. Together, they assure the continuing reliance on faster turnarounds and shorter press runs. As well, marketers have expressed a preference for shrink-wraps and seamed sleeves over wrapped labels, and that creates another boom chance for packaging industries to shine. A 2003 report by Keymark Associates tracking converter sales shows that the $1 billion market for wrapped labels of carbonated beverages and waters is expected to grow slowly at 3 percent. Sales of shrink labels and seamed sleeves—already a $413-million market ($324 million in beverages)—will grow more than 20 percent. According to Keymark, this explosive growth is led by single-serve milk (33% growth), ready-to-drink (RTD) coffee (19%), water (17%), RTD tea (15%) and sports drinks (11%).

6.0 **Conclusion**

As markets evolve, with ever more pressure on costs, changing customer needs and new marketing strategies, packaging of sports beverages will continue to be challenged. Technological progress and revolutionary changes are taking place at a very fast speed in beverage packaging industry. Packaging functionality is becoming a point of differentiation in product choice. Consumer acceptance of new packaging concepts will continue to grow with the increasing pressures for convenience foods. New concepts, materials, designs, machinery, printing and labelling and computer software are revolutionising the packaging sector. Software packages are now available which automate the process of designing a food package on the basis of composition, cost and film requirements in terms of barrier properties.

7.0 **Suggested Readings**


1.0 Introduction

Milk fatty acids had always had a bad literature for its relative high content of some saturated fatty acids, which may increase plasma cholesterol associated to LDL. However, recently much attention has been directed toward an unusual fatty acid, conjugated linoleic acid (CLA), which is naturally present in milk and dairy products. The acronym CLA refers to various biochemical and geometrical isomers of linoleic acid in which the two double bonds have a conjugated arrangement instead of methylene interruption. CLA is produced in ruminants as an intermediate in the biohydrogenation of dietary linoleic acid by a linoleic acid isomerase from the rumen bacteria *Butyrivibrio fibrisolvens*. During this process vaccenic acid is also formed, which may be converted to CLA in all organisms that possess delta 9 desaturase. Therefore vaccenic may serve as a precursor of CLA. Furthermore, it has been demonstrated that manipulating cow’s dietary PUFA intake it is possible to modulate milk CLA content. Therefore, ruminants are the major reservoir for this fatty acid. The CLA content of dairy products varies from 0.6 to 30 mg/g fat. The biological properties of dietary CLA are currently attracting considerable interest. CLA is not only a powerful anticarcinogen, it also has antiatherogenic, immunomodulating, growth promoting and lean body mass enhancing properties.

Even though the presence of CLA in milk was known since the ‘30s, only 60 years later it has been discover that it possesses biological activities (Table 1). Since the first report of on CLA antimutagenic activity, there has been an explosion of interest in CLA research. In fact, among 28 possible positional and geometrical isomer of CLA, only two have been thoroughly studied (c9, t 11 and t10, c12). Studies on pure single isomers showed that they might have some differences in biological activities in regards to decrease body mass and in general on adipogenesis. On the other hand, the anticarcinogenic and the antiatherogenic effects seem to be exerted by both isomers.

2.0 CLA has anticarcinogenic effect

It has been shown that dietary CLA inhibited mammary tumours induced by 7, 12-dimethylbenz(a)anthracene (DMBA) in rats in a dose dependent manner up to 1.0% CLA in the diet. No further protection was seen at CLA levels above 1% in the diet. Further, at a lower dose of DMBA (5 mg/animal), as little as 0.1% CLA in diet was sufficient to reduce significantly the number of tumours. It is also evident that CLA provided a life-long protection from mammary cancer, when fed for a short period of about 3 weeks from weaning, before initiation of tumours by carcinogen. When CLA was removed from the diet, CLA returned to basal levels in about 4 and 8 weeks, respectively. The rate of disappearance of neutral lipid-CLA (rather than phospholipid-CLA) subsequent to CLA withdrawal paralleled more closely the rate of occurrence of new tumours in the target tissue. The neutral lipid-CLA might be more sensitive marker of tumour protection than phospholipid-CLA.
The efficacy of CLA inhibition of mammary tumourigenesis is independent of the amount or type of dietary fat. Inhibition of DMBA-induced mammary tumours by 1% dietary CLA was virtually the same in rats fed either 10 or 20% fat in the diet. The extent of tumour inhibition by CLA was also the same in rats fed 20% maize oil diet or 8% maize oil + 12% lard diet. Also, the amount of linoleate present in the diet (2 or 12%) did not affect the inhibitory potential of CLA. CLA was equally effective against the action of a direct acting carcinogen methyl nitrosourea, as it was against DMBA (a carcinogen which requires metabolic activation), thus suggesting that it may have a direct modulating effect on susceptibility of the target organ to neoplastic transformation).

The effect of CLA on 2-amino-3-methyl-imidazo [4,5-f] quinoline (IQ) induced colon carcinogenesis in male F344 rats was studied. CLA or safflower oil was given to rats on alternate days at levels designed to reflect 5 g CLA/kg in the diet. The carcinogen was administered orally at weeks 3 and 4 (100 mg/kg body weight). CLA treatment was discontinued after 4 weeks and the rats killed 12 weeks later. The number of aberrant crypt foci per rat were 4.3 in the IQ-fed controls; 3.2 in rats given IQ + safflower oil; and 1.1 in rats given IQ + CLA. The number of aberrant crypts per foci in the three groups were 14.2, 12.0 and 4.0, respectively.

In a study on effects of increasing levels of dietary CLA on phorbol ester (12-O-tetradecanoyl phorbol-13-acetate) induced promotion of skin tumours in mice, tumour yield fell significantly with increasing levels of dietary CLA, from 6.71 in controls to 5.92, 4.83 and 4.67 in mice fed 5, 10 or 15 g CLA/kg diet, respectively.

The severe combined immunodeficient (SCID) mouse provides a model for studying the growth of human tumour cells. Subcutaneous inoculation of human tumour cells into the SCID mouse leads to tumour growth at the site of injection and metastatic spread as well. SCID mice were fed on a diet containing 10 g CLA/kg for 2 weeks and then inoculated with 10⁷ human breast adenocarcinomas cells (MDA-MB468). CLA feeding was continued for 14 weeks. The tumour weight and volume were significantly reduced in CLA treated mice and systemic spread of the tumour into the lungs, peripheral blood and bone marrow was abrogated completely. In another study, the effect of CLA and linoleic acid was compared wherein SCID were inoculated subcutaneously with 5x10⁶ human prostatic cancer cells (DU145). CLA and linoleic acid were fed (1%) in diet beginning 2 weeks before inoculation of the DU145 cells, and the mice were observed for another 12 weeks. Tumour volume was significantly higher in linoleic acid fed mice then in CLA fed mice and tumour mass followed the same pattern. Metastatic spread of the tumour to the lungs was observed in 80 to 100% of the control or linoleic acid fed mice and in only 10% of the mice fed on CLA.

3.0 Fat Reducing Effect of Conjugated Linoleic Acid (CLA)

Dietary CLA has been shown to affect body composition (reduction in body fat, enhancement of fat-free mass) in mice and rats. CLA added to the culture medium of mouse 3T3-L1 adipocytes reduced lipoprotein lipase activity as well as the concentration of intracellular triacylglycerol and glycerol. Additionally, skeletal muscle from mice fed CLA exhibited elevated carnitine palmitoyltransferase (CPT) activity. Based on these findings it was proposed that the physiological mechanism of body fat reduction in mice by CLA involved inhibition of fat storage in adipocytes coupled with both elevated β-oxidation in skeletal muscle and an increase in skeletal muscle mass.
There is evidence indicating that the CLA-associated body composition changes in mice result from feeding the trans-10, cis-12 CLA isomer. Body composition changes, exhibited as reduced body fat, enhanced body water, enhanced body protein and enhanced body ash, were associated with feeding the trans-10, cis-12 CLA isomer. Similar findings have been reported for hamsters. In cultured 3T3-L1 mouse adipocytes, the trans-10, cis-12 isomer of CLA reduced lipoprotein lipase activity as well as the concentration of intracellular triacylglycerol and glycerol. By contrast cis-9, trans-11 and trans-9, trans-11 CLA isomers did not affect these biochemical activities.

There are indications that the trans-10, cis-12 CLA isomer decreased the expression of hepatic stearoyl-CoA desaturase mRNA in mice, whereas enzymatically synthesized cis-9, trans-11 was not active in this regard. The trans-10, cis-12 CLA isomer depressed milk fat synthesis in cows.

CLA is potent in reducing the fat body mass in different species. Among the identified causes, a greater channeling of fatty acids toward β-oxidation (higher CPT-I activity), a lower adipose fatty acid uptake and greater adipose tissue lipolysis and an overall increased metabolic rate are responsible for body fat reduction in mice. A possible impairment of triacylglycerol synthesis in the adipocyte cannot be ruled out as well. CLA affect lipid metabolism in young animals, not in adults.

### 4.0 Possible mechanisms by which CLA exerts its activities

CLA may act through different pathways (Table 1) and probably influence each tissue differently. In the early reports it was hypothesized an antioxidant activity as a mechanism of action, which has successively been confuted. The discovery that CLA can be elongated and desaturated as a regular PUFA to conjugated (CD) 18:3, CD 20:3 and CD 20:4 brought a new possibility that the activity of CLA may be related to its metabolism and possible competition with the other PUFA families and in particular way with n-6 for the formation of arachidonic acid. Its incorporation into rat liver lipids is similar to that of oleic acid, and occurs preferentially into neutral lipids. Furthermore, CD 18:3 and CD 20:3 are incorporated similarly to CLA and very differently from their non-conjugated parent compounds 18:3n-6 and 20:3n-6. On the other hand CD20:4 is mainly incorporated into phospholipids. The preferential incorporation of CD 18:3 and CD 20:3 into neutral lipid fraction allows these metabolites to be stored in adipose tissue. This may give an advantage in the competition with arachidonic acid since its precursors 18:3n-6 and 20:3n-6 are mostly incorporated into phospholipids. Interestingly, in mammary and adipose tissues it seems that the decrease of 20 carbon atom polyunsaturated fatty acids i.e. 20:4 and 20:3, substrates of cyclooxygenase and lipoxygenase pathways, are replaced by CLA isomers CD 18:3 and CD 20:3, that have been demonstrated to inhibit both pathways of eicosanoid biosynthesis. It would be reasonable to expect that the biosynthesis of eicosanoids will be affected by the reduced availability of arachidonic acid. In addition to the activity of cancer prevention, CLA is known to modulate immune functions, and atherogenesis (Table 1).

It has also been shown that CLA is a good ligand of nuclear receptors called peroxisome proliferator activated receptors (PPARs). CLA seems to be active on two isoforms alpha and gamma. The isoform alpha is mainly located in liver and works as trancription factor for many enzymes linked to fatty acid oxidation and particularly to peroxisomal beta-oxidation. In fact induction of key enzymes of peroxisomal beta oxidation
by CLA has been reported as a consequence of activation of peroxisome proliferator activated receptors (PPAR) alpha by CLA.

One more biological effect that may be linked to PPAR alpha activation is the ability of dietary CLA to induce an increase of free retinol in different tissues. This increase may be due to an increase of the level of the carrier protein of retinol (CRBP), mediated by an activation of PPAR alpha known to be a transcription factor for CRBP. The activation of PPAR gamma, which is mainly present in adipose tissue, may well explain its activities on adiposity and diabetes. In fact PPAR gamma regulates adipocyte differentiation and glucose tolerance. Furthermore, it has recently been shown that blocking the delta 9 desaturase the CLA activity on PPAR gamma was significantly lower, implying that some CLA metabolites could contribute in a substantial way on PPAR gamma activation. The inhibition of delta 9 desaturase by CLA has also important implication in terms of triacylglycerol synthesis and thereby on accumulation of triacylglycerol in adipocytes. It is now demonstrated that one specific isomer t10,c12 is able to inhibit delta 9 desaturase in vivo, even though it has been recently shown that on human breast cancer cells both isomers c9,t11 and t10,c12 have inhibitory activity.

Other biological activities that may be a consequence of the biochemical changes described above, and that may easily explain the preventive activities in several pathological states, especially on cancer, are the inhibition of proliferation with concomitant induction of apoptosis of mammary epithelial cells. Also the very recent report that dietary CLA is able to inhibit angiogenesis in vivo in mice, may well explain the capacity of CLA to inhibit metastasis, showing that CLA may inhibit each phase of carcinogenesis.

5.0 Human studies

Human studies are not numerous as animal studies even though many are in progress. From human studies is emerging that CLA may be beneficial in preventing some diseases by modulating lipid metabolism and immune function, thus establishing stable conditions which may delay or inhibit the onset of important disease such as cancer, atherosclerosis and diabetes. However no significant changes have been detected by CLA administration in normal conditions, while data are more encouraging when CLA is given to patients showing risk factors such as obesity (19) or hyperglycaemia. Probably, in some pathological states where lipid metabolism is impaired such as diabetes or obesity, it is likely that CLA is more effective. Another important discover is that CLA is metabolized similarly in humans and rat. Therefore, if CLA metabolism is able to influence lipid metabolism in humans as it has been demonstrated in rats, it should exert similar activities. By measuring plasma levels of CLA and metabolites in humans taking 6 g/d of CLA and comparing them to plasma levels of rats fed increasing amounts of CLA from 0-2% in diet, resulted that 6 g in humans corresponded to about 0.4% of dietary intake in rats, slightly less than the range of 0.5-1% within which CLA exerts most of its activities. It is not surprising therefore that lower dietary intake to healthy humans showed no significant effects. However, in particular chronic pathological conditions where lipid metabolism is impaired probably lower levels but for very long periods may exert beneficial effects. Another important finding is that an increase in CLA level is possible by way of delta 9-desaturase activities that convert vaccenic acid to CLA, thus determining an increase of tissue CLA. This should be taken into account when planning intervention studies on CLA or vaccenic acid activities.
6.0 Enrichment of dairy products with CLA

Dairy products can be enriched with CLA through in vivo and in vitro approaches. An eight fold increase CLA content of milk has been reported by feeding cows with diet containing 5.3% sunflower oil. Alternatively, CLA can be synthesized in the laboratory by refluxing linoleic acid with sodium hydroxide dissolved in ethylene glycol at 180°C under the atmosphere of N₂ for 2h. While 80-90% CLA present in milk is c9, t11-isomer, the CLA prepared by alkali treatment contained 42.5% of c9, t11- and t9, c11-isomers and 43% t10, c12-isomer.

Conclusions

CLA has been clearly shown to be powerful as a preventive agent against different pathologies in experimental models. Furthermore, the inclusion of CLA among “dangerous” trans fatty acids is not substantiated by any reference on possible adverse effects that may justify any limitation of CLA intake. The same is true for vaccenic acid, which has been recently discovered that is efficiently converted to CLA either in humans or rodents (Table 2) where it has been shown to be able to reduce preneoplastic lesions in rat mammary gland. The use of t10, c12 may have some adverse effect in lactating women for its activity in decreasing milk fat. The supplementation of t10, c12 CLA for decreasing body weight may be advised in people that already possess risk factors such as obesity, hyperlipidemia and diabetes. The c9, t11 CLA can safely be vehicled with food, since no adverse effects have been reported so far. For this reason, naturally CLA enriched dairy products could represent a good way to deliver CLA and vaccenic acid in a certain amounts.

6.0 References


Table 1 Major CLA activities on experimental models “in vivo”

**Biological activities of CLA**

- Antimutagenic activity
- Anticarcinogenic activity in mouse fore stomach
- Mammary cancer prevention in rat
- Anticarcinogenic activity in colon
- Anticarcinogenic activity in skin
- Antiatherogenic activity in rabbits
- Reduction of body fat
- Antidiabetic activity in rat
- Inhibition of metastasis in mice

**Biological activities linked to possible mechanisms of action**

- Metabolism similar to linoleic acid
- Decrease of arachidonic acid
- Activity as PPARs ligand
- Inhibition of proliferation of mammary epithelial cells
- Inhibition of delta 9 desaturase
- Modulator of immunoglobulins
- Increase of free retinol in rat tissues
- Induction of apoptosis premalignant lesions in the rat mammary gland
- Induction of both proliferation and apoptosis in rat liver

**Studies on single isomers**

- Anticarcinogenic activity of CLA enriched butter in rat
- Vaccenic acid is desaturated to CLA in mice
- t10,c12 isomer inhibits delta 9 desaturase in rats
- Suppression of development of premalignant lesions in the rat mammary gland by vaccenic acid
- t10,c12 and c9,t11 are both effective in mammary cancer prevention in rats
- Both t10,c12 and c9,t11 are both anti-atherogenic in rabbits

Table 2 Major CLA activities in humans

**Changes induced by dietary CLA**

- Reduction of body fat mass in overweight and obese
- Reduction in serum lipids and body fat
- Reduction of abdominal adipose tissue in obese middle-aged men with signs of metabolic syndrome
- Reduction of body fat in healthy exercising humans
- Effects on fatty acid and glycerol kinetics
- Maternal supplementation with CLA decreases milk fat
- Increase of immune response

**Conversion of vaccenic acid to CLA**

- Increase of CLA by dietary trans fatty acids
- Vaccenic acid is converted to CLA
- Conversion of vaccenic acid to CLA is about 20%
SAFETY AND STABILITY OF ARTIFICIAL SWEETENERS

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1.0 Introduction

The role sweeteners play in the diet is constantly debated. Sweeteners of one kind or another have been found in human diets since prehistoric times. Terms such as sugar free, sugar alcohols, sucrose, corn sweeteners, etc. can be confusing. Each of the sweeteners available to consumers has specific applications and certain limitations. A variety of sweeteners exist to help consumers satisfy their desire for sweetness. Sweeteners are used in foods for several reasons, besides adding sweetness. Sugar is used as a preservative in jams and jellies; it provides body and texture in ice cream and baked goods; it aids in fermentation in breads and pickles. Sweeteners that supply energy (calories) are referred to as nutritive sweeteners even though they lack other nutrients essential for growth and health maintenance. Nutritive sweeteners provide four calories per gram. For most sugars this is about 17 calories per teaspoon. Sweeteners that do not supply calories are referred to as non-nutritive sweeteners. Synthetic (artificial) sweeteners may be nutritive or non-nutritive. Low calorie artificial sweeteners are used to aid in the management of diabetes, aid in the management of dental caries, assist weight control and create a variety of good tasting foods and beverages.

The Indian government has permitted use of artificial sweeteners in about 25 food items including carbonated water, soft drink concentrate, sugar/sugar free confectionery, chewing gum, biscuits, breads, cakes and pastries, traditional sweets like halwa, gulab jamun and roshogulla, and others. India was perhaps the only country where use of ‘artificial sweeteners’ in foods items was regulated till now. According to a notification amending the Prevention of Food Adulteration (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 and under proper label declarations. Marketing of any food article containing artificial sweeteners was hitherto regulated by the standards prescribed in the PFA Rules, 1955. However, in case of carbonated water and soft drink concentrates, the use of a combination of two artificial sweeteners ‘aspartame’ and ‘acesulfame’ was allowed in 1999. The present notification permits use of four artificial sweeteners like saccharin, aspartame, acesulfame and sucralose in combination, within prescribed limits (Table 1). The change in the food law opens up a vast untapped market of sugar-free food products including sugar-free confectionery. The ideal sweetener does not exist. It would be at least as sweet as sucrose and provide the same properties to a product as sucrose, with processing parameters similar to those of sucrose so that existing equipment can be used. It would be colorless, odorless, and noncariogenic, with a clean, pleasant taste, and have immediate onset and not much lingering. Solubility and stability are important. The ideal sweetener must be compatible with a wide range of food ingredients because sweetness is but one element of a complex food flavor system. Even sucrose is not perfect, being unsuitable for some applications. There are therefore real advantages to having a number of sweeteners available. With several available, food manufacturers can use sweeteners in the applications for which they are best suited, and limitations of individual sweeteners can be overcome by using them
in blends. Most sweeteners, including the polyols, are synergistic, so the sweetness of sweetener blends is greater than the sum of the individual parts.

Table 1: Notification issued by Ministry of health and family welfare on 25th June, 2004.

<table>
<thead>
<tr>
<th>Name of artificial sweetener</th>
<th>Article of food</th>
<th>Maximum limit of artificial sweetener (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharin Sodium</td>
<td>Sweets (carbohydrates based and milk products based):-Halwa, Mysore Pak, Boondi ladoo, Jalebi, Khoya burfi, Peda, Gulab Jamun, Rasogolla and similar product based sweets sold by any name.</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Aspartame (methyl ester)</td>
<td></td>
<td>200 ppm</td>
</tr>
<tr>
<td>Acesulfame potassium</td>
<td></td>
<td>500 ppm</td>
</tr>
<tr>
<td>Sucralose</td>
<td></td>
<td>750 ppm</td>
</tr>
</tbody>
</table>

2.0 Approved High intensity Sweeteners

2.1 Acesulfame potassium

Acesulfame K 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, and is very stable, with virtually no change in concentration observed in the pH range common for foods and beverages after several months. ADI of 15 mg/kg Body weight. The approximate sweetening power is 200 times that of sucrose. Beverages containing acesulfame K can be pasteurized under normal pasteurization conditions without loss of sweetness. Decomposition in baked goods is only found at temperatures well over 200°C. It blends well with other sweeteners and is especially synergistic with aspartame and sodium cyclamate but less so with saccharin. It is noncaloric and has a taste closer to sucrose when combined with other noncaloric sweeteners. The substance is not considered to be carcinogenic and mutagenic (Lipinski, 1985). The Food and Drug Administration states that there is no evidence of cancer risk and that the product is safe. Pharmacokinetik studies have shown that acesulfame potassium is not metabolized and is rapidly excreted unchanged in the urine (Higton and Thurgood 1994).

2.2 Aspartame

Aspartame occurs as off-white, almost odorless crystalline powder with an intensely sweet taste. The approximate sweetening power is 200 times that of sucrose. It is slightly soluble in water and sparingly soluble in alcohol. ADI of 50 mg/kg body weight/day. Degradation occurs by hydrolysis and during prolonged heat treatment. The use of aspartame has been of some concern due to the formation of the potentially toxic metabolites, methanol, aspartic acid and phenylalanine (Higton and Thurgood 1994). Although it technically has 0.4 kcal/g, as used it provides essentially no calories because it is 200 times sweeter than sugar. 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. Aspartame is slightly soluble in water, sparingly soluble in alcohol, but not soluble in fats or oils. Under dry conditions, it has good stability. In liquids under certain conditions of moisture, temperature, and pH, it may hydrolyze, resulting in loss of sweetness. No adverse health effects related to aspartame have been demonstrated.

2.3 Saccharin

Saccharin occurs as white, crystals or a white crystalline powder, is odorless, or has a faint aromatic odor. It is slightly soluble in water, sparingly soluble in alcohol and soluble at 0.05% in a fixed oil. It is about 500 times as sweet as sucrose. ADI of 15 mg/kg body weight/day. In its bulk form, saccharin and its salts have been shown to be stable for several years. In aqueous solutions, saccharin demonstrates high stability over a wide pH range. Saccharin has been available for more than 100 years. Saccharin is stable under the normal range of conditions employed in 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. 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.

2.3.1 Saccharin Sodium

Saccharin sodium, USP occurs as white crystals or a white, crystalline powder. It is odorless or has a faint aromatic odor and an intensely sweet taste even in dilute solutions. It is about 500 times as sweet as sucrose. 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. Saccharin sodium is stable under the normal range of conditions employed in formulations.

2.4 Sucralose

Sucralose 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 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. It’s excellent chemical and biological stability, both dry and in aqueous solution, allows for its use essentially anywhere sugar is used, including cooking and baking. The solubility and aqueous stability of the sweetener allow for sucralose to be provided as a liquid concentrate for industrial use. This product provides an extremely stable ingredient system compatible with most food operations. Studies in model food systems, confirmed by actual product use, 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. Sucralose is being used in a
broad range of products. The actual use level varies with the sweetness level desired and the other ingredients and flavor system used in the specific formulation. It has been thoroughly tested in more than 100 studies over a 20-year period and found to be safe. It passes rapidly through the body virtually unchanged.

3.0 Low-Calorie Sweeteners Pending Approval

Three additional low-calorie sweeteners—alitame, cyclamate, and neotame—are pending approval by the Food and Drug Administration (FDA) for use in the U.S.

3.1 Neotame

Neotame is both a low-calorie sweetener and a flavor enhancer. It is structurally similar to aspartame but 30–60 times sweeter—or 7,000–13,000 times sweeter than sucrose. Neotame is stable across a wide range of applications. ADI of 18 mg/person/day (mg/p/d). It is similar in stability to aspartame but has greater stability in baked and dairy products. Neotame’s clean, sweet taste is maintained over the range of concentrations required for numerous food and beverage applications. It extends both sweetness and flavor in confectionery applications, such as sugar-free chewing gum, and its onset and linger are similar to those of sucrose in applications such as powdered soft drinks. It is soluble in ethanol, and its solubility increases in both water and ethyl acetate with increasing temperature. It dissolves rapidly in aqueous solutions, since very little is needed as a result of its intense sweetness. Extensive research has been conducted confirming neotame’s safety for human consumption.

3.2 Alitame

Alitame is formed from the amino acids L-aspartic acid and D-alanine, with a novel amide moiety (formed from 2,2,4,4-tetramethylthienylamine). This novel amide is responsible for the intense sweetness of alitame, 2,000 times that of sucrose. Alitame’s sweetness is described as sucrose-like, without bitterness or metallic notes. It is a crystalline, odorless, nonhygroscopic powder that is very soluble in water at the isoelectric pH. Excellent solubility is seen in other polar solvents, as well. This sweetener is sufficiently stable for use in hard and soft candies, heat pasteurized foods, and neutral-pH foods processed at high temperatures, such as sweet baked goods. Alitame is compatible with a wide variety of freshly prepared foods. It can undergo chemical reactions with certain food components. For example, high levels of reducing sugars, such as glucose and lactose, may react with alitame in heated liquid and semi liquid systems, such as baked goods, to form Maillard reaction products. Similar reactions may be observed when high levels of aldehydes are present. Prolonged storage in liquid beverages may result in off-flavors in the presence of hydrogen peroxide, sodium bisulfite, ascorbic acid, and some type of caramel color at pH values below 4.0.

3.3 Cyclamate

Cyclamate is almost always used in combination. It is considered noncaloric and may be used to impart sweetness in combination with other sweeteners. It has a favorable flavor profile and is better able to mask bitterness than sugar. It is compatible with most food ingredients, natural and artificial flavoring agents (enhancing fruit flavors), and chemical
preservatives. It is extremely stable at both high and low temperatures, over a wide pH range, as well as in the presence of light, oxygen, and other food ingredients. Cyclamate is particularly well suited for fruit products because it enhances fruit flavors and even at low concentrations can mask the tartness of some citrus fruits. Canned fruit is one product in which it has been used as the sole sweetener. Cyclamate solutions used for such products have a lower specific gravity and osmotic pressure than sucrose syrups and therefore do not pull water out of the fruit. It is suitable for tabletop sweeteners and beverages. Before it was banned in the U.S., cyclamate was responsible for the popularity of several brands of diet soft drink. Cyclamate can also be used in gelatins, jams, jellies, and low calorie salad dressings. With the proper combination of other ingredients to provide bulk and texture, it can be used in baked goods. It also has been used in cured meats. It has a higher melting point than sugar and does not caramelize when fried.

3.4 Stevioside

*Stevia rebaudiana* is a small, shrub-like plant, indigenous to Paraguay. It derives its sweetness from a glycoside called stevioside. The herb, especially its leaves, is ten to 15 times sweeter than table sugar. Extracts of stevia (stevioside) usually range anywhere from 250 to 300 times sweeter than sugar. Studies have shown that stevia does not affect blood-sugar metabolism. The extract is non-nutritive—it does not contribute calories to the diet. It is freely soluble in water and soluble in alcohol. Stevia can be heated to 195°C with no adverse reactions. It is not destroyed by heat, so it can be used in cooking and baking (Lawson, 2000). It is classified as a food ingredient. Stevia extract contains approximately 90% stevioside and occurs as a white crystalline powder. Stevioside is mentioned here because of the considerable attention it receives from those promoting “natural.” Stevioside may be used as a dietary supplement in the U.S., but it is not approved as a sweetener and no reference to sweetness should be made.

4.0 Low Calorie Bulk Sweeteners

4.1 Sugar Alcohols or Polyols

Polyols are often used in combination with other sweeteners to provide bulk and improve texture and mouthfeel. Use of these sugar-free products will contribute to improved oral health.”Non-carbohydrate sweetener that are alcohols with several hydroxyl groups enabling them to be used as sweeteners. Used in sugar free products as they are absorbed much more slowly than sugars. Prolonged time in the gut allows for microorganisms to metabolize them and produce gas.

4.1.1 Erythritol

It is the newest polyol. It is an odorless white crystalline powder with a clean sweet taste. It is approximately 70% as sweet as sugar but provides only 0.2 kcal/g. It is nonhygroscopic and moderately soluble in water. It is stable at high temperatures and over a wide pH range and has a mild cooling effect in the mouth. It is suitable for use in a number of food products, including chewing gum, candies, chocolate, lozenges, fondant, fudge, bakery products, beverages, and tabletop sweeteners.

4.1.2 Hydrogenated starch hydrolysates.

Hydrogenated starch hydrolysates including maltitol syrups, sorbitol syrups, and hydrogenated glucose syrups, are a family of products found in a wide variety of foods. The
term hydrogenated starch hydrolysate can correctly be applied to any polyol produced by the hydrogenation of the saccharide products of starch hydrolysis. In practice, however, certain polyols such as sorbitol, mannitol, and maltitol are referred to by their common chemical names. The term hydrogenated starch hydrolysate is more commonly used to describe the broad group of polyols that contain substantial quantities of hydrogenated oligo- and polysaccharides in addition to any monomeric polyols (e.g., sorbitol or mannitol) or dimeric polyols (e.g., maltitol). The broad term does not differentiate polyols with different sweetness levels, and it does not identify the principal polyol in the hydrogenated starch hydrolysate. Common names have, therefore, been developed for major subgroups. These common names are usually based on the most common polyol in the hydrogenated starch hydrolysate. For example, polyols that are 50% or more sorbitol are known as sorbitol syrups, and those that are 50% or more maltitol are called maltitol syrups. Polyols that do not have a majority component are referred to by the general term hydrogenated starch hydrolysate. Hydrogenated starch hydrolysates are 40–90% as sweet as sugar and serve a number of functional roles, including use as bulk sweeteners, viscosity and bodying agents, humectants, crystallization modifiers, cryoprotectants, and rehydration aids. They can also carry flavors, colors, and enzymes. Since they are excellent humectants which do not crystallize, they can be used in the production of sugar-free confections with the same cooking and handling systems used to produce sugar candies. Their excellent humectancy also makes them suitable for baked goods. Also, they do not have reducing groups, thereby minimizing Maillard browning reactions. They are also used to replace sugar in a variety of frozen desserts, since they do not form crystals.

4.1.3  **Isomalt**

Isomalt has a sweet taste similar to sucrose and reinforces flavor transfer in foods. It is 0.45–0.6 times as sweet as sucrose and, unlike most polyols, does not produce a cooling effect. Isomalt can replace sugar in many foods, using existing processing equipment without major changes. Hard-boiled candies made with it, for example, can be stamped, filled, pulled, combed, and molded and have very good shelf life. Minor changes in the production of these products are required, since isomalt has lower solubility, a higher melting point, lower viscosity of the melt, and a higher specific heat capacity than sucrose or corn syrup. Isomalt can also be used in pan-coated products, chewing gum, chocolate, low-boiled candies, ice cream, jams and preserves, fillings, fondant, and baked goods. “Light” baked goods with isomalt require only minimal formulation modifications. Isomalt’s low solubility, low hygroscopicity, and browning reaction should be considered. The final baked product containing isomalt has a sugar-like taste with a long shelf life. Isomalt cookies absorb less water than sugar formulations, so are crisper.

4.1.4  **Lactitol**

Lactitol has a clean taste about 0.4 times as sweet as sucrose, without an aftertaste. A low-calorie sweetener also may be needed in some applications to provide the desired sweetness. Lactitol is nonhygroscopic, making it suitable for all applications in which water absorption is a critical issue, such as bakery products, tablets, and panned confections. Since its molecular weight is similar to that of sucrose, its influence on water activity is also similar to that of sucrose (on a dry-weight basis). It is stable in acidic and alkaline conditions and under the high temperatures of food processing. Lactitol is suitable for a wide range of products, from baked goods to frozen dairy desserts. The prebiotic effects of lactitol have
been studied. Lactitol reaches the colon untouched and can be used as an energy source by intestinal microflora. In-vitro studies show that lactitol stimulates the growth of Lactobacillus and Bifidobacteria.

4.1.5 Maltitol

It has many attributes that allow its use in a wide variety of food applications. It is a white crystalline powder with a sweetness profile similar to that of sugar, is substantially nonhygroscopic, and is thermostable. It exhibits a negligible cooling effect and can be used to replace fat as well as sugar, since it provides a creamy texture to brownies, cakes, and cookies. This attribute also facilitates its use in sucrose-free chocolate. Maltitol’s anhydrous crystalline form, low hygroscopicity, high melting point, and stability allow it to replace sucrose in high-quality chocolate coatings, confectionery, bakery chocolate, and ice cream. It is also suitable for granola bars, jams with no added sugar, pie fillings, salad dressings, and spreads. Although maltitol works well with other sweeteners, the use of a low-calorie sweetener is not required because maltitol is nearly as sweet as sucrose.

4.1.6 Mannitol

It has long been used in food and pharmaceutical products. It is nonhygroscopic, so it is often used as a dusting powder for chewing gum to prevent the gum from sticking to the manufacturing equipment and wrappers. It also is used a part of the plasticizer system to help maintain the soft texture of the gum. Because of its high melting point (165–169°C), it is used in chocolate-flavored coating agents for ice cream and confections. It has a pleasant taste and does not discolor at high temperatures. FDA requires the statement “Excess consumption may have a laxative effect‖ on the label of a food if the daily consumption of mannitol in the food might exceed 20 g. Mannitol is used in tableting applications as a diluent or filler. Because of its chemical inertness, it is one of the most stable tablet diluents available and is most often used in chewable tablets because of its pleasant taste and mouthfeel, as well as its ability to mask the bitter taste of vitamins and minerals, herbs, or active pharmaceutical ingredients.

4.1.7 Sorbitol

It occurs as an odorless, white or almost colorless, crystalline, hygroscopic powder. It is a hexahydric alcohol. Sorbitol has a pleasant, cooling, sweet taste and has approximately 50% to 60% of the sweetness of sucrose. It is very soluble in water and slightly soluble in alcohol. Sorbitol is absorbed more slowly from the gastrointestinal tract than sucrose and is metabolized in the liver to fructose and glucose. Sorbitol is better tolerated by diabetics than sucrose and is widely used in many sugar-free liquid vehicles (Nash, 1994). However, it is not considered to be unconditionally safe for diabetics has been used in processed foods for half a century as a sweetener, humectant, and texturizing agent. It has a smooth mouthfeel, is 0.6 times as sweet as sucrose, and has a cool, pleasant taste. Its moisture-stabilizing and textural properties are important to the production of confectionery, baked goods, and chocolate, where products tend to become dry or harden. It is very stable and chemically unreactive. It can withstand high temperatures and does not participate in Maillard reactions. It combines well with other food ingredients, including sugars, gelling agents, proteins, and vegetable fats. In addition to the products mentioned above, it functions well in chewing gums, frozen desserts, icings, and fillings. Sorbitol is affirmed by FDA as Generally Recognized As Safe (GRAS).
4.1.8 Xylitol

Xylitol is used mainly in noncariogenic confections such as chewing gum, candies, chocolates, and gum drops. In both clinical and field studies, xylitol use between meals is associated with significantly reduced formation of new caries, even when participants were already practicing good oral hygiene. Xylitol is as sweet as sucrose. Crystalline xylitol provides a significant cooling effect. The cooling effect enhances the perception of mint flavor but is most notable in sugar-free chewing gum, compressed candies, and chewable vitamins. The cooling effect is not perceived in jellies or boiled, transparent candies. Xylitol’s solubility is similar to that of sucrose. It is chemically inert and does not participate in Maillard reactions.

5.0 Other New Sweeteners

There are two new sweeteners also of interest—tagatose and trehalose.

5.1 Tagatose

It occurs naturally in dairy products, but the commercial product is made via a patented process. It has the bulk of sugar, is almost as sweet, but provides only 1.5 kcal/g. It has the potential for use in many products where sucrose is currently used, such as confections, ice cream, soft drinks, cereals, and meal replacements. It is synergistic with other sweeteners and can be used with low-calorie sweeteners to improve texture and mouthfeel. Its solubility in water is similar to that of sucrose. It is nonhygroscopic, with lower water activity than sucrose. Tagatose containing products “brown” more readily than sucrose containing baked goods. It has also been shown to have prebiotic properties.

5.2 Trehalose

It is a multifunctional sweetener found naturally in honey, mushrooms, lobster, shrimp and food produced using baker’s and brewer’s yeast. It is commercially made from starch by an enzymatic process. It is metabolized much like other disaccharides. Trehalose protects and preserves cell structure in foods and may aid in the freezing and thawing process of many food products by assisting in maintaining the desired texture. It is also heat stable. It may be used in beverages, purees and fillings, nutrition bars, dehydrated fruits and vegetables, and white chocolate for cookies or chips. Because it provides 4 kcal/g and is only half as sweet as sucrose, it is more likely to be used for cell preservation than for sweetness.

6.0 Sweetener Blends For Low Calorie Products/Beverages

There are significant benefits to be gained from customizing sweetener blends when developing new beverages or reformulating existing beverages. The role of the sweetener has progressed beyond that of a “calorie-reducing agent” to an ingredient which can add real value in influencing and optimizing taste and stability as well as economics. However, by creating new blends of sweeteners or modifying the proportions of current blends, manufacturers can optimize the sweetening systems for specific types and flavors of beverages. The first commercial sweetener blend was saccharin and cyclamate. The primary advantage of this blend was that saccharin (300 times sweeter than sucrose) boosted the sweetening power of cyclamate (30 times sweeter than sucrose), while cyclamate masked the aftertaste some people associate with saccharin. Today, blends are frequently used. For
example, in the United States, diet fountain soft drinks are generally sweetened with a combination of aspartame and saccharin, while in other parts of the world soft drinks may contain as many as four sweeteners. Sugar-free gums and candies contain combinations such as saccharin/sorbitol and acesulfame potassium/isomalt. When choosing sweeteners, many things need to be considered. What is the goal? For example, are you trying to reduce calories, increase sweetness, reduce sweetness, or replace the sugar in a product? Is labeling, such as “reduced calorie” or “sugar free,” important? Do you want the finished product to have essentially the same taste and appearance as a traditional product? If so, not only taste, but also texture and bulk are especially important. How long a shelf life is required? Some sweeteners may not hold up well in an acidic product over time. Will the product require baking or heating? Some sweeteners may break down at prolonged high temperatures, while others may develop a metallic taste when heated. What other ingredients does the product contain that might interact with the sweetener? Some sweeteners enhance fruit flavors. Is this important to your new product? It is important to remember that in developing low-calorie products, low calorie sweeteners cannot be simply substituted for sugar. Products must be reformulated. The various sweeteners interact differently with other food ingredients, so the flavoring acid/sweetness ratio may require modification.

7.0 Conclusion

With increased knowledge about taste and technology, the food and beverage industry is on the verge of developing a wider variety of good-tasting, low-calorie and low-fat products to meet the growing needs and demands of consumers. A wide variety of low-calorie ingredients provides products with improved taste and texture, increased stability, lower manufacturing costs, and ultimately, more choices for the consumer. It is important to remember that in developing low-calorie products, low calorie sweeteners cannot be simply substituted for sugar. Products must be reformulated. The various sweeteners interact differently with other food ingredients, so the flavoring acid/sweetness ratio may require modification. Degradation and hydrolysis of sweeteners during prolonged heat treatment has been of concern due to the formation of some potentially toxic metabolites. The potential of low calorie sweeteners has been demonstrated but not fully realized.

8.0 Suggested Readings


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; Peterson et al., 2000; Pariza et al., 2001). The various health effects of CLA may relate to specific CLA isomers. However, it has been established that the cis-9, trans-11 CLA is anticarcinogenic when included in the diet as a natural component of food (Lp et al., 1999), and cis-9, trans-11 C_{18:2} is the major CLA isomer in dairy products (Parodi, 1997). The major source of cis-9, trans-11 CLA in milk fat is endogenous synthesis by Δ⁹-desaturase with the precursor being trans-11 C_{18:1}, an intermediate formed in rumen biohydrogenation of polyunsaturated fatty acids (Griinari et al., 2000; Corl et al., 2001).

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. Some researchers have also reported variation associated with breed (White et al., 2001) found that Holstein cows tended to have a higher concentration of CLA in their milk than Jersey cows. In another study, milk from Brown Swiss cows was reported to contain more CLA than Holstein milk, although Brown Swiss milk appeared to be less responsive to dietary manipulation (Whitlock et al., 2002). Variation in Δ⁹-desaturase may explain much of this difference between breeds. Age of the dairy cow and stage of lactation may also influence the milk CLA content to some degree but the effect of these parameters has not been well characterized. 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.

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>
</tbody>
</table>
2.0 Estimation Of CLA Content:

Conjugated linoleic acids are estimated in food products at two levels: i) total conjugated linoleic acid ii) conjugated linoleic acid isomers. The various methods followed are as:

2.1 Spectrophotometric Method

This method is used for the estimation of total conjugated linoleic acid content in milk and milk products AOAC (1995). According to this method, weight accurately 0.01 g of milk lipids in 25 ml volumetric flask and dissolve in 10 ml iso-octane [2,2,4-trimethylpentane, spectrophotometric grade) make the volume to 25 ml with iso-octane and mix thoroughly. Measure the absorbance at 233 nm in the UV region of spectrophotometer by taking the diluted sample in 1 cm cell length cuvette against matched cell containing diluent iso-octane as a blank. The amount of conjugated linoleic acid can be calculated by following formulae:

Absorptivity $a_{233} = A/bc$,
where $A = $ observed absorbance; $b = $ cell length in cm, and $c = $ g sample/litre, final dilution used for $A$ measurement.

Absorptivity at 233 nm corrected for background absorption by acid groups

$$a_2 = a_{233} - a_0$$
where, $a_0 = 0.03$ for fatty acids

Therefore, percentage of conjugated linoleic acid = $0.91 \times a_2$

Thus, final formulae for % conjugated linoleic acid = $(A - 0.03)/g \times df \times 0.9$

where, $A = $ absorbance at 233 nm, $g = $ g of sample taken for analysis and $df = $ dilution factor

2.2 Chromatographic Methods

These methods are based on the principal of chromatographic separation of fatty acids. The methods in vogue are:

<table>
<thead>
<tr>
<th>American Processed Cheese</th>
<th>5.0</th>
<th>93</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat (uncooked)</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>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>

Total fatty acid analysis by capillary GC: Ideally, this should be with a column suited to the analysis of trans fatty acids, e.g. CP-Sill 88 (100 m).

Concentration of CLA isomers by reversed-phase HPLC and silver ion chromatography, prior to GC or GC-MS analysis.

GC-MS analysis in the form of the 4,4-dimethyloxazoline derivatives and/or 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) adducts.

Silver ion HPLC of isomers (very few have this facility).

All the above techniques require pre treatment of fats like extraction from milk and milk products and their derivitization.

2.2.1 Lipid Extraction

Extract total lipids with chloroform : methanol (2 : 1, v/v). Use 20 ml of solvent mixture per gram of dry solids by continuous stirring for 3 h. Repeat the extraction twice. The last extraction is accomplished by a mixture of chloroform : methanol in the ratio of 7 : 1 (v/v) saturated with concentrated NH₃ (about 5.5% v/v). Combine the extracts and transfer to separating funnel. Add a solution of KCl (0.88%) amounting to 25 per cent of the extract. Shake the mixture thoroughly and allow to settle. Collect the lower layer and add a mixture of water and methanol (1:1, v/v) amounting to 25 per cent of the lower layer. Repeat the washing twice. Collect the bottom layer containing the purified total lipids and pass through anhydrous sodium sulphate. Evaporate the solvent by a flash evaporator at low temperature of 40°C and passing nitrogen gas to further evaporate residual solvents. (Folch, 1957). Store the extracted lipid samples under refrigeration temperature in screw capped tubes till analyzed.

2.2.2 Preparation of Fatty Acid Methyl Esters (FAME) For HPLC and GLC Analysis Of CLA and Fatty Acids

2.2.2.1 Esterification method

The methyl esters of fatty acids for HPLC and GLC analysis of CLA are prepared by the method of Christoperson and Glass (1971) with slight modifications. The method, in brief, is as follows:

2.2.2.2 Basic Methylation

Take fat solution (10% fat in petroleum ether) of 1.9 ml in screw capped test tube (10 ml capacity) and to this add 0.1 ml of methanolic solution of 2 N KOH and keep at 50°C / 10 min in water bath, cool in refrigerator for about 10 min before using it for further acidic methylation.

2.2.2.3 Acidic methylation

Further acidic methylation is done by adding 1 ml of 5 per cent methanolic HCl and keeping at 80°C /10 min in water bath. Then cool in refrigerator for 10 min before using it for further analysis.

2.2.3 Extraction of FAME

Add about 2 ml of HPLC grade hexane to the cooled methyl ester and centrifuge at 200 rpm for 10 min, to make sure the complete solubilisation of FAME in hexane. Keep at
refrigeration temperature for about 30 min, then with the help of syringe, add to 1 ml ampolles, seal and use for GLC analysis.

Transfer one ml of FAME extract to 50 ml flat bottom flask. Evaporate the solvent hexane with rotary evaporator (Metrex Scientific Instruments Pvt. Ltd., New Delhi) at low temperature (40 to 45°C), then dissolve the FAME in 1 ml acetone (HPLC grade) and use for the HPLC analysis.

2.2.4 Identification and Quantification of CLA Isomers By Gas Liquid Chromatography (GLC)

2.2.4.1 GLC Method

The gas liquid chromatographic (GLC) analysis of FAME are carried out on Hewlett Packard (USA) HP 6890 series gas chromatograph GLC system equipped with flame ionization detector and fused silica capillary column (30 m x 0.25 mm i.d., phase thickness 0.25 µm, non-bonded; biscyanopropyl polysiloxane, Supelco Inc., U.S.A.).

Inject the fatty acids methyl esters (FAME) (0.5 µl) (prepared by the method given in section 3.7.1), onto the injection port of the GLC equipment through microsyringe. Programme the column temperature from 70°C to 230°C at the rate of 5°C/min and hold at 230°C for 20 min with the total run time of 52 min. Other parameters to be followed are: Split injection; carrier gas (Nitrogen flow rate 1 ml/min), injection port temperature, 240°C detector temperature 240°C

2.2.4.2 Identification And Quantification Of CLA Isomers

Standard CLA methyl esters (Sigma Chemical Co., USA) diluted in hexane are run in GLC under the same conditions as followed for FAME of milk fat. By comparing the retention time of standard CLA peaks with milk fat FAME, the CLA isomer peaks can be identified. Further, the CLA peaks are confirmed by mixing methyl esters of CLA standard with milk fat FAME, ran under similar conditions. The proportionate ratio of the CLA isomers are calculated as follows

Propionate ratio of isomer = \( \frac{X}{A} \times 100 \)

where, X = particular isomers peak area, A = total peaks area

3.5.1 HPLC Method

Silver-ion High-Performance Liquid Chromatography is the technique, which is used to resolve the CLA isomers on the basis of chain length, double bond configuration and position of the conjugated diene functional group in the fatty acid chain. For each CLA positional isomer, the corresponding cis/trans or trans/cis pair of geometric isomers are not separated by silver – ion HPLC (Sehat et al., 1998).

The separation of the fatty acid methyl esters can be achieved on any good HPPLC machine with the following:

**Column:** An analytical column of dimensions (4.6mm i.d X 250nm stainless steel; 5 µm particle size) silver impregnated column.
**Mobile Phase:** The mobile phase 0.1% acetonitrile in hexane (all HPLC and U.V grade) is used in the separation. The mobile phase is prepared fresh daily. Operate the column isocratically at room temperature for 1hr prior to the initial injection of the sample. Set the flow rate of the mobile phase 1.1 ml/min.

**Detector:** U.V detector is used for the identification purpose at 233nm.

**Sample volume:** Inject 5-15µl of the FAME

**Regeneration:** If the regeneration of the HPLC column is required, flush it with 1% acetonitrile for 4 hrs, followed by 1h with 0.1% acetonitrile.

### 3.0 Suggested Readings


1.0 Introduction

Changes in lifestyle, dietary habits and rapid urbanization are leading to multifarious multiplication of health enigmas like obesity, diabetes and heart related diseases, particularly in the affluent stratum of the world population. Obesity is a highly complex, chronic disorder with a multifactorial etiology. In reality, obesity is a disorder that differs in the amount of excess adiposity, the regional distribution of that excess adipose tissue, and its concomitant physiological consequences. Developing countries like India are also now increasingly facing these evils. Recent findings reveal that one third males and half the females in urban areas are suffering from obesity in our country. In addition, India is reported to have 40 million diabetics, the highest in any country and current trends indicate that this silent killer would slap a monstrous 57 million lives in India by 2025 (Anon, 2002).

Obese people need to achieve a negative energy balance to maintain ideal body weights by cutting down their calorie intake or by burning the deposited fat. Diet is most easily modifiable factor of lifestyle without many efforts by the subject. Based on the success of dietary therapy to tackle a number of lifestyle disorders, existing foods may be reformulated or new food formulation can be done to suit the requirements of a large segment of the world populace suffering from Obesity and overweight.

2.0 Developing Thermogenic and Lipotropic Foods: Rationale to Reality

Overweight, obesity and cardiovascular diseases, etc are major public health problems round the world especially in America where obesity in adults has doubled since 1980, while over weight among adolescents has tripled. Approximately 280,000 to 3,25,000 deaths each year are attributed to obesity (Allison et al., 1999). Health care costs associated with obesity are estimated to amount to nearly $ 100 billion a year and Americans spend about $ 40 billion annually on weight control products and services (Expert Panel, 1998 and Wickelgren, 1998). In 2001, weight loss pills and liquid meal replacements grew by 20 per cent and 11 per cent, respectively contributing $ 3.9 billion to the weight loss market (Ohr, 2002). Now days, obesity, an established health hazard, is considered as a highly complex, chronic disorder with a multifactorial etiology (Vaughan, 2002).

Energy balance in the body depends on energy input and output. When energy input exceeds output, the excess is stored in the body as fat and body weight increases. Fat burners are the hottest supplements on the market today, whether being used by athletes or non-athletes and can provide many benefits to training and performance including increased energy and stamina. People suffering from high blood pressure and heart disease, for example, should be cautious with a fat burner that contains natural sourced caffeine. As long as fat burners are used correctly, they can provide many benefits for the athlete as well as overweight persons wishing to loss fat. The foods with fat burners to result thermogenesis are probably the most proliferated dietetic foods amidst the affluent society in the present decades.
3.0 Thermogenesis/Fat Burning: Approaching towards Definitions

Thermogenesis is activated by supplements, nutrients, exercises and exposure to cold (Sharma et al., 2004). Thermogenesis is the process by which the body generates heat or energy by increasing the metabolic rate above the normal. This rise in metabolic rate is referred to as the thermogenic effect, thermogenic response or Specific Dynamic Action (SDA). Thermogenesis is activated by a few different mechanisms including supplements, nutrition, exercise and exposure to cold. Among the nutritional activators, the various macronutrients have different effects on the thermogenic response. In the case of protein, energy is required to process the protein, which is then used for tissue growth and repair. On the other hand, carbohydrates and fat function primarily as fuel and are used more efficiently as such by the body. Carbohydrates and fat, therefore, have a much lower thermogenic effect than protein.

There are five pathways of stimulating thermogenesis in humans. Diet-Induced thermogenesis triggers a catalytic response, which actually causes the body to burn its "fat fuel" at a higher rate (Makhal and Kanawjia, 2003). Thermogenic aids increase metabolism by increasing the body's production of heat-energy by nutritional and biological means, i.e. the use of caffeine and ephedra combinations. Fat burners have become the dominant supplement sold on the market today and are typically used to increase energy and stamina as well as promote thermogenesis.

4.0 Lipotropic versus Thermogenic Foods

Lipotropic and thermogenic fat burners are two burgeoning sub-categories within the larger weight loss category. In the simplest of terms, lipotropics are any products or ingredients that have the ability to dissolve and metabolize fats. In essence, lipotropic foods emulsify stored fats and dump them into the blood stream to make them more available for the body to use as an energy source (Makhal and Kanawjia, 2003). The mode of action of thermogenic fat burners involves the creation of heat to increase metabolism and burn fat. Thermogenic products usually raise the body temperature by about one degree, increasing the metabolism to burn fat more effectively and act as an overall energizer. They may also have the side effect of depressing the appetite. How these two categories work depends on the goal of the consumers.

5.0 Thermogenic Ingredients

Fortunately for the consumers, there is a wide array of other thermogenic products to choose from the market. Typical thermogenic ingredients include Guarana, Citrus aurantium, Asian ginseng, Cayenne pepper, Coleus forskholii, and Green tea extract, etc.

5.1 Spices and Herbs

Some spices can help the body to lose weight. Commonly used spices like hot peppers, mustard, celery, chili powder, cinnamon, ginger, garlic, peppermint, as well as herbs like parsley have shown to induce thermogenesis by various ways. They regulate fat burning along with certain other important physiological functions. Here is some of the most potent example of spices to be used as thermogenic ingredients in formulation of thermogenic foods.

Cayenne Pepper It contains capsaicin that stimulates the metabolism of fat approximately 20 per cent. It not only stimulates the body's metabolic rate, but also cleans fat out of the arteries. Cinnamon, cloves, turmeric and bay leaves actually have drug-like
properties that help in stimulation of insulin activity, which means the body can process sugar more efficiently and therefore needs less insulin. By raising body temperature, *ginger* helps stimulate the body’s fat burning mechanisms. *Garlic* is another potent diuretic and an excellent weight loss aid. *Peppermint* is carminative, meaning it reduces gas build up, antispasmodic, anti-emetic and a cholagogue, which promotes bile secretion helping in fat digestion and elimination.

*Parsley* is primarily used to stimulate the circulatory system, increase energy and fight water retention. *Coleus forskholii* is an ancient herb from Ayurvedic medicine that has gained attention lately as a possible fat burner. It has incredible ability to raise levels of a particular enzyme called adenylate cyclase. This enzyme raises the conversion rate of ATP to cyclic adenosine monophosphate (cAMP) in the body. cAMP is known as a key regulator of the metabolism and fat burning. An active principle called forskolin present in *Coleus* has been found to be beneficial in its ability to burn fat. It is a safe fat burning ingredient that may be used as such or in formulation of thermogenic foods for a wide group of people ranging from obese to athletic persons.

The herb *Ephedra*, until recently, had been one of the most popular ingredients in thermogenic supplements. *Ephedra*, a central nervous system stimulant, was used as an energy tonic and metabolic enhancer. While it was not suitable for people with heart problems or high blood pressure. *Guarana*, a wild herb from the Amazon region of South America, is a very popular ingredient for fat burning and increases body's metabolic rate. The active constituent of guarana, guaranine, is nearly identical to caffeine and has become popular to the athletes and overweight persons looking to improve stamina, increase endurance, and burn fat. *Guarana* also contains the alkaloid compounds theobromine and theophylline, which may help curb appetite and increase weight loss. As a weight loss aid, *white willow* bark extracts offers little to no benefits by itself. In combination with other dietary supplements.

Asian ginseng has been a part of Chinese medicine for over 2,000 years and was traditionally used to improve mental and physical vitality. The active constituents of *ginseng*, the ginsenosides, are believed to increase energy and enhance physical performance. Studies conducted at the University of Munich, German found that *ginseng* ingestion with a 4 per cent ginsenoside extract had a positive effect on the performance of athletes.

### 5.2 Whole Vegetables

Certain vegetables or their extracts possess molecules with proven fat or calorie reducing ability. *Brussels sprouts*, broccoli, cauliflower and many berries are the examples of this category.

Berries have fructose as principle sugar to satisfy the cravings for sweets. British researchers found that the high content of insoluble fiber found in most berries and vegetables reduces the absorption of fat from foods enough to promote weight loss, without hampering nutrition. Berries are also an excellent source of potassium, which is effective for blood pressure control. Brindle berry helps suppress the appetite and block carbohydrates being converted into fats.

### 5.3 Proteins, Peptides and Amino acids

Research has shown that soy is a low-calorie, low-carbohydrate food that possesses the ability to decrease fat storage, increase fat cell metabolism, and process lean muscle mass. (Ohr, 2002) That’s why soy has become an important ingredient in many weight-loss
programs. Soy help to retain lean muscle mass, which in turn leads to increased burning of calories and decreased fat. Whey protein is another lean protein popular in weight-loss products. The whey peptide glycomacropeptide has been reported to stimulate the release of cholecystokinin (CCK). CCK has been shown to be an important regulator of appetite in humans, working directly on the appetite control center of the brain. Following food consumption, CCK is released from cells in the small intestine. Its release reduces appetite by slowing the movement of food from the stomach to promote the feeling of fullness. It also acts on nerves in the stomach lining that tell the brain that the stomach is full (Ohr, 2003). Many workers have reported that dieting is much easier when using a properly prepared whey protein formula. L-methionine is an essential amino acid. It assists in the breakdown or fats, preventing the build-up of fat in the liver and arteries, which obstructs blood flow to the brain, heart and kidneys. Methionine helps in the synthesis of the L-Carnitine.

Carnitine emerges as a great thermogenic supplement: it increases energy, burns fat making it an excellent addition to a weight loss program, and improves heart and liver health all at the same time. L-Carnitine is not an amino acid in the strict sense. It is not used as a neurotransmitter or in protein synthesis; however it bears many resemblances to amino acids and is usually grouped under this heading. L-Carnitine also helps the body in a function called lipolysis. When exercising, body uses carbohydrates for energy. L-Carnitine enables our body to burn fat for energy before burning carbohydrates, thus increasing fat burning ability.

Tyrosine is an amino acid, which acts as an appetite suppressant as it's also a precursor to CCK, the 'satiety hormone'. Tyrosine is also a powerful potentiator of the appetite suppressing effects of ephedrine-based supplements. It helps to maintain a higher metabolic rate on a reduced calorie diet. Probably, the cheapest of the best fat burners is tyrosine. It act as precursor for the synthesis of norepinephrine and dopamine. These two chemicals aid in the release of stored body fat, regulate metabolism and control appetite.

5.4 Fatty Substances as Thermogenic and Lipotropic Ingredients

The vast majority of studies have indicated that essential fatty acids, namely ω-3 and ω-6 fatty acids are very effective for weight loss. Aside from a multitude of other health benefits, ω-3 fatty acids block fat storage, increase fat burning and thermogenesis of the body. Rich in lecithin, which provides the building materials for healthy cell membranes, they also contain medium chain triglycerides used directly by our cells as a source of energy without increasing fat deposition. Clinical expertise has advised to take a high potency antioxidant formula to maximize the benefits of an ω-oil blend. The effectiveness of ω-fatty acids as fat burning agents shows good promise in formulation of effective thermogenic foods by its incorporation. Omega 3 fatty acids, found in salmon, mackerel, tuna and other fatty fish, can increase the metabolic rate; rid the body of excess fluids and increase energy levels. Other essential fats for a healthy metabolism are Omega 6 fatty acids, especially Gamma Linolenic Acid (GLA). Good sources are evening primrose oil, borage seed oil and black current oil, fish oil and flaxseed (either in seed form or as oil) are great sources of Omega 3 essential fat. Lecithin is known as a lipotropic, an agent that helps the liver converts fat stores into energy. Lecithin helps burn this extra fat and is also prescribed to lower blood cholesterol levels, increase energy and endurance, and improve short-term memory (Love, 2001).
5.5 Micronutrients in Thermogenic and Lipotropic Formulations

Inositol, also a part of the B vitamin complex, is closely associated with choline. Like choline, inositol as phosphatidylinositol is also found in lecithin, though in lesser amounts than choline, and acts as a lipotropic agent (milder than choline) in the body, helping to emulsify fats. Studies have demonstrated that chromium picolinate in humans improves the HDL/LDL ratio and that it increases muscle mass while decreasing the percentage of body fat. Niacin bound chromium is a biologically active form of chromium. Choline bitartrate is widely available vitamin in food but is sensitive to water and may be destroyed by cooking, food processing, improper food storage, and the intake of various drugs including alcohol, estrogen, and sulfa antibiotics. Choline is the active component of it and one of the "lipotropic" B vitamins, Choline is easily absorbed from the intestines and is one of the only vitamins that cross the blood-brain barrier into the spinal fluid to be involved directly in brain chemical metabolism. Choline is referred to as the "memory" vitamin, as it is an important part of the neurotransmitter acetylcholine.

5.6 Alkaloids as Thermogenic Ingredients

Ephedrine, caffeine, and aspirin are some of the alkaloids that exhibited fat regulating metabolisms. Ma Huang—Ephedrine a traditional herb has been shown to stimulate the nervous system and increase the rate of metabolism by stimulating the thyroid gland, thereby effectively burning calories at a faster rate through thermogenesis. Ephedrine raises body’s Resting Metabolic Rate (RMR) and increases the output of noradrenaline, a fight or flight hormone. Caffeine and aspirin minimize the body's attempts to neutralize the extra noradrenaline. Multiple human studies have showed that ECA (ephedrine/caffeine/aspirin) is one of the best fat burners for the majority users with minimal side effects. One of the best fat burners in the ephedrine category is Xenadrine RFA1, which contains herbal forms of ephedrine, caffeine and aspirin in optimum proportions. Special mention must be made of ephedrine free Xenadrine EFX, which has been reported to outperform ephedrine-based products in clinical studies. It's thought that Caffeine is FDA approved thermogenic ingredient. It is present in high amount in the coffee, green tea, kola nut, guarana etc. Caffeine stimulates the nervous system and metabolism, thereby burning calories at a faster rate through thermogenesis.

6.0 Thermogenic Foods/Drinks

Virtually, all foods are thermogenic viz. the body burns energy to digest foods. Some foods appear to burn more energy than they contain. Recent research has revealed that thermogenic foods can be formulated and its effectiveness can be increased by 100 per cent through enzyme manipulation. Thermogenic foods contain some thermogenic ingredients, which increase the rate that the body burns the stored fat to release energy. As the calories are burnt, the body has more energy for physical activities, which in turn burn more calories. The followings are the example of some thermogenic foods or drinks that can be used by athletes or overweight persons to help maintain their health (Makhal and Kanawjia, 2003).

6.1 Ice Water

It has been proved that high water intake reduces fat deposits and rids the body of toxins. Ice water will burn more calories since body’s metabolism will increase to warm the water to body temperature. It is suggested not to drink Ice Water with meals as it dilutes digestive enzymes. A little warm water or herb tea is a good choice then.
6.2 Green Tea (Iced or Hot)

*Green Tea* increases resting energy expenditure by 4 per cent, which in turn burns more calories. Green tea extract contains a considerable amount of catechin polyphenols that work with other chemicals to upgrade levels of fat oxidation and thermogenesis. Thermogenesis, by green tea extract, does not raise heart rates or blood pressure. This makes green tea superior to ephedra or other stimulants, which can have negative cardiac effects, especially in overweight individuals with hypertension.

Studies have shown that green tea that contains both Cannatic extract and Gymnemic acid, reduces absorption of sugar into the blood, and lessen the craving for sweets. Green tea extract has recently become a popular ingredient as fat burners. Many fat burners add green tea to their product not only for its overall health benefits but also for its natural source of caffeine. Recent studies show that green tea helps to liberate significant amounts of stored body fat as fuel without affecting lean body tissue.

6.3 Guggul-Phosphate Formula

*Guggul* sterones have been shown to trigger the output of thyroid hormones T₄ and T₃. The stimulation of these two hormones prevents the loss in metabolic rate while dieting. This process also influences the body to maintain normal or higher thyroid output, which positively affects the body's metabolic rate. The higher a person's metabolic rate will equate to more calories that will be burned and greater fat loss. When restricting calories by dieting, the body responds by lowering body's metabolic rate thus reducing the effectiveness of diet. However, when *Guggul* lipids and phosphates are combined to form a *Guggul*-phosphate formula, the effect is found to be significantly greater. The *Guggul*-phosphate formula, “Metabolic Thyrolean” is therefore worthy of a place in best fat burners.

6.6 Citrus Fruits

*Garcinia* is a type of citrus fruit found in Asia that is similar to our domestic fruit like oranges, lemons and grapefruit except that it contains (-) hydroxycitric acid (HCA). HCA acts as an appetite suppressant. It also has the ability to inhibit an enzyme responsible for the conversion of carbohydrates into fat. The conversion of dietary starches and sugars to body fat depends on a process known as the "Krebs Cycle". HCA interferes with the "Krebs Cycle" by effectively competing with the enzyme, ATP-citrate lyase necessary to produce fat by reducing the availability of acetyl-CoA, the building block for fatty acid and cholesterol synthesis. This effect has been demonstrated in animal studies. Scientists speculate that HCA works the same way in humans, and regular drinking of *Garcinia* juice will be an effective means to tackle the problem of overweight. A small amount of vinegar or lemon juice, has profound effect on the body's digestion rate. Because, its acidity has a powerful slowing effect on stomach emptying, thereby slowing downs the rate of starch digestion. Apple cider vinegar, an excellent fat burner, assists in the reduction of excess body weight. Bitter orange is a caloric expenditure and fat burner. It also increases the body’s metabolic rate and promotes weight loss and increases energy level. Bitter Orange, commonly known as *Zhi Shi*, has been used for thousands of years to promote weight loss by enabling the body to accelerate the removal of fat stores. Grapefruit (*Citrus paradisi*) extract is used to increase physical energy and a sense of well being in people suffering from obesity. Grapefruit is a concentrated source of fiber and vitamin C and both nutrients are role in fat metabolisms.
## 7.0 Thermogenic Supplements or Formulae: Commercial Scenario

With the phenomenal advances in food science and technology, a number of thermogenic foods and/or supplements have evolved with great deal of fanfare in the international supermarkets to meet the growing need of the modern health conscious consumers. Followings are the example of some them:

<table>
<thead>
<tr>
<th>Name</th>
<th>Active components</th>
<th>Physiological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitroPhen Enzyme</td>
<td>Ephedra, caffeine and white willow bark</td>
<td>Dynamic acceleration of thermogenesis, reduces appetite</td>
</tr>
<tr>
<td>BioDiet Plus TM Herbal</td>
<td>Guggul extract (Guggulsterone)</td>
<td>Metabolise deposited fat, increase Basal Metabolic rate, stimulate thyroid activity</td>
</tr>
<tr>
<td>Thyrox T-3</td>
<td>Guggul extract (Guggulsterone)</td>
<td>Metabolise deposited fat, increase Basal Metabolic rate, stimulate thyroid activity</td>
</tr>
<tr>
<td>Metabo Trim</td>
<td>Ginseng, Vitamin-C &amp; E, licorice root extract, atractyloses extract, holine, lipase, L-carnitine, arginine, taurine, glycine, DMAE, citric acid and green tea</td>
<td>Reduces cholesterol, metabolise stored fat</td>
</tr>
<tr>
<td>Gin –Tea</td>
<td>Ginseng, Vitamin-C &amp; E, licorice root extract, atractyloses extract, holine, lipase, L-carnitine, arginine, taurine, glycine, DMAE, citric acid and green tea</td>
<td>Reduces cholesterol, metabolise stored fat</td>
</tr>
<tr>
<td>Gin-G</td>
<td>Ginseng, garlic herbs and vitamins</td>
<td>Reduces cholesterol, metabolise stored fat</td>
</tr>
<tr>
<td>Gin-E</td>
<td>Ginseng, Vitamin E &amp; C, Schisandra, choline</td>
<td>Metabolism of stored fat</td>
</tr>
<tr>
<td>Gin-Sandra</td>
<td>Ginseng, Ma Huang, Schisandra, niacinamide, lipase, arginine, taurine, propylene glycol, DMAE, citric acid and pyridoxine</td>
<td>Reduces cholesterol, metabolise stored fat</td>
</tr>
<tr>
<td>Fast-Oxi</td>
<td>Arginine, atractyloses extract, Vitamin-E, C, B_3, B_6, B_12, betaine, glycine, L-carnitine, taurine, choline</td>
<td>Reduces cholesterol, metabolise stored fat, inhibit absorption of fat</td>
</tr>
<tr>
<td>Thin</td>
<td>Picolinate, L-carnitine, amino acids, green tea, Atractyloses extract, “B” vitamins, choline and chromium picolinate</td>
<td>Metabolise stored fat</td>
</tr>
<tr>
<td>Amplify</td>
<td>Ma Huang, atractolydoes extract, Vitamin B-12, licorice root extract, white willow, Garcinia and DMAE</td>
<td>Control appetite</td>
</tr>
<tr>
<td>Smart-Life</td>
<td>Ginseng, Vitamin B, C, E, licorice root extract, DMAE and choline</td>
<td>Reduces cholesterol, metabolise stored fat</td>
</tr>
<tr>
<td>Fast-Burn</td>
<td>Amino acids, B- vitamins, lipase and L-carnitine</td>
<td>Reduces cholesterol, metabolise stored fat</td>
</tr>
<tr>
<td>Metabolic Tropin-EF Fat Burner</td>
<td>Zhi Shi, Greentea, L-tyrosine, HCA or <em>Garcinia cambogia and Guggulsterone</em></td>
<td>Metabolise stored fat</td>
</tr>
</tbody>
</table>
8.0 Milk: A Weight-Loss Drug?

Dairy products contain literally hundreds of compounds that all have a positive effect on human health and enhance the fat-burning machinery. Lack of dairy products from the diet, send the body a signal -- to make more fat and the body becomes deprived of calcium, it begins conserving calcium. This mechanism prompts the body to produce higher levels of a hormone “Calcitriol” which promotes increased production of fat cells in the body (Zemel et al., 2000, 2002). Extra calcium in the diet suppresses this hormone. Milk, cheese, and yogurt are much more effective than calcium supplements or calcium-fortified foods. Dairy products are a complex collection of compounds. They are not classically nutrients, but are recognized as having beneficial effects.

Studies are showing that high calcium increases fat oxidation or fat burning, and those results in greater fat loss and weight loss if it's a reduced-calorie diet. Heany (2003) estimated that the incidence of overweight and obesity may be reduced by as much as 60-80 per cent if dietary calcium intakes were shifted upward to the recommended of 1000 mg/day for women ages 19-50. Teegarden (2003) found that increased calcium intake may help increase weight and fat loss.

9.0 Thermogenic Foods in India: Necessity and Futuristic Vision

There are a number of thermogenic diets or formulas available; some of them have found their position in Indian market also. A number of companies emerge to manufacture and launch a variety of thermogenic supplements and/or formulae to help protect the health conscious people from the problems of Obesity and overweight. However, because of their unpalatable taste and inherent consumers’ disregard towards drugs as such, these supplements and/or formulae are not well accepted. Hence, the development of thermogenic foods to provide an alternative solution seems to be an exciting frontier in the field of health foods. So there is an urgent need to reformulate the existing foods with the incorporation of thermogenic ingredients so that this vulnerable section of our society can entertain the benefits of natural thermogenesis from their food itself. As a result, there is a potential market for thermogenic foods in our country. Therefore as per the present scenario, it is a challenge before the food industries to develop thermogenic foods or supplements. There is an excellent potential for India to formulate thermogenic foods because of its rich floral kingdom and improved age-old Ayurvedic technology. The recent food-pharma alliance may provoke interest to our scientist community to extract these thermogenic ingredients from the floral sources and incorporate them to some selected existing foods to make them thermogenic.

10.0 Conclusion

Thermogenic foods, some of which have been now launched in the market shelves of the international supermarkets, are scientifically engineered to force the body to burn stored fat tissue at the high rate, until there is no more fat tissue left to burn. The prevention of obesity is more desirable than the treatment of the condition after it has become entrenched. Thermogenic foods help ensure the health and fitness level while allowing the body to loose weight fast and achieve goals of fat loss. Such kind of foods force our body to find it much harder to burn protein and carbohydrates and much easier to burn fat tissue for energy. Recently, advances in human nutrition and food science make possibility to design such kind
of thermogenic foods, which force our body to switch from burning protein and carbohydrates to burning fat tissue.

11.0 References

1.0 Introduction

Chhana, for which about 4% of total milk produced in India is utilized, is a product obtained by acid coagulation of hot milk it is used as a base for production of a wide variety of traditional Indian dairy sweets. Production of chhana involves destabilization of casein by addition of dilute acid to milk at relatively higher temperature. Acid affects the stability of casein directly by disturbing the charges carried by the particles and indirectly by releasing the calcium ion from colloidal calcium-caseinate-phosphate complex. The destabilization results in formation of large structural aggregates in which milk fat, serum proteins and other constituents get entrained. Whey is obtained as by-product, as is the case during paneer, cheese and casein production. About 80% of the total whey produced in India comes from chhana and paneer production alone. According to one estimate, (Khamurai, 2000), in India more than three million tons of whey is produced annually which contain about two lakh tons of valuable nutrients in it. There is an increased awareness all over the world on the potential utilization of whey, primarily because of stringent pollution prevention regulations and secondly due to salvaging the unique components of whey. Recently quite a few attempts have been made in the direction of processes for chhana making and chhana and whey-based product development. Some of these developments are mentioned below

2.0 Newer Developments and Opportunities in Chhana

2.1 Continuous Chhana Making:

To overcome the problems associated with small-scale production, attempts have been made to mechanize chhana-making process and a prototype chhana-making machine, with a capacity 40 kg chhana per hour, has been developed.

2.2 Chhana from Concentrated and Dried Whole Milk

Good quality of chhana from concentrated and dried whole milk after reconstituting it to 15 per cent TS has been produced.

2.3 Chhana by Ultra Filtration

Good quality chhana, using skim milk ultra filtered-diafiltered retentate and plastic cream. Skim milk, heated to 95°C for 5 min, is ultrafiltered (26% TS). The retentate is diafiltered (23% TS) with equal amount of water to reduce lactose. For preparation of chhana the retentate is mixed plastic cream to a protein/fat ratio of 0.722. The mixture is heated to 85-90°C/ 5 min and coagulated with dilute lactic acid to develop the characteristic grain. The granular mass is subsequently pressed to remove free moisture, yielding chhana. The process is reported to yield about 18-19 percent extra product and also no significant difference in flavour, body and texture and appearance compared to traditional method. High yield, easy
automation and flexibility in operation are emphasized as advantages of this method for adoption of large-scale production.

2.4 Chhana Powder

Method for production of chhana powder has been standardised (Tewari and De, 1976) The method consists of standardization of cow milk to 4.0% fat, boiling, addition of 0.2% sodium citrate, holding for 2+1/2 hrs. at 80+2 °C, effecting coagulation by adding 1-2% citric acid solution, draining of whey, making slurry of coagulum by adding equal amount of water and mixing thoroughly in an electric mixer. The slurry thus prepared is spray dried under standard conditions. Chhana powder is reported to contain: moisture-3.5%; fat 41.05%; protein- 45.37%; lactose- 4.22%. The powder is reported to be suitable for preparation of Sandesh however; rasogolla could not be made from it.

2.5 Dried Chhana Like Product

Dried chhana-like product, for use as a base for production of ‘Dried Rasogolla Mix’ has been prepared using membrane filtration approach. Cow skim milk is ultrafiltered to 3-fold concentration. The retentate thus obtained is diafiltered to reduce the mineral and lactose to almost to same level as in chhana. Subsequently pasteurised cream is added to diafiltered retentate, mixed thoroughly and spray dried under standard conditions.

The dried chhana-like powder obtained by this process was successfully used for making ‘Dried Rasogolla Mix’ after blending it with certain additives.

2.6 Chhana Based Low Fat Sweetened Dairy Spread

A process for production of a ‘Low Fat Sweetened Dairy Spread’ has been standardized, by using chhana as a base material. Other ingredients used in the product development included skim milk powder, sucrose, maltodextrin, and diacetyl. The chemical composition of the product was: TS, 78.8; fat, 34.4; protein, 10.1; carbohydrate, 32.6 ash, 2.2 percent. The calorific value (kcal / 100 g) of the product was) 495. The self-life of the product, with and without preservative was 36 and 16 days respectively.

3.0 Newer Opportunities in Whey Utilization

Whey contains almost half of the total solids of milk and is a source of precious nutrients like whey proteins, lactose, milk salts and water-soluble vitamins. Utilization of whey have been studied extensively world over as a possible means of diverting whey-solids in human chain. Utilization of whey for preparation of various vegetable based soups and fruit beverages is one of the most attractive way of avenue creation.

3.1 Whey Based Soups

Whey has successfully been utilized for the manufacture of soups with mixed vegetable, mushroom, tomato and spinach and beverages with fruit like mango, lemon, banana and pineapple (Singh, et al., 1994a,b). The common sequence of operation for in the development of whey-based soup involves blending of vegetable in whey and cooking of corn flour followed by heat processing. A proper time / temperature combination for cooking of vegetables, corn and seasoning is important step for proper dispersion of vegetables, gelatinisation of starch and flavour development in the finished soup. The flow diagram for the manufacture of whey-based soup is given in Fig- 1. Preparation of long shelf life soup involves proper sterilization of the prepared soup. Whey based soup in powder form can be
manufactured by cooking of vegetables in concentrated whey, blending gelatinised starch, mixing thoroughly to get a homogenous mass followed by spray drying.

Frying of seasoning
Cooking of corn flour
Cutting into uniform size
Vegetables
Vegetables
cleaning in water
Cooking into whey
Vegetables-whey soup mix
Blending into whey
Addition of salt
Boiling of liquid soup
Sterilization of soup

FIG. 1 Flow diagram for manufacture of whey based soup

3.2 Whey-based Fruit Beverage

Whey based fruit beverages are produced by mixing fruit pulp/juice or juice concentrate with processed whey. Quite often flavour / flavour blends and mineral addition is also included in the formulation. A brief process outline for production of whey-based beverage is given in Fig. 2

Clarified whey → Addition of sugar → Filtration → Addition of fruit pulp/juice
Sterilization ← pH adjustment ← Addition of synthetic flavour

FIG. 2 Flow diagram for manufacture of whey based beverage

4.0 Other Applications

4.1 Whey Products in Sweet Snacks

Sweet whey and whey protein concentrates are widely used in sweet and baked snacks. Such snacks included cereal bars (granola, muesli, rice), a wide variety of cookies, biscuits and wafers as well as nutrition, sport and candy bars. In general, whey products are used because of the mild and smooth dairy flavor they provide. In baked snacks, a typical level of 5% (flour basis) is recommended for achieving the functional benefits of whey products. Crust color and gloss are typically improved with the addition of whey and dough stiffness or stickiness reduced. Whey protein concentrates and isolates are also used as partial replacement of egg white or gelatin and as gelling/ binding agents. Whey ingredients are multi-functional and cost-efficient. In confections, such as nougat and malted milk balls, the aeration properties of whey proteins help control ingredient costs. The protein network within the foam structure helps stabilize the final aerated product. In the syrup or continuous phase of the confection, the protein network surrounds each of the air cells and prevents
collapse. Whey protein concentrates are also used in toffee, caramel, fudge and other confections as a partial casein replacement.

In addition to widespread use in seasonings and extruded snacks, whey products are increasingly used in crackers and mesa dough, and new applications include their utilization as coating for nuts and other snacks. Another key reason why manufacturers around the world select whey products as ingredients in snacks is that they provide valuable nutrients. Whey proteins are exceptionally rich in branch-chain amino acids, important nutrients in sports nutrition and they are rated among the top quality proteins available. Whey products are also a source of highly bioavailable dairy calcium, other minerals and vitamins. Whey protein concentrates and whey protein isolates are the ingredient of choice in healthy and sports snacks.

4.2 Whey-Functional Applications

Whey proteins have many valuable functional properties desired in egg less cakes, vegetarian sausages, sauces & soups, spreads, salad dressings, meat products, dairy products, bakery and confectionery products, etc. Whey proteins act as excellent emulsifiers, whipping agents, viscousifiers, water retention aids, texture aids and texture improvers. Whey proteins and whey powders as food ingredients can modify organoleptic, visual, hydration, surfactant, structural, textural and rheological properties of food, resulting in improved consumer acceptance of the finished product. In many cases, whey proteins serve more than one functional purpose in foods. For example, as whey proteins remain soluble over a wide pH range, and in particular near pH 4.5, they may be used in acidic drinks as protein fortifiers. They may also bring emulsifying properties to these products and add turbidity. Whey powders with lower protein concentration can be successfully used in desserts, soups, sauces, meats, baby food, ice cream, bakery, fermented products, and chocolate as substitute to skimmed milk powder/milk powder, with an attractive price differential.

4.3 Whey-nutraceutical Applications

It is reported that whey proteins are digested much faster than less advanced proteins. Increased rate of digestion has been shown as results in increased plasma amino acid and protein synthesis attributes, which makes it particularly attractive in the fields of clinical nutrition.

Whey proteins are studied by various nutritionist and it is of great interest that whey proteins are very useful in postoperative care of patients as most digestible proteins. It is highly recommended in body cell repairing & growth as well as provides all essential Amino Acids in sufficient amount to provide the best possible solution for formulation of clinical foods. Whey proteins consist of various minor proteins like lactoalbumin, lactoferrin, immunoglobulin, and serum albumin, lactoperoxidase, etc. Having high therapeutic values. These constituents have special medical application in dietetic products to treat & prevent a large number of clinical problems such as:

- Special formulation for diabetic patients
- For cardiac ailments
- For high cholesterol patients
- For liver, arthritis patients
- For burnt patients
- To deal with situation like renal failure, gout, trauma etc.
Recent researches have shown better efficacy of dietary whey proteins over other proteins including casein, meat & soya, because of special attributes in whey protein. Some of the details are as under

4.4 Immune-enhancing Properties - for H.I.V. & AIDS Patients

Recently it is discovered by research workers in Canada that whey proteins contain the most critical three bio active proteins required for the regeneration of intracellular stores of glutathione (IGSH). These three proteins are alpha-lactalbumin, lactoferrin and thermolabile serum albumin. They facilitate cellular GSH replenishment or maintenance required for the immune system of the body in case of HIV or AIDS infections.

4.5 Anti-cancer Effects of Whey Proteins

The clinical trials conducted have demonstrated better efficacy of total dietary Whey Proteins in preventing or retarding the development of chemically induced colon cancer and improving the immunocompetence, as compared to other dietary proteins including casein, meat & soya.

4.6 Anti-tumor Effects of Dietary Whey Proteins

Whey protein has been shown to stimulate cell mediated and humoral immunity, improve the body’s nutritional status in stressed individuals, have an antioxidant role by increasing tissue glutathione and, perhaps because of these, to have an inhibitory effect on the growth of several types of tumors including head and neck cancer.

4.7 Biopreservatives in Whey Proteins

Whey contains several proteins that display antimicrobial and in some cases antiviral activity. These components contribute to the natural defence system following birth and during development. Proteins from whey, which possess bio-preservative activity include lactoferrin, lactoperoxidase, lysozyme and immunoglobulins. The power of these defence systems in part in part arises from the synergism between these proteins, which function by distinct but complementary mechanisms without harming the body. Lactoferrin binds iron very strongly and renders this essential component unavailable to bacteria. In contrast to lactoferrin, lactoperoxidase inactivates or kill a board spectrum of microorganisms through an enzymatic reaction.

5.0 Selected Readings

Functional, Industrial and nutraceutical application of whey proteins.  www.mahaanfood.com
1.0 Introduction

Proteins are usually divided into two main categories, complete and incomplete. Complete proteins contain the entire amino acid spectrum including the nine essential amino acids that the body cannot synthesize, whereas incomplete proteins lack certain amino acids. The sources of complete proteins in diet are meat, fish, egg and milk products. Proteins from vegetables, beans, lentils and nuts usually lack one or other amino acids and hence termed as incomplete. These elements are vital to balanced diets to successfully build muscle as quickly as possible. Amino acids – the building blocks of proteins – are formed mostly of carbon, hydrogen, oxygen, and nitrogen.

The number of these amino acids is about 20 and although they are all important, 11 of these amino acids are considered non-essential amino acids because the human body can synthesize these. Nine that cannot be synthesized by the body are known as essential amino acids. These amino acids are also classified as semi-essential. This means that they must be derived from essential amino acids if insufficient amounts are consumed. When this occurs, the body’s supply of certain essential amino acids is depleted. Researchers now suggest that some other non-essential amino acids assume a more essential status when the body cannot readily generate them. This occurs during some illness. ‘Glutamine’ may assume an essential status in traumatic injury, especially in the period after intestinal surgery, and ‘arginine’ is essential for children born preterm.

2.0 Whey Proteins

During the manufacture of cheese, casein, paneer and shrikhand, the biologically high-grade whey protein is transferred to the whey. Whey, in the past, used to be considered a waste product that was at best used for animal nutrition. Recently, however, considerable efforts have been made to explore technological possibilities by which this valuable product could be made available for human nutrition. Whey protein is particularly rich in the sulphurous amino acids methionine and cystine. Whey protein concentrates, which can now be abstracted in an extremely pure form from whey, are therefore particularly suited for the protein enrichment of several articles of food. The supplementary value of whey proteins can be rated quite high on account of its extraordinarily high percentage of essential amino acids. This applies particularly to mixtures with vegetable protein.

2.1 Nutritional Aspects of Whey Proteins

The high nutritional value of a whey protein is based on its high concentration of essential amino acids. A group of FAO/WHO experts have worked out data for the ideal concentrations of essential amino acids in a dietary protein to meet the human requirements. WPC has all the essential amino acids in excess of FAO Standards; of particular importance are isoleucine, lysine, threonine and tryptophan. Whey protein has an even higher
concentration of tryptophan, leucine, threonine and lysine than whole egg protein, which, on the other hand, is richer in methionine and cystine as well as in phenylalanine and tyrosine. This whey protein fraction has a relatively low content of methionine, which, however, is compensated for by the high concentration of cystine. Its valine content is only slightly below the value of the reference protein. The true digestibility of WPCs has been found to be 97-100%, that of lactalbumin somewhat lower with values of 94-96%.

3.0 Whey Proteins - Nutraceutical Application

Whey proteins are termed as “wonder proteins” and may find applications in nutritional, functional, dietetic, sports and infant foods because of best possible amino acids profile, high PER and biological value, easier digestibility and assimilability, natural taste in native form and excellent functional properties.

3.1.1 Nutritional & Dietetic Foods

Whey proteins provide excellent nutritional values, in nutrition foods formulated for kids, adults and old aged people as growth tonic for body health maintenance. The major products are general health food products as well as protein supplements as under:

Nutritional supplements for all age groups containing USP as high protein foods, low fat foods, low salt foods etc.:
- For lactose intolerance for all ages
- Geriatric food formulation – for old aged people with low digestibility
- For pregnant, lactating and nursing women
- Pediatric foods for growth of children, for anemic children, for memory boosting of children
- For faster post-operative recovery or recovery from illness
- For general health beverages, stamina building & vitality

Whey proteins fulfill the need of essential amino acids for growing kids as they require constant supply of high amount of essential as well as non-essential amino acids. The excellent PER value gives the regular weight gain as per their growth requirements. For adults, whey proteins are highly recommended as these proteins facilitate the weight control management of body. Lactoferrin present in whey protein improves iron absorption in body.

3.1.2 Clinical Foods

It is established that whey proteins are digested much faster. Increased rate of digestion results in increased plasma amino acid and proteins synthesis attributes, which makes it particularly attractive to the field of clinical nutrition. Whey proteins are very useful in post operative care of patients. It is highly recommended in body cell repairing and growth as it provides all essential amino acids in sufficient amount and serves as the best possible solution for formulation of clinical foods.

Whey Proteins consist of various minor proteins like: α-lactalbumin, lactoferrin, immunoglobulin, serum albumin, lactoperoxidase etc. having high therapeutic values. These constituents have special medical application in dietetic products to treat & prevent a large number of clinical problems such as:
- Special formulations for cancer / tumor patients
- Special formulations for diabetic patients
For cardiac ailments
For high cholesterol patients
For liver, arthritis patients
For burnt patients
To deal with situation like renal failure, gout, trauma etc.

3.1.3 Infant Food Formulations

Formulation of physiologically suitable infant foods necessitates reduction of protein and mineral levels from bovine milk – rich in lactose and whey proteins and containing appropriately low levels of essential minerals. Demineralised whey is an ideal ingredient for infant formula. Lactose and its breakdown products, glucose and galactose have several metabolic and developmental functions in infants. Whey proteins extracted from milk are universally known for their immunological values for infants. That is the most important factor in mother’s milk considered the best for babies. In low birth weight baby formulation, the level of whey proteins is now made mandatory in the ratio of 60:40 with casein; hence fortification with whey proteins is the only solution to achieve this.

3.2 Sports Foods

The importance of whey protein supplementation is critical to any individual looking forward to build and maintain muscle mass. Whey proteins have been enriched by nature with branched chain amino acids (BCAA), namely L-isoleucine, L-leucine, L-valine. These branched chain amino acids must be present in the muscle cell to promote protein synthesis. These BCAA helps increase the bioavailability of high complex carbohydrate intake and are absorbed by muscle cells for anabolic muscle building activity.

The current theory is that during prolonged exercise, the BCAAs are released from skeletal muscle, the carbon part is used as fuel and the nitrogen part is used to make the amino acid alanine, which then goes to the liver where it is turned into glucose for energy. So for athletes who want to protect their existing mass, the idea is to take a BCAA source before and after the exercise. The issues being investigated are whether BCAAs reduce muscle breakdown and act as an energy source during this period. While maintaining exercise performance and delaying exertion, BCAAs are very important for muscle growth. Whey proteins also help in speedy repairing of injured and torn muscles during practice and performance.

4.0 Recent Research Findings

4.1 Anti Cancer Effects of Dietary Whey Proteins

Recently, it has been recognized that several of the whey proteins confer antibacterial and immune associated protection to the neonate against disease and that these and other proteins also have putative biological effects when ingested, including anti-cancer action. This putative activity provides the rationale for further investigation of therapeutic benefits elicited by whey proteins when included in the diet, and the basis, if substantiated, for development of whey protein–based foods for the expanding functional food market. Scientific efforts have focussed primarily on the roles of dietary fat, fiber and carbohydrate in promotion of carcinogenesis, with less consideration for the role of dietary proteins in cancer.
etiology. The anti-cancer activity of dietary whey proteins has been investigated in an attempt to substantiate their role in disease prevention. The work has focused on the impact of whey proteins on colon cancer susceptibility and immune status using an established animal model. Feeding trials have been conducted to compare the efficacy of dietary proteins in preventing or retarding the development of chemical-induced colon cancer in laboratory rats, and the influence of dietary protein on animal immune competence as measured by thymus development.

Total dietary whey proteins was demonstrated to have a protective effect against the development of dimethyl-hydrazine-induced colon cancer in the rats when compared with other common dietary protein, including casein, meat and soy proteins. Chemical analysis of tissues from the animals also indicated that the whey protein-fed rats have the highest antioxidant status (as measured by tissue glutathione content), and the lowest content of fecal fat. Low antioxidant status and high levels of fat in the feces are recognized risk factors for colon tumorigenesis. In complementary and parallel study, the same diets were compared in a rat model of immune status. Results showed that when all other dietary components were equal, the consumption of milk proteins (whey, casein) was associated with the highest animal immune-competence, as measured by thymus growth and retardation of thymus involution. It has been also observed that age is not a barrier to the potential health benefits associated with consumption of whey protein. It has also been demonstrated that diet supplemented with lactoferrin enhance the protective effect of total whey protein and reduce the number of aberrant crypt cells in the colon of animals consuming proteins associated with poor protection in the colon cancer model.

4.2 Immune-enhancing Properties of Whey Protein Isolated in HIV Patients

It has been demonstrated that the ability of lymphocytes to offset oxidative damage (during their oxygen-requiring colonel expansion and following that expansion in the product of antibodies), is measured by determining the capacity of these cells to regenerate intracellular stores of glutathione (GSH). Therefore allowing them to respond more fully to the antigenic stimulus.

More evidences for the involvement of GSH in the modulation of immune function have come from studies on HIV patients. HIV-infected individuals have lower GSH concentrations in their blood lymphocytes. Moreover, a recent study indicates that the more GSH the patients carry in their CD4 helper T-Cells, the longer these patients are likely to survive.

When GSH stores are “used up” or depleted, the bioactive proteins present in the WPC help maintain GSH levels, thus supporting an optimal immune response. Optimization of the immune response in animal fed with specially prepared isolates is attributed to greater production of GSH in their lymphocytes through continuous dietary provision of supplementary doses of GSH precursors.

Moreover, a Canadian clinical trial with the whey protein isolate was conducted in children with AIDS and Wasting Syndrome over a six-month period. Patients who started the study with low blood-lymphocyte GSH exhibited a substantial increase in GSH content.

4.3 Biopreservatives from Whey Proteins

In studies with mice, it has been shown that whey proteins enhance humoral immune response. The sulphhydryl containing amino acids, cysteine and glutathione are related to
immune response. Whey proteins are rich in cysteine. \( \beta \)-Lactoglobulin contains 33mg of cysteine per g protein, while \( \alpha \)-Lactalbumin and bovine serum albumin contain 68 and 69 mg cysteine/g protein, respectively. The \(-\)SH compounds are also involved in quenching toxic free radicals. \( \alpha \)-Lactalbumin is a calcium binding protein and thereby enhances calcium absorption. It is an excellent source of essential amino acids, tryptophan and cysteine. Tryptophan regulates appetite, sleep-waking rhythm, and pain perception. Cysteine is important in function of \(-\)SH compounds. \( \alpha \)-Lactalbumin interacts with galactosyl transferase enzyme to promote transfer of galactose from UDP – galactose to glucose to form lactose in the mammary gland.

The immunoglobulins in milk are also important for importing immune defence to the host. Approximately 75% of the total immunoglobulin protein in milk is Ig G1. Milk contains 0.6gm/litre of Ig G1, whereas colostrum has it at a substantially higher level of 48 gm/litre. Other fractions are Ig G2, Ig A, Ig M, all of which provide passive immunity.

Lactoferrin has a role in non-specific defence of the host against invading pathogens. It is active against several Gram positive and Gram-negative bacteria, yeasts, fungi and viruses. Its iron – binding characteristic aids in enhancing iron absorption. It stimulates and protects cell involved in host defence mechanism. Furthermore, it controls cytokine response.

Lactoperoxidase, an enzyme, oxidizes thiocyanate (SCN) to a number of short-lived anti-bacterial components (hypothiocyanate, cyano sulphurous acid and cyano sulphuric acid) through the breakdown of hydrogen peroxide. Therefore, it has been recommended as a biopreservatives for preservation of raw milk under field conditions. Another use of lactoperoxidase is to control the acid development in stored yoghurt. It is also incorporated in toothpaste to prevent cavities.

Lysozyme has antimicrobial activity against Gram positive bacteria and acts by digestion of bacterial cell walls.

Bifido bacterial flora of colon impacts health promoting properties and health gut ecology in the host. Bifidogenic activity of milk is ascribed to \( \alpha \)-Lactalbumin, lactoferrin and glyco macro-peptide derived from the breakdown of k-casein during digestion.

5.0 Conclusion:

Whey proteins are the “Wonder Proteins”. Recent medical researches on whey proteins have established the fact that this amazing protein synthesis makes a much powerful immunity shield to keep away many diseases and illness that we would be exposed to. The main applications are in clinical nutrition for preparation of special formulations for patients suffering from diabetics, cardiac ailments, high cholesterol, arthritis, serious burns, renal failures, gout etc. Whey proteins are also valuable in developing infant foods including low birth weight formulae (LBW) and dietetic & nutritional foods for all age groups like lactose intolerance, pregnant or lactating women, pediatric foods, post-operative recovery, general health beverages, stamina building etc. The most recent medical research reports have shown better efficacy of dietary whey proteins over other proteins as immune enhancing proteins for HIV and AIDS patients, anti-cancer effects, anti-tumor effects, anti-microbial & anti-viral effects etc.
1.0 Introduction

Milk proteins have unique role in human nutrition. Besides the supply of nitrogen and essential amino acids to the human beings, they also have physiological activities through bioactive sequences. Preventing disease by food interventions, based on a thorough understanding of the underlying mechanisms, is the most challenging field of research. Here, food and pharma gets connected and interface exists for bringing know-how from both sides together. Several diseases such as diabetes, obesity, cardiovascular disorders and allergy are related to food intake. The various risk factors identified for these diseases may be reduced by adaptation of diet and the consumption of functional foods or nutraceuticals. The development of products with specific health benefits requires a combination of skills that go beyond those of the traditional food technologists. The competence of formulating tasteful food products that are eaten up with pleasure has to be combined with the ability of designing meaningful clinical trials that prove efficacy and safety of the incorporated bioactive peptides. The development process will be significantly expensive than the traditional way of new product formulation and the final product will consequently be more expensive.

2.0 Physiological role of milk protein based bioactive peptides

Biologically active peptides have been characterised in milk protein following

- their in-vitro digestion with both specific and crude proteinase and exopeptidase preparations,
- their in vivo digestion with gastrointestinal proteinase/peptides,
- the action of bacterial proteinases and peptidases activity during the generation of fermented milk products.

Furthermore, the structure and biological activity of various peptides corresponding to specific sequences of milk proteins have been determined and confirmed by peptide synthesis studies.

3.0 Casein derived bioactive peptides

Opioid peptides are those having pharmalogical similarities to opium (morphine) and are derived from casein called casomorphins. The major opioid peptides derived from bovine milk are fragments of β-casein. The opioid peptide fragments of β-casein are called β-casomorphins due to their morphine like behavior. All κ-casein fragments known as casoxins, behave as opioid antagonists. Further, opioid peptides are two α-c-casein exorphins (α-casomorphins), which were found in pepsin hydrolysates of α-casein. A tetrapeptide amide, morphiseptin is the most active opioid agonist in bovine β-casomorphin group. Two opioid antagonist, casoxin C and D also belong to this group. All bioactive peptides derived from bovines’ α-casein behave as opioid antagonists. Opioid peptides released from casein during digestion showed gastrointestinal motility. Some of these peptides have been found to
affect gastrointestinal transit time. Casomorphins have been found to prolong gastrointestinal transit time and exert antidiarrhoeal action. Some peptides show antihypertensive activity. These peptides are referred to as casokinins. The angiotensin I converting enzyme (ACE) hydrolyses largely inactive angiotension I to the octapeptide angiotension II, which increases blood pressure. This enzyme also hydrolyses bradykinin, which is a hypotensive. Thus ACE inhibitors are antihypertensive peptides, other antihypersensitive peptides are located in the primary sequence of bovine β-lactoglobulin (β-lactorphins) and human β- and κ-casein. Some specific inhibitors of ACE are proven antihypertensive drugs (Meisel & Schlimme 1994). The antihypertensive effect of orally administered doses of Calpis sour milk or peptides (Val-Pro-Pro or Lie-Fro-Pro) on spontaneous hypertensive rats was studied by Nakamura et al. (1995). The sour milk or peptides decreased systolic blood pressure 6-8 hr post administration. Several immunomodulating peptides resulting from casein have been detected including immunopeptides from α-casein and β-casein. In vitro activity of immunomodulating peptides resulting from tryptic and chymotryptic hydrolysates α-casein and β-casein has been reported to stimulate the macrophage activity against red blood cells (Parker et al. 1984). The peptide may stimulate the proliferation and maturation of T cells and natural killer cells for the defense of the newborn against a large number of bacteria, particularly enteric bacteria. Lahov & Regelson (1996) have reported antibacterial activity of isracidin, the 1-23 fragment of αs1-casein obtained from the action of chymosin, against Staphylococcus aureus and Candida albicans. The injection of isracidin into the udder of sheep and cow gave protection against mastitis. Casein phosphopeptides (CPPs) can be released in vitro and in vivo by gastrointestinal trypsin from α- or β-casein. CPPs are not unpalatable and can be used as anticariogenic additive. Severe heat treatment of milk may cause dephosphosylation of phosphoseryl residues and may affect the bioavailability of CPP. The casein phosphopeptides prevent the precipitation of calcium phosphate and increase the concentration of soluble calcium in vitro and also in the lumen of small intestine of rat (Naito et al. 1972). Thus, the physiological role of CPPs may be due to inhibition of precipitation of calcium phosphate. Some peptides have shown antithrombotic activity. There are a number of similarities between clotting of blood and that of milk. Renin or chymosin hydrolyses the peptide bond between residues 105 and 106 (Phe-Met) of κ-casein. There are structural and functional homologies between the dodecapeptide of fibrinogen and the 106-110 sequence of κ-casein (called casopastrin). Casopastrin has been shown to have anti thrombotic activity as this peptide inhibits binding of fibrinogen on platelets (Fiat et al. 1993). The involvement of free radicals, primary and secondary products formed during lipid oxidation, and protection against their deleterious consequences in many biological processes and diseases and during processing and storage of food products have now been largely recognized. Many biological materials such as muscles, milk, eggs and blood contain protein or peptides, which can exhibit an antioxidant activity. The antioxidant activity of milk proteins such as lactoferrin and casein has been demonstrated in various systems. Casein hydrolysates and casein derived peptides have been shown to inhibit enzymatic and non-enzymatic lipid peroxidation. Caseinophosphopeptides were demonstrated to exhibit antioxidant properties in oil in water emulsion. Iron chelation, scavenging of free radicals and reactions of some chains of amino acids with hydroperoxide or secondary oxidation products, such as malonaldehyde and other aldehyde are the main mechanism involved in the antioxidant activity of amino acids peptides and proteins.
3.1 Whey protein derived peptides

The bioactive peptide sequences are in an inactive state inside the polypeptide chain of the intact whey protein. These peptides released during intestinal digestion of whey proteins, may be involved in regulation of nutrient entry and influence the postprandial metabolism via stimulation of the secretion of hormones (Yamaguchi, 1992). Some of the bioactive peptides obtained from whey protein include α-lactorphin, β-lactorphin, albutensin, α- and β-lactotensin. Lactoferricin, a peptide derived from bovine lactoferrin due to action of pepsin has been found to have antimicrobial activity against various bacteria and Candida albicans. It is a single peptide consisting of 25 amino acid residues. A single active peptide consisting of 47 amino acid residues has been obtained from human lactoferrin. Recently, it has been shown that α-lactalbumin and β-lactoglobulin hydrolysate lowers the metabolic performance of recombinant E.coli strain. The highest bacteriostatic effect was observed upon hydrolysis with enzyme combination of pepsin followed by trypsin. Some whey proteins are known to contain bioactive peptides with weak opioid activity including serorphin and albutassin from the serum albumin fraction, lactoferroxin from lactoferrin and lactorphin from α-lactalbumin. The two peptides, α-lactorphin and β-lactorphin have shown to show contraction of smooth muscles similar to morphine. Albutensin and β-lactotensin cause contraction of guinea-pig ileum longitudinal muscle. Whey proteins including α- and β-lactophin and albutensin appear to have ACE inhibitory activity.

3.2 Technological Challenges

Four different technological hurdles have to be overcome before a product containing bioactive substances is ready to consider marketing:
- Isolation of the desired components,
- Pre-establishment of the biological activity,
- Incorporation of the bioactive components into a formulated product,
- Verification of efficacy and safety of final product.

Such a sequence of experimental events is also required for the introduction of new food additives; additional is a thorough proof of the claimed benefit of the bioactive components. This applies especially when the bioactive component is a completely new substance never consumed before in significant amounts. Due to the economy at scales in dairy industry, it is worthwhile to consider the commercialization of minor components in milk with (potential) biological activity. Separation, purification and production of an industrial level of these peptides must be thought in terms of integrated and high added value use of milk components that is use of controlled separation technologies preserving researched properties, preventing microbial spoilage and taking in account potential value of co-products generated at each step of the used process. Membrane and chromatographic separation techniques are preferred over solvent extraction technologies. Membrane technologies (MF, UF, NF) provide key opportunities to prepare well-defined protein substrates in a native state. Oriented release of specific peptides can be achieved, in spite of the lack of basic knowledge on enzyme kinetics under heterogeneous conditions, by controlling environmental parameters of proteolysis in a membrane reactor. The technologies can only be feasible on large scale at locations where substantial quantities of milk are processed. These methods can also be applied to fractionate specific bioactive peptides set free by food grade enzymes from major dietary protein like
caseins. Here, a well-balanced trade off between product purity and manufacturing costs is even more important.

Once a bioactive component can be efficiently produced on pilot scale, questions about the relevant properties for application become prominent. It is obvious that there is a desire to have an application range as broad as possible. The component will be envisaged in several end products with often completely different product matrices and manufacturing process. To effectively incorporate the bioactive components in a product, the prediction of its properties during processing, storage and finally consumer use is required to prevent multiple failures during application development. The first step in this process is the establishment of the key physical and chemical properties of the purified bioactive component. This will bring the essential information on parameters such as solubility, stability, heat and pH sensitivity. Step two is the incorporation in the final formulation in a way that the food product is tasteful and the bioactivity maintained in an effective dose. Step three is the ultimate proof that the expected efficacy and safety of the final product can carry the beneficial claims implicitly or explicitly connected to the product.

3.3 Status of Bioactive peptides in fermented products

Many industrially used dairy starter cultures are highly proteolytic. This property is traditionally exploited by 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 LAB such as *Lactococcus lactis*, *Lactobacillus helveticus* and *Lactobacillus delbrueckii var. bulgaricus* is already well known. The release by microbial fermentation of various bioactive peptides from both casein and whey proteins has been reported in many studies. Nakamura *et al.* (1995) reported the presence of two angiotensin converting enzyme (ACE) inhibitory peptides (Val-Pro-Pro and Ileu-Pro-Pro) in sour milk, which was fermented with a starter culture composed of *L.helveticus* and *Saccharomyces cerevisiae*. This enzyme plays a crucial role in the regulation of blood pressure in mammals. Immunostimulatory peptides have also been detected in milk fermented with a *L.helveticus* strain. Yoghurt contains peptides in the range of 500 - 10,000 Da, which in vitro reduce the risk of cancer. A recent study revealed that when adults ingested 500 ml of milk or yoghurt, certain functional peptides could be identified in their stomach, duodenum and blood notably caseinoglycopeptides with an antithrombotic sequence. It is interesting to note that the level of these peptides is higher after ingesting yoghurt rather than milk, which suggested the role of LAB in the formation of the functional peptides in fermented milk products. Table 1 shows a number of bioactive peptides with their functional activities derived from different fermented dairy products. Various studies have reported on the casmorphins, ACE inhibitory peptides and phosphopeptides found in cheese. Cheese contains phosphopeptides as natural constituents and extensive proteolysis during cheese ripening leads to the formation of other bioactive peptides such as ACE-inhibitory, peptide and precursors of β-casomorphins having several healthy attributes. The ACE-inhibitory activity in medium aged gauda was about double that of long ripened gauda. A αs-casein derived peptide isolated from 6 month ripened Parmesan cheese was not detectable after 15 months of ripening. Products having a low level of proteolysis (Quarg) have a low ACE inhibition index. These results indicate that the bioactive peptide liberated by proteolytic enzymes from LAB during cheese ripening were degraded further to inactive fragments as a result of further proteolysis (Meisel et al. 1997). Table 2 presents different
bioactive peptides detected and identified in a number of cheese by a number of workers. ACE inhibitory peptides have been separated from several Italian cheese characterized by short (Crescenza and Italico) and medium (Ghorgonzola) ripening period (Smacchi and Gobbeti 1998). Another bioactive peptide containing the β-casomorphin –7 sequence, showed an antihypertensive activity has been isolated from Crescenza and Cheddar cheese. Proteolytic enzymes of LAB produce caseinophosphopeptides during ripening of cooked curd cheese like Comte or Grana Padano. Peptides derived from cheese slurry prepared using Lactobacillus lactis sub sp. lactis as starter culture has anticarcinogenic effect (Kin et al. 1995).

Table 1. Bioactive peptides identified in fermented milk products

<table>
<thead>
<tr>
<th>Product</th>
<th>Bioactive peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sour milk</td>
<td>Phosphopeptide, ACE-inhibitor, β-casomorphin-4</td>
</tr>
<tr>
<td>Fermented milk (Treated with pepsin &amp; trypsin)</td>
<td>ACE-inhibitory, Immunomodulatory</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>ACE-inhibitory, Immunomodulatory, Antihypertensive, Antiamnesic, Microbicidal &amp; Antithrombotic</td>
</tr>
</tbody>
</table>

Table - 2 Bioactive peptides identified in several cheese

<table>
<thead>
<tr>
<th>Product</th>
<th>Bioactivity observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarg</td>
<td>ACE inhibitor</td>
</tr>
<tr>
<td>Parmesan, Reggiano</td>
<td>β-casomorphin precursors</td>
</tr>
<tr>
<td>Comte</td>
<td>Phosphopeptides</td>
</tr>
<tr>
<td>Cheddar</td>
<td>Phosphopeptides</td>
</tr>
<tr>
<td>Edam, Emmental, Gouda, Roquefort, Tilsit</td>
<td>ACE inhibitory</td>
</tr>
<tr>
<td>Mozzarella, Crescenza, Italico, Gorgonzola (Italian varieties)</td>
<td>ACE inhibitory</td>
</tr>
<tr>
<td>Edam, Emmental, Cheddar, Turunmaa, Festivo (Finnish varities)</td>
<td>ACE inhibitory</td>
</tr>
<tr>
<td>Cheddar, Edam, Swiss, Feta, Camembert</td>
<td>Immunomodulatory</td>
</tr>
<tr>
<td>Blue vein (Australian varieties)</td>
<td>Antiamnesic, Opioid agonist</td>
</tr>
<tr>
<td>Gauda, Emmental, Havarti</td>
<td>ACE inhibitory</td>
</tr>
</tbody>
</table>

4.0 New dairy products with bioactive peptides

The starter culture applied in the manufacture of ‘Festivo’ cheese, a novel bioactive cheese is a mixture of commercial starter cultures containing 12 different strains of the following genera or species: Lactococcus sp. and Leuconostoc sp. (BD type cultures), Propionibacterium sp. Lactobacillus sp. as well as Lactobacillus acidophilus and Bifidobacterium sp. It has been reported that the ACE-inhibitory peptides increases during ‘Festivo’ cheese ripening and decreases when proteolysis exceeds a certain level during the storage period. These results would suggest the ACE inhibitory peptide and probably other biologically active peptides as well are naturally found in cheese. This called for the development of totally new type of cheese with the health attributes of derived bioactive peptides. The new Festivo cheese based on an innovative concept in the production of
healthy foods has been commercially developed (Ryhanen et al. 2001). Festivo is now manufactured commercially in Finland and has attracted growing interest among health aware consumers.

Valio Ltd has investigated the blood pressure effects of lactobacillus helveticus LBK-16H fermented milk product, Evolus ® both in animal models and in humans. The composition of the Evolus® product has been optimized to hypertensive subjects by optimizing the fermentation conditions to increase the production of the antihypertensive peptides VPP and IP using living bacteria and optimizing the mineral composition. In mild hypertensive subjects, this milk product containing bioactive tri-peptide Val-Pro-Pro and Ile-Pro-Pro reduced blood pressure in an eight week long study. In another study the same milk product tended to lower blood pressure in moderately hypertensive subjects.

A clinical study presented at American Diabetes Association Scientific Sessions showed that hydrolyzed whey proteins significantly reduced blood pressure (Nutra ingredients.com 2004). A team from the university of Minnesota led by Joel Pins studied the effects of an enzymatically prepared hydrolysed whey protein supplement on multiple heart disease risk factors, including ACE activity and bradykenin, which increases vascular permeability and dilates blood vessels. The trial enrolled 30 generally healthy individuals with mild/moderate hypertension. Participants were randomized to 20gm/day of either hydrolysed whey protein or unmodified whey protein and were asked to not make lifestyle changes throughout the six-week trial. Treatment with the hydrolysed whey protein resulted in a significant reduction in blood pressure levels by the end of the first week of treatment, this effect was maintained throughout the study. The hydrolysed whey protein treatment also significantly reduced ACE activity and increased bradykenin, while levels were not significantly changed by the unmodified protein control.

5.0 Conclusion

Milk derived bioactive peptides unquestionably, represent a fascinating opportunity for the dairy industry to be a major actor of the nutraceutical market but their common use for preservation of health and even treating chronic illness requires a deep and constant exchange between protein biochemists, technologist and nutritionists in order to demonstrate their physiological activity on humans.

6.0 Suggested Reading

1.0 Introduction

Natural antioxidants present in food and other biological systems are receiving considerable interest in recent times due to their role in human nutrition and health. The main characteristic of an antioxidant is its ability to react with free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases. Antioxidants scavenge free radicals such as peroxides, hydroperoxides or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Some of the major health benefits of natural antioxidants are:

- Counteraction of the damaging action of low-density lipoproteins (LDLs) thereby protecting the arteries from the effects of atherosclerosis.
- Protecting the endothelial cells of the arteries from the free radical damage, permitting them to be compliant and reactive rather than rigid and dysfunctional.
- Decrease in platelet aggregation, thus protecting vascular system from clot formation responsible for heart attack.
- Counteraction of the oxidation promoting effects of stress hormones, such as the catecholamines (epinephrine and norepinephrine) often secreted in high amounts during high chronic stress.
- Counteraction of free radical damage to many cells of the body that could potentially trigger un derived proliferations leading to cancer.
- Protection against damaging effects of aberrant metabolism, which could trigger type II diabetes.
- Protection of important connective tissues of the body to counteract age related degeneration.
- Enhancement of immune responses against viral infections and protection from the formation and spread of many forms of cancer.
- Counteraction of damaging actions of inflammatory responses in body including joints (Arthritis) and brain (Alzheimer’s disease)
- Protection against degenerative processes in brain, which could lead to specific neural damage, associated with Parkinson’s and Alzheimer’s diseases.

Antioxidants depending on their chemical structure could be divided into polyphenols (flavonoids, anthocyanins, phenolcarboxylic acids, and coumarins), carotenoids (carotenes and xanthophylls), and tocopherols (vitamin E). Ascorbic acid (vitamin C) and selenium also posses strong antioxidant activity. Some of the common antioxidants and their sources are listed in Table 1.
Table 1 Common Antioxidants and their sources

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astaxanthin</td>
<td>Algae, yeast, shellfish, salmon and trout</td>
</tr>
<tr>
<td>Bixin</td>
<td>Seed coats of anatto</td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td>Salmon, trout and egg yolk</td>
</tr>
<tr>
<td>α-carotene</td>
<td>Many of the sources of β-carotene, apples, corn, peaches, potatoes,</td>
</tr>
<tr>
<td></td>
<td>water melon, palm oil</td>
</tr>
<tr>
<td>β-carotene</td>
<td>Common in green leaves, pumpkin, mangoes, apricot, carrot, spinach and</td>
</tr>
<tr>
<td></td>
<td>parsley</td>
</tr>
<tr>
<td>δ-carotene</td>
<td>A precursor of α-carotene and lutein, many yellow-red fruits &amp;</td>
</tr>
<tr>
<td></td>
<td>vegetables and corn</td>
</tr>
<tr>
<td>δ-carotene</td>
<td>A precursor of β-carotene and lutein, apricots, carrots, corn, sweet</td>
</tr>
<tr>
<td></td>
<td>potatoes, tomatoes, watermelon and palm oil</td>
</tr>
<tr>
<td>Catechins</td>
<td>Red wine and tea</td>
</tr>
<tr>
<td>Cryptoxanthins</td>
<td>Red capsicum, pumpkin and mangoes</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Tea, green tea, citrus fruits, red wine, onion and apple</td>
</tr>
<tr>
<td>Indoles</td>
<td>Cruciferous vegetables such as broccoli, cabbage, and cauliflower</td>
</tr>
<tr>
<td>Isoflavonoids</td>
<td>Soybeans, tofu, lentils, peas and milk</td>
</tr>
<tr>
<td>Lignans</td>
<td>Sesame seeds, bran, whole grains and vegetables</td>
</tr>
<tr>
<td>Lutein</td>
<td>Leafy greens like spinach and corn</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Tomatoes, pink grapefruit and watermelon</td>
</tr>
<tr>
<td>Manganese</td>
<td>Seafood, lean meat, milk and nut</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Thyme and oregano</td>
</tr>
<tr>
<td>Selenium</td>
<td>Seafood, offal, lean meat and whole grains</td>
</tr>
<tr>
<td>Vitamin-C</td>
<td>Oranges, blackcurrant, kiwifruit mangoes, broccoli, spinach, capsicum</td>
</tr>
<tr>
<td></td>
<td>and strawberries</td>
</tr>
<tr>
<td>Vitamin-E</td>
<td>Vegetable oils (such as wheat germ oil), avocados, nuts, seeds and</td>
</tr>
<tr>
<td></td>
<td>whole grains</td>
</tr>
<tr>
<td>Zinc</td>
<td>Seafood, lean meat, milk and nut</td>
</tr>
</tbody>
</table>

2.0 Polyphenolic Compounds

Polyphenols also referred as polyhydroxy phenols have characteristic six-carbon ring and more than one –OH group attached to it. Flavonoids are the most important polyphenolic compound and account for nearly 2/3 of the polyphenols we obtain in our diet. Together these represent more than 8000 different phytochemicals, about half of which are flavonoids. Major flavonols represent quercetin, kampferol and myricetin while the important flavones are luteolin and apigenin.

The term flavonoids represent some 4000 compounds that impart the colourful pigments to fruits, vegetables and herbs. Table 2 represents antioxidant activity and total phenolic content of popular vegetables. They are also found in legumes, grains and nuts. While research reports suggest tremendous healing potential of flavonoids, only a few of them have been thoroughly investigated. Among them are genistein, found in soyabean and some other legumes; quercetin, found in apple and onion; PCOs (Procyanidolic oligomer, also known as proanthocyanidins) found in grape seed extract and red wine; citrus flavonoids
including rutin and hesperidin, found in oranges, grapefruits, tangerines and other citrus fruits; and EGCG (Epigallocatechin gallate) found in green tea.

### Table 2

**Anti-oxidant activity and total phenolic content of vegetables**

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Total phenolics [mg/100gm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kachnar</td>
<td>275.0±10.1</td>
</tr>
<tr>
<td>Aonla</td>
<td>348.8±13.4</td>
</tr>
<tr>
<td>Beet root</td>
<td>323.0±11.7</td>
</tr>
<tr>
<td>Black carrot</td>
<td>350.5±12.9</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>217.5±8.9</td>
</tr>
<tr>
<td>Ginger</td>
<td>221.3±9.4</td>
</tr>
<tr>
<td>Palak</td>
<td>196.3±8.1</td>
</tr>
<tr>
<td>Cabbage</td>
<td>92.5±2.4</td>
</tr>
<tr>
<td>Carrots</td>
<td>55.0±0.9</td>
</tr>
<tr>
<td>Bathua</td>
<td>253.5±9.8</td>
</tr>
<tr>
<td>Potato</td>
<td>149.8±6.3</td>
</tr>
<tr>
<td>Turnip</td>
<td>127.0 ±1.8</td>
</tr>
<tr>
<td>Garlic</td>
<td>145.0±5.9</td>
</tr>
<tr>
<td>Onion</td>
<td>56.8±1.1</td>
</tr>
<tr>
<td>Green chillies</td>
<td>115.0±1.2</td>
</tr>
</tbody>
</table>

### 2.1 Health Benefits of Flavonoids

As antioxidants, flavonoids offer several health benefits.

- **Lower cancer risk** — A high intake of Polyphenols and quercetin has been found to lower stomach, pancreatic, lung and breast cancer. Genistein, a phytoestrogen that acts as a weak form of hormone estrogen, reportedly helps in preventing breast cancer and other hormone related cancers, including prostate cancer because it binds with estrogen receptors in the body’s cells.
- **Reduced risk of CHD** — A diet high in flavonoids particularly quercetin and PCOs may prevent blood clots and blocked arteries.
- **Protection against age related vision disorders and macular degeneration** — Flavonoid quercetin inhibits build up of sugar sorbitol in the eye’s lens responsible for development of cataracts. Grape seed extracts also help combat cataracts and prevent macular degeneration because it improves blood circulation in the eyes.
- **Relieve hay fever, sinusitis, and asthma symptoms** — Quercetin is known to have significant anti-inflammatory properties and reduce inflammation in the lungs and other air passages.
- **Alleviate inflammatory skin conditions, such as eczema and bug bites** — Quercetin has anti-inflammatory properties which can treat skin irritations.
- **Reduce inflammation in joints and rheumatoid arthritis** — Quercetin is particularly effective in treating these conditions.
- **Minimize menopausal hot flashes** — Genistein, reduces the symptoms of hot flashes as it can mimic the effects of estrogens in the body.
- **Shink hemorrhoids and reduce varicose veins** — Citrus flavonoids and PCOs help repair hemorrhoids and varicose veins by strengthening blood vessels.
• Battle viral infection — Flavonoids, in general, boost immunity and thus help the body to speedily recover from illness.

3.0 Carotenoids

Carotenoids are the family of compounds also called tetraterpenes. Carotenoids include the carotenes: \(\alpha\), \(\beta\), \(\delta\) and \(\gamma\). Compounds such as lycopene, lutein and astaxanthin are also carotenoids but not carotenes. Some of the carotenoids are further classified as ‘Xanthophylls’ - which are ‘oxygenated carotenoids’ and include one or more oxygen atoms in their structure. Compounds such as lutein, astaxanthin, violaxanthin, zeaxanthin and capsanthin are example of xanthophylls. Carotenoids are colourful fat/oil soluble pigments found in green leaves and yellow, orange and red fruits and vegetables such as sweet potatoes, carrots, apricots, mangoes and tomatoes. Astaxanthin is a carotenoid from marine sources like algae, salmon and trout.

Vitamin E and Vitamin C are considered very important antioxidant vitamins. The term Vitamin E is used as a generic description for all tocol and tocotrienol derivatives exhibiting the biological activity of \(\alpha\)-tocopherol. There are at least eight E vitamers (four tocopherols and four tocotrienols). All tocopherols and tocotrienols have vitamin E activity. The biopotency of \(\alpha\)-tocopherol is the highest, while those of \(\beta\), \(\gamma\) and \(\delta\)-tocopherols are 50%, 10% and 3% of that of \(\alpha\)-tocopherol. Tocotrienols have lower Vitamin E activity than the respective tocopherols.

3.1 Health Benefits

Carotenoids are oil soluble and protect the lipid component in a way similar to the oil soluble Vitamin E. They help to prevent the lipid peroxidation (oxidative damage to the lipid components in cell membrane and in circulating blood), which is associated with atherosclerosis and cardiovascular disease. Carotenes are, however easily oxidisable and should be therefore consumed along with other antioxidants so that they can act synergistically with the carotenes to protect them. However, all antioxidant vitamins work singly as well as synergistically to prevent or delay oxidative reactions that lead over time to degenerative diseases including cancer, cardiovascular disease, cataracts etc. Table 3 gives an assessment of scientific evidences supporting role of other antioxidant vitamins in prevention of major chronic diseases, which are associated with free radical cause.

Table.3 Scientific evidence for health benefits of antioxidant vitamins in chronic disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
<th>(\beta)-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Cancer</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cataracts</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Immune function</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Arthritis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

- Little or no evidence of relationship
+ Some evidence of relationship
++ Good evidence of relationship
+++ Excellent evidence of relationship
4.0 Lycopene

Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. It is a carotenoid, an acyclic isomer of β-carotene. It is one of the most important anti-oxidant, with a singlet oxygen quenching activity twice as high as that of β-carotene and 10 times higher than that of α-tocoferol. Red fruits and vegetables including tomatoes, watermelon, pink grapefruit, apricot and pink guava contain lycopene (Table 4). Ripe tomatoes are one of the richest sources of lycopene as it represents nearly 80-90% of the total carotenoids present in the common varieties of tomatoes.

<table>
<thead>
<tr>
<th>Material</th>
<th>Lycopene content mg/100 g, Wet Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>0.72-20</td>
</tr>
<tr>
<td>Watermelon</td>
<td>2.3-7.2</td>
</tr>
<tr>
<td>Guava (pink)</td>
<td>5.23-5.50</td>
</tr>
<tr>
<td>Grapefruit (pink)</td>
<td>0.35-3.36</td>
</tr>
<tr>
<td>Papaya</td>
<td>0.11-5.30</td>
</tr>
<tr>
<td>Rose hip</td>
<td>0.68-0.71</td>
</tr>
<tr>
<td>Carrot</td>
<td>0.65-0.78</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>0.38-0.46</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>0.02-0.11</td>
</tr>
<tr>
<td>Apple pulp</td>
<td>0.11-0.18</td>
</tr>
<tr>
<td>Apricot</td>
<td>0.01-0.05</td>
</tr>
</tbody>
</table>

4.1 Health Promoting Effects

Lycopene has been found to be extremely effective in the prevention of varieties of ailments in human including cancer and cardiovascular diseases. In contrast to carotenoids such as β-carotene, the biological activity of which is related to its ability to form vitamins within the body, lycopene lacks the β-ionone ring structure and cannot form Vitamin-A. Its biological effect in humans has therefore been attributed to mechanism other than Vitamin-A. Dietary lycopene possibly increases the lycopene status in the body and acting as an anti-oxidant, may trap reactive oxygen species, increase the overall antioxidant potential or reduce the oxidative damage to lipid (lipoproteins, membrane lipids), proteins (important enzymes) and DNA, thereby lowering oxidative stress. This reduced stress may lead to reduce risk for cancer and cardiovascular disease. Alternatively, the increased lycopene status in the body may regulate gene functions, improve intercell communication, modulate hormone and immune response, regulate metabolism, thus lowering the risk for chronic disease. These mechanisms could be interrelated and may operate simultaneously to provide health benefits.

5.0 Glutathione

Glutathione is a tripeptide composed of the amino acids cysteine, glycine and glutamic acid (gamma-glutamyl-cysteinyl-glycine). Cysteine is toxic outside of the cells, and is not readily transported into cells. Inside cells, cysteine alone does not increase synthesis of glutathione as much as the dipeptide glutamyl-cysteine. Dietary glutamyl–cysteine is also
believed to increase cellular cysteine much more than dietary cysteine. The whey proteins β-lactoglobulin, serum albumin and lactoferrin are especially rich in glutamyl-cysteine. Whey proteins contain 8 times more of the amino acid cysteine than casein. The highest concentration of glutathione is found in the liver which is the principal organ involved in the detoxification and elimination of toxic materials. Glutathione also acts to reconstitute the antioxidant vitamin C and E after they have been oxidized and therefore plays a determinant role in their functions.

5.1 Health Benefits

Low glutathione levels are found in immune compromised individuals, neuro degenerative diseases such as multiple sclerosis, ALS, Alzheimers and Parkinson’s disease, atherosclerosis, male infertility, pregnancy complications, cataracts, damage from many pharmaceutical drugs, cancer and poor survival rates for patients with AIDS. High levels of glutathione appear to protect against dangers of cancer, heart disease, premature ageing, autoimmune disease and chronic illness.

6.0 Effect of Processing on Functionality of Natural Antioxidants

Like most other food components, antioxidants present in foods also change during processing and storage. The most important losses of antioxidant activity occur as a result of chemical changes in antioxidant present in food materials. The most pronounced changes are brought about by oxidative reactions occurring rapidly on heating or slowly in storage. Antioxidants are oxidized either by lipid oxidation product (mainly hydroperoxidases) or directly by oxygen, either dissolved in lipidic or aqueous phases or adsorbed from the air. The effects of various processes involved in processed food manufacture on the stability of natural antioxidants are delineated below.

6.1 Heating with Water as Medium of Heat Transfer

Heating of foods at temperatures above ambient conditions result in loss of nutritional quality as well as of antioxidant activity. While these changes are generally moderate at temperatures below 100°C, higher temperatures and longer heating times result in significant losses.

6.1.1 Changes During Pasteurization

Transformations of tocopherols (Vit E) are moderate during pasteurization. Losses of ascorbic acid are mainly due to thermal destruction and to a lesser extent due to oxidation. In fruit juices, enzymatic browning of polyphenolics, catalysed by polyphenoloxidases in the presence of dissolved oxygen results in colour deterioration. So, rapid inactivation of polyphenoloxidases is essential for preservation of phenolics. Deaeration minimizes losses of ascorbic acid and carotenes as well.

6.1.2 Effect of Blanching

Rapid heating of the food material deactivates enzymes, such as lipoxigenases, which catalyse lipid oxidation. The primary products of lipoxygenase catalysed oxidation-lipid hydroperoxides-may partially destroy natural antioxidant. The deactivation of polyphenoloxidases is also helpful in protection of phenolics against enzyme-catalysed
oxidation into the respective quinones, which have very low or non-existent antioxidant activity.

6.1.3 Effect of Sterilization
The severity of heat treatment during sterilization results into substantial losses of water-soluble vitamins and more importantly ascorbic acid. Deaeration or removal of oxygen prior to heating is generally helpful in reducing subsequent losses.

6.1.4 Effect of Boiling
Boiling affects antioxidant activity quite similar to sterilization. The heat denaturation of haeme pigments in foods of animal origin may increase the prooxidative effect of iron and thus reduce the antioxidant activity. During boiling, antioxidants are partially extracted and remain in boiling water and if the boiling water is to be discarded substantial antioxidants are lost.

6.1.5 Effect of Evaporation
Thermal decomposition during evaporation results in loss of antioxidants. Reduced pressure, rapid rate of heat transfer and efficient vapor-liquid separation could minimize their losses.

6.1.6 Effect of Extrusion Cooking
Losses of ascorbic acid and vitamin A are up to 50% depending on the moisture content in food and the residence time at elevated temperature. Losses of antioxidants rise moderately with increasing temperature and water content in the barrel. In contrast, losses in cold extrusion process are minimal.

6.2 Heating with Air as the Medium of Heat Transfer
Air being a poor medium of heat transfer, higher temperatures and longer processing times are often used. Changes are more intensive on the surface than in the inner layer so that antioxidants are formed or destroyed at or near the surface.

6.2.1 Effect of Roasting and Baking
During these operations, while the outer layer temperatures are in the range of 120-200° C or more, the innermost layer temperature may not exceed 100° C. Antioxidants and other food components in the inner layer undergo changes identical to those occurring during boiling; but in the outer layers, pyrolysis, caramelisation and maillard reactions may transform antioxidants into less active or sometimes into more active products. Some intermediary maillard products such as reductones have high antioxidant activities in aqueous solutions or emulsions. Volatile products originating in various side reactions are further oxidized using oxygen present in the systems and the oxidation of lipids or antioxidants is thus prevented. Maillard products also possess some chelating activity. By binding heavy metal ions or active complexes into complexes possessing no oxidation promoting activity, they partially protect other antioxidants against destruction. Thus, although natural antioxidants are partially lost during heating, the overall antioxidant properties of heated foods are maintained or even enhanced by the development of new antioxidants; such as maillard type of reaction products. Tocopherols and tocotrienols present in the flour are partially destroyed (25% in case of α-tocopherol) during cooking and baking.
6.2.2 Effect of Drying

During the drying process, lipid oxidation is limited because of short residence time and the presence of water vapor in the atmosphere. Antioxidants are usually not damaged during drying and their evaporation is only moderate.

6.3 Microwave Heating

Studies on the effect of microwave heating on oilseeds indicate that tocopherols and other antioxidants are partially destroyed. Tocopherols are destroyed in the order of δ-tocopherol > β-tocopherol > γ-tocopherol > α-tocopherol.

6.4 Frying in Oil

Losses of natural antioxidants during deep fat frying are only small as their volatility is much lower than that of common synthetic antioxidants. Carotene is rapidly oxidized with full access of oxygen but at low oxygen pressure, which generally occurs during deep fat frying, carotenes act as free radical scavengers. Tocopherols in frying oils are decomposed by both direct oxidation with oxygen and by reaction with oxidized fatty acids. The more unsaturated the frying oil, the more rapid the destruction of tocopherols under frying conditions. The stabilization of frying oils with flavonoids or phenolic acids is less efficient as they are rarely soluble in oil and their antioxidant activity is thus evident only on interface of frying oil and fried material. During deep fat frying, heavy metal derivatives are partially released into frying oil thus enhancing deterioration. It is therefore advisable to add some metal scavengers like citric acid to the frying oil.

6.5 Fermentation Processes

Fermentation processes are enzyme driven and progress at around room temperatures so that lipids are damaged to a negligible extent by oxidation processes and thus antioxidants are not damaged by oxidation as well. Furthermore, oxygen is generally replaced by carbon dioxide in most fermentation processes. Hydrolytic processes may cause cleavage of esters or glycosides of phenolic antioxidants into the respective acids or aglycones. They are generally more active as antioxidants than the original compounds, such as quercetin and myricetin, which are more active than the respective glycosides. Some free amino acids released during proteolysis act as synergists to the stability of fermented foods against oxidation.

7.0 Suggested Readings

1.0 Introduction

The nutrients are metabolized within the human body by various biochemical means. Many of these nutrients are metabolized into simpler compounds, absorption through intestinal linings and then act as precursor for the synthesis of certain other molecules vital for the human life. The figure summarizes the metabolic pathways of carbohydrate, protein and fat metabolism.

2.0 Pyruvic Acid - Cross Roads Compound

Depending upon the conditions and the organism under consideration, a variety of metabolic fates await pyruvic acid.

2.1 Acetyl CoA

If the conditions are aerobic, pyruvic acid is converted into acetyl CoA for entry into the citric acid cycle.

2.2 Gluconeogenesis

Pyruvic acid can be converted back to glucose or glycogen by the process of gluconeogenesis.

2.3 Alanine

Pyruvic acid can be converted into the amino acid alanine by the process of transamination to be described later.
2.4 Anaerobic Synthesis of Lactic Acid

If conditions are anaerobic, pyruvic acid is converted into lactic acid. This occurs under strenuous exercise conditions in the muscles. The vigorous exercise soon strips the cells of available ATP (energy). Oxygen cannot be resupplied to the cells at a fast enough rate, therefore, an oxygen debt or anaerobic conditions result.

2.5 Fermentation - Ethanol

Yeast and several other microorganisms undergo glycolysis or fermentation to produce ethanol from sugars. This is the process to make wine, beer, and other alcoholic products from grains and fruits. The reactions of glycolysis are identical to those involved in the production of pyruvic acid. The difference is in the fate of pyruvic acid. In fermentation, pyruvic acid is converted first to ethanal (acetaldehyde) and then to ethanol.

2.6 Formation of Acetyl CoA from Pyruvic Acid

Under aerobic conditions the end product of glycolysis is pyruvic acid. The next step is the formation of acetyl coenzyme A (acetyl CoA) which is the initiator of the citric acid cycle. In carbohydrate metabolism, acetyl CoA is the link between glycolysis and the citric acid cycle.

3.0 Carbohydrate Metabolism

3.1 Glycogenesis Glycogenolysis and Gluconeogenesis

The goal of glycolysis, glycogenolysis, and the citric acid cycle is to conserve energy as ATP from the catabolism of carbohydrates. If the cells have sufficient supplies of ATP, then these pathways and cycles are inhibited. Under these conditions of excess ATP, the liver will attempt to convert a variety of excess molecules into glucose and/or glycogen.

3.1.1 Glycogenesis

Glycogenesis is the formation of glycogen from glucose. Glycogen is synthesized depending on the demand for glucose and ATP (energy). If both are present in relatively high amounts, then the excess of insulin promotes the glucose conversion into glycogen for storage in liver and muscle cells. In the synthesis of glycogen, one ATP is required per glucose incorporated into the polymeric branched structure of glycogen. Actually, glucose-6-phosphate is the crossroad compound. Glucose-6-phosphate is synthesized directly from glucose or as the end product of gluconeogenesis.

3.1.2 Glycogenolysis

In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1-phosphate and then into glucose-6-phosphate. Two hormones, which control glycogenolysis, are a peptide, glucagon from the pancreas and epinephrine from the adrenal glands. Glucagon is released from the pancreas in response to low blood glucose and epinephrine is released in response to a threat or stress. Both hormones act upon enzymes to stimulate glycogen phosphorylase to begin glycogenolysis and inhibit glycogen synthetase (to stop glycogenesis). Glycogen is a highly branched polymeric structure containing glucose as the basic monomer. First individual glucose molecules are hydrolyzed from the chain, followed by the addition of a phosphate group at C-1. In the next step the phosphate is moved to the C-6 position to give glucose 6-phosphate, a cross road compound. Glucose-6-
phosphate is the first step of the glycolysis pathway if glycogen is the carbohydrate source and further energy is needed. If energy is not immediately needed, the liver converts the glucose-6-phosphate glucose for distribution in the blood to various cells such as brain cells.

3.1.3 Gluconeogenesis:
Gluconeogenesis is the process of synthesizing glucose from non-carbohydrate sources. The starting point of gluconeogenesis is pyruvic acid, although oxaloacetic acid and dihydroxyacetone phosphate also provide entry points. Lactic acid, some amino acids from protein and glycerol from fat can be converted into glucose. Gluconeogenesis is similar but not the exact reverse of glycolysis, some of the steps are identical in reverse direction and three of them are new ones. Oxaloacetic acid is synthesized from pyruvic acid in the first step. Oxaloacetic acid is also the first compound to react with acetyl CoA in the citric acid cycle. The concentration of acetyl CoA and ATP determines the fate of oxaloacetic acid. If the concentration of acetyl CoA is low and concentration of ATP is high then gluconeogenesis proceeds. Also notice that ATP is required for a biosynthesis sequence of gluconeogenesis. Gluconeogenesis occurs mainly in the liver with a small amount also occurring in the cortex of the kidney. Very little gluconeogenesis occurs in the brain, skeletal muscles, heart muscles or other body tissue. In fact, these organs have a high demand for glucose.

4.0 Lipid Metabolism

4.1 Lipids as an Energy Reserve

Nearly all of the energy needed by the human body is provided by the oxidation of carbohydrates and lipids. Whereas carbohydrates provide a readily available source of energy, lipids function primarily as an energy reserve. The amount of lipids stored as an energy reserve far exceeds the energy stored as glycogen since the human body is simply not capable of storing as much glycogen compared to lipids. Lipids yield 9 kcal of energy per gram while carbohydrates and proteins yield only 4 kcal of energy per gram.

It is interesting to compare the relative amounts of energy provided by various biochemicals in a typical 154 lb male. The free glucose in the blood provides only a 40 kcal energy reserve -- only enough to maintain body functions for a few minutes. Glycogen remaining stored in the liver and muscles after an overnight fast, amounts to about 600 kcal energy. Glycogen reserves can maintain body functions for about one day without new inputs of food. Protein (mostly in muscle) contains a substantial energy reserve of about 25,000 kcal. Finally, lipid reserves containing 100,000 kcal of energy can maintain human body functions without food for 30-40 days with sufficient water.

The following diagram summarizes the metabolic fate of fat as well as interconversion of various non fat metabolites to fat under various conditions.

4.1.1 High carbohydrate-low fat diets

Note that a high carbohydrate diet will not free you from fat accumulation. Glucose can be converted to fatty acids and glycerol via acetyl CoA and glyceraldehyde 3 phosphate respectively:
4.1.2 Starvation diets

On the other hand starvation (or a high fat/low carbohydrate/low protein diet) will cause other problems: It is indicated from the above diagram, that for fats to be converted to citric acid, oxaloacetate must be available. Without oxaloacetate, citric acid cannot be formed and acetyl-CoA cannot be further processed. Oxaloacetate can be produced from the breakdown of certain amino acids. However a person on a high fat/low carbohydrate/low protein diet, will need to use the oxaloacetate for gluconeogenesis. Fatty acid breakdown however must still occur for ATP generation and acetyl-CoA will need to be processed through an alternative pathway. This pathway leads to the formation of ketone bodies. The amino acids will need to be derived from muscle tissue, leading to muscle wastage.

4.1.3 High protein, low fat, low carbohydrate diets:

High protein, low fat, low carbohydrate diets offer a mechanism for fat reduction. The amino acids in the diet provide the oxaloacetate required for gluconeogenesis. Body fat and the amino acids provide ATP. An example of such a diet would be a diet based around fish meat. The suitability of such a diet for fat reduction is probably not a straightforward matter as other complications may arise. The best strategy for burning fat is a low fat diet coupled with exercise.

4.1.4 Ketone bodies:

Ketone bodies are water-soluble acetate derivatives. These derivatives include, acetone, acetoacetate and β-hydroxybutyrate. Ketone bodies are produced in small quantities in healthy persons. However under some conditions, eg. fasting or starvation conditions, excess levels of ketone bodies can accumulate to high levels. The production of ketone bodies under these conditions is an important survival mechanism used by cells under such conditions.
conditions. Ketone bodies are produced in the liver. The liver does not use them but acetoacetate and β-hydroxybutyrate can be used by the heart, skeletal muscle and kidney. During starvation conditions, the form a valuable energy source for the brain. Ketone bodies are synthesized from acetyl-CoA and not surprisingly the excessive accumulation of acetyl-CoA will lead to higher levels of ketone bodies in the liver and blood stream. Acetoacetate and β-hydroxybutyrate are converted back to acetyl CoA during their degradation. Acetone is not used but is expelled via the lungs. One indication that a person is producing ketone bodies at a higher rate is the presence of acetone in the person's breath.

Acetyl-CoA will accumulate during starvation or fasting conditions. As discussed above under these conditions, oxaloacetate is required for gluconeogenesis and is not available for the conversion of acetyl-CoA to citrate. As a consequence, Acetyl-CoA derived from fatty acid and amino acid breakdown will be converted to ketone bodies. As acetoacetate and β-hydroxybutyrate are both acids, there is risk that high levels of ketone bodies can lead to acidosis of the blood.. Acidosis (or keto-acidosis) can also occur in untreated diabetics.

4.1.5 Essential Fatty Acids and Eicosanoid Metabolism:

The two essential fatty acids namely Linolenic Acid (18:2 n-6) (LA) and α-Linolenic Acid (18:3, n-3) (ALA) are the precursors of eicosanoids, the derivatives of 20- carbon fatty acids. The eicosanoids consist of the prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs). The PGs and TXs are collectively identified as prostanoids. Prostaglandins were originally shown to be synthesized in the prostate gland, thromboxanes from platelets (thrombocytes) and leukotrienes from leukocytes, hence the derivation of their names. The eicosanoids produce a wide range of biological effects on inflammatory responses (predominantly those of the joints, skin and eyes), on the intensity and duration of pain and fever, and on reproductive function (including the induction of labor). They also play important roles in inhibiting gastric acid secretion, regulating blood pressure through vasodilation or constriction, and inhibiting or activating platelet aggregation and thrombosis. The following diagram describes in short the elongation of LA and ALA. Their respective elongation products namely Arachidonic Acid (AA) and Eicosa Pentaenoic Acid (EPA) are further converted to various eicosanoids.

![Eicosanoid Metabolism Diagram](image-url)
Since the enzymes involved in elongation and desaturation of both the pathways are same, there is competition between the two fatty acids which act as substrates for these enzymes, α-Linolenic Acid being the preferred substrate. Moreover, the eicosanoid products of n-3 fatty acids as well as other elongation products such as EPA and DHA have more health promoting benefits than those of n-6 fatty acids. Therefore, there is a need for restoring the balance of n-6:n-3 fatty acids in diet. The current dietary pattern of affluent Indian as well as American population shows a ratio of 40:1 to 10:1 n-6:n-3 fatty acids which needs to be reduced as one of our dietary goals.

5.0 Protein Metabolism

Nitrogen metabolism is no less important than carbohydrate and lipid metabolism. Proteins make up the structural tissue for muscles and tendons, transport oxygen or hemoglobin, catalyze all biochemical reactions as enzymes, and regulate reactions as hormones. Our bodies must be able to synthesize the many proteins, amino acids, and other non-protein nitrogen containing compounds needed for growth, replacement, and repair. Proteins in excess are used to supply energy or build reserves of glucose, glycogen, or lipids. The "nitrogen or amino acid pool" is a grand mixture of amino acids available in the cell derived from dietary sources or the degradation of protein.

Since proteins and amino acids are not stored in the body, there is a constant turnover of protein. Some protein is constantly being synthesized while other protein is being degraded. For example, liver and plasma proteins have a half-life of 180 days or more, while enzymes and hormones may be recycled in a matter of minutes or hours. Each day, some of the amino acids are catabolized producing energy and ammonia. The ammonia is converted to urea and excreted from the body and represents a drain on the nitrogen pool. A nitrogen balance is achieved by a healthy person when the dietary intake is balanced by the excretion of urea wastes. If nitrogen excretion is greater than the nitrogen content of the diet, the person is said to be in negative nitrogen balance. This is usually interpreted as an indication...
of tissue destruction. If the nitrogen excretion is less than the content of the diet, a positive nitrogen balance indicates the formation of protein.

5.1 Transamination Reaction

Transamination as the name implies, refers to the transfer of an amine group from one molecule to another. A family of enzymes called transaminases catalyzes this reaction. Actually, the transamination reaction results in the exchange of an amine group on one acid with a ketone group on another acid. It is analogous to a double replacement reaction. The most usual and major keto acid involved with transamination reactions is alpha-ketoglutaric acid, an intermediate in the citric acid cycle. A specific example is the transamination of alanine to make pyruvic acid and glutamic acid. Other amino acids which can be converted after several steps through transamination into pyruvic acid include serine, cysteine, and glycine.

5.2 Other Transamination Reactions:

Aspartic acid can be converted into oxaloacetic acid, another intermediate of the citric acid cycle. Other amino acids such as glutamine, histidine, arginine, and proline are first converted into glutamic acid. Glutamine and asparagine are converted into glutamic acid and aspartic acid by a simple hydrolysis of the amide group. All of the amino acids can be converted through a variety of reactions and transamination into a keto acid which is a part of or feeds into the citric acid cycle.

5.3 Urea Cycle

Urea is the major end product of nitrogen metabolism in humans and mammals. Ammonia, the product of oxidative deamination reactions, is toxic in even small amounts and must be removed from the body. The urea cycle or the ornithine cycle describes the conversion reactions of ammonia into urea. Since these reactions occur in the liver, the urea is then transported to the kidneys where it is excreted. The overall urea formation reaction is:

\[ 2 \text{Ammonia} + \text{carbon dioxide} + 3\text{ATP} \rightarrow \text{urea} + \text{water} + 3\text{ADP} \]

Urea is routinely measured in the blood as: Blood Urea Nitrogen (BUN). BUN levels may be elevated (a condition called uremia) in both acute and chronic renal (kidney) failure. Various diseases damage the kidney and cause faulty urine formation and excretion. Congestive heart failure leads to a low blood pressure and consequent reduced filtration rates through the kidneys, therefore, BUN may be elevated. Urinary tract obstructions can also lead to an increased BUN. In severe cases, hemodialysis is used to remove the soluble urea and other waste products from the blood. Waste products diffuse through the dialyzing membrane because their concentration is lower in the dialyzing solution. Ions, such as Na⁺ and Cl⁻, which are to remain in the blood, are maintained at the same concentration in the dialyzing solution - no net diffusion occurs.

As stated previously, high ammonia levels are toxic to humans. A complete block of any step in the urea cycle is fatal since there is no known alternative pathway for the synthesis of urea. Inherited disorders from defective enzymes may cause a partial block in some of the reactions and results in hyperammonemia which can lead to mental retardation. Extensive ammonia accumulation leads to extensive liver damage and death. Liver cirrhosis caused by alcoholism creates an interference in the enzymes which produce carbamyl phosphate in the first step on the cycle.
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 gastroenteritis and more chronic disorders like inflammatory bowel 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 phyco-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 pancreatic amylase and gastric juice. This particular property makes them an ideal “prebiotic” substance for various food applications.
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 “bifidogenesis” 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 Du 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 *bifidobacteria* 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. Immunomodulation 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 specially 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 cancogenesis 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 xylo- 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 furanucosidase 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 fructooligosaccharides 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 (GF₂), nystose (GF₃) and 1⁵-β-fructofuranosylnystose (GF₄). 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 rotilline 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 CO₂. 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 up to 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 up to 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 Latulose

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 169⁰C. Lactulose is water-soluble and solubility increases with increases with temperature, exhibiting 86% solubility at 90⁰C. 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 during
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 Europian dairy manufacturers, have developed a prebiotic ingredient named “Elix’or”.
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 transferse 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 hydroxylic 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 oligosaccharids 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 baceteroidaceae 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. Addtion 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/feremtned 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 cariogenesity, 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 specially those obtained from plant sources have been investigated for their fat replacing ability and gaining popularity as “fat mimetic”. 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: Milk, the ‘Elixir’

Milk is the only food product, which is considered nature’s almost perfect food. It is a treasure of unlimited nutrients. It is considered best and ideal food by virtue of possessing almost all the nutritional factors viz. fats, proteins, carbohydrates, mineral and vitamins. They are not only of higher quality but are present in milk in such a form and proportion that their digestion and assimilation in the body is very efficient. Milk also plays a supplementary role in our nutrition. If it is included in our diet, it enhances digestion and assimilation of the constituents of other food products. This is the reason that it is kept high esteem as a food product and is called ‘Elixir’ of this creation.

2.0 Milk, the Medicine

Milk provides a highly versatile medium, which can be easily manipulated to result in precious food products viz. with fortification and enrichment through various minerals and vitamins. Milk has a long intrinsic association with useful microbes. It provides a superb medium where lactic acid bacteria (LAB) find their natural habitat. If fresh milk after milking is kept undisturbed, it is the LAB, which grow and proliferate suppressing other contaminants. This property of milk has been harnessed in the form of natural souring giving rise to various fermented milk products.

The role of fermented milk and milk products in human nutrition has been well documented since the time antiquity. Fermentation has occupied a place of complacency for the food preservation. It improves and enhances nutritional value and enriches flavour, texture and rheology of the product. Fermented milk products have come to be enjoyed ubiquitously in the world for their characteristic energizing taste. This characteristic as a preserved food is one reason for their success, but also with the diversification of the diet in recent years and their image as health foods, not only plain fermented milks but also fermented milks in liquid and frozen forms and with all kinds of fruit juices or fruit added have appeared in the market.

In early days, these foods were produced by natural fermentations with the sole objective of preserving milk for future use. In Europe, Asia and Africa, sour milk was known for its better keeping quality, stability, digestibility and other beneficial attributes as compared to the fresh milk. In Indian subcontinent, the conservation of milk by souring with the leftover of previous day’s sour milk has been a common practice ever since the Aryans inhabited the land. Today the practice of preserving the milk by fermentation has become a fascicle household technology and fermented milks have become almost a dietary adjunct in this part of the subcontinent. Dahi, the indigenous fermented milk invariably figures as a regular component in our menu. The Indian medical treatise, “Sushruta Samhita” describes ‘Dahi’ as a food promoting appetite and strength. Since the antiquity as depicted in our old
testaments, our country serves to be the origin of a number of fermented milk products like Dahi, Lassi, buttermilk, Shrikhand, etc and the most important food delivery systems for beneficial probiotic cultures have been the fermented milk products, probably because of their intrinsic and traditional association with health and since they already harbour viable microorganisms. Today India is producing a diverse sort of fermented milks and other cultured dairy products, i.e. Dahi market in India alone is worth around 52,00,000 tonnes a year or about 15,000 crores annually, roughly the same as milk market growth.

The use of fermented milks in the human diet was also first advocated this Nobel Prize winning Russian scientist, i.e. Elie Metchnikoff. He believed that when consumed, the fermenting bacillus (Lactobacillus) positively influenced the microflora of the colon, decreasing toxic microbial activities. He further postulated that Lactobacilli delbrueckii subsp. bulgaricus possessed a therapeutic weapon to combat disease-causing germs in the human intestine. Later, several other types of fermented milk products like acidophilus milk, yoghurt, Kefir, Koumiss, cheese, Shrikhand, etc made their appearance among the multitude of fermented milks. The list of such products has expanded enormously during the last decade precisely as a result of better understanding of the fermentative characteristics of LAB that serve as starter cultures in the preparation of such foods and due to new innovations and advancements made in dairy processing technologies.

3.0 Fermentation

As per IDF (1969) specifications, fermented milks are defined as the products prepared from milks—whole, partially or fully skimmed, concentrated milk or milk substituted from partially or fully skimmed dried milk homogenized or not, pasteurized or sterilized and fermented by means of specific organisms.

Currently, about 2.3 per cent of the total dairy product market is in the form of cultured dairy products and their production has been showing a marked increase. With the advent of biotechnological techniques used for the genetic manipulation of LAB to improve their fermentative characteristics, the popularity and consumption of these high value products has improved dramatically during the last couple of decades all over the world. A variety of such products are now available for consumption of a wide section of consumers depending upon the individual’s liking. As a result of better understanding of their beneficial effects on human nutrition and health, these products are now in great demand all through the world. The great popularity of fermented milks is also attributed to their attractive taste as well as extended shelf life at low temperatures at which survival of pathogenic contaminating microflora is considerably diminished at low pH. The major advantages of fermented milks include their relatively easy production, better keeping quality, better nutritive value, easier digestibility and strong therapeutic potential. Fermented milk products have been well recognized to have therapeutic, anticholesterolemic, anticarcinogenic, anticariogenic properties etc beyond their basic nutritive value.

4.0 Cultured Milk

Cultured milks are products made by use of special cultures. They fall into two broad categories. Those made by use of LAB, which grow well at ambient temperature (25-30°C). Such bacteria are known as mesophilic starter cultures. The other type of cultured dairy product is the one made by use of LAB, which grow well under warm conditions (38–45°C).
The LAB used are technically known as thermophilic starter cultures. Yoghurt or yoghurt like products belongs to this group.

There is a long history of health claims concerning living microorganisms in food, particularly LAB. In a Persian version of the Old Testament, it states that "Abraham owed his longevity to the consumption of sour milk. In 76 BC, the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis. Since the advent of the microbiology era, some investigators, particularly Elie Metchnikoff attributed such health effects to shifts of the intestinal microbial balance. Interest continues today as recent technological advances have enabled microorganisms to be isolated and colonized to determine their specific therapeutic properties. Studies show these microflora are capable of providing numerous health benefits beyond basic nutritional value.

5.0 Growing Market of Fermented Dairy Products Harbouring beneficial Microbes

Food products containing probiotic bacteria are almost exclusively dairy products, capitalizing on the traditional association of LAB with fermented milk. Yoghurt and fermented milks have received the most attention as carriers of live probiotic cultures. Probiotic bacteria used in these products include Lactobacillus and Bifidobacterium species, among others. A variety of fermented dairy products and supplements containing viable microorganisms with specific therapeutic and health attributes are commercially available in the market (Table 1).

Table 1. Some commercial probiotic dairy products

<table>
<thead>
<tr>
<th>Product</th>
<th>Culture</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidophilus Bifidus yoghurt</td>
<td>A+B+C</td>
<td>Germany</td>
</tr>
<tr>
<td>Bifidus milk</td>
<td>B. bifidum or B. longum</td>
<td>Germany</td>
</tr>
<tr>
<td>Bifidus yoghurt</td>
<td>B. longum + C</td>
<td>Many countries</td>
</tr>
<tr>
<td>Bifighurt</td>
<td>B. longum + S. thermophilus</td>
<td>Germany</td>
</tr>
<tr>
<td>Bifilak (c) t</td>
<td>A + B</td>
<td>Russia</td>
</tr>
<tr>
<td>Biobest</td>
<td>B. bifidum or B. longum + C</td>
<td>Germany</td>
</tr>
<tr>
<td>Biomild</td>
<td>A + B</td>
<td>Germany</td>
</tr>
<tr>
<td>Mil-Mil</td>
<td>A + B + B. breve</td>
<td>Japan</td>
</tr>
<tr>
<td>Cultura</td>
<td>A + B</td>
<td>Norway, Denmark</td>
</tr>
<tr>
<td>Kyr</td>
<td>A + B + C</td>
<td>Italy</td>
</tr>
<tr>
<td>Ofilus</td>
<td>A + B + S. thermophilus</td>
<td>France</td>
</tr>
<tr>
<td>Biogarde</td>
<td>A + B + S. thermophilus</td>
<td>Germany</td>
</tr>
<tr>
<td>Actimel</td>
<td>L. casei</td>
<td>Germany</td>
</tr>
<tr>
<td>Vifit</td>
<td>L. casei GG L</td>
<td>Germany, UK</td>
</tr>
<tr>
<td>Primo</td>
<td>Bacto Lab Cultures</td>
<td>Germany</td>
</tr>
<tr>
<td>Zabady</td>
<td>B. bifidum + C</td>
<td>Egypt</td>
</tr>
<tr>
<td>BA live</td>
<td>A + B + C</td>
<td>U.K.</td>
</tr>
<tr>
<td>Femilact</td>
<td>A + B + Pediococcus acidilactici</td>
<td>Czechoslovakia</td>
</tr>
<tr>
<td>Philus</td>
<td>A + B + S. thermophilus</td>
<td>Sweden</td>
</tr>
<tr>
<td>Fysig</td>
<td>L. acidophilus</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Gaio</td>
<td>E. faecium + S. thermophilus</td>
<td>Denmark</td>
</tr>
<tr>
<td>Stoneyfield</td>
<td>L. reuteri</td>
<td>Sweden</td>
</tr>
<tr>
<td>Bio K+</td>
<td>L. acidophilus+ L. casei</td>
<td>Canada</td>
</tr>
<tr>
<td>Yakult</td>
<td>L. casei Shirota</td>
<td>Japan (23 countries)</td>
</tr>
<tr>
<td>LC1 (Nestle)</td>
<td>L. johnsonii LJ</td>
<td>European countries</td>
</tr>
</tbody>
</table>
6.0 Fermented Milks with Beneficial Microbes: Role in Health

For centuries, folklore suggested that fermented dairy products containing live active cultures are healthful. Recent controlled scientific investigation supports these traditional views, suggesting that probiotics are a valuable part of a healthy diet. In addition, the emergence of some new public health risks suggests an important role for effective probiotics in the mitigation of illness. For example, the ability of probiotic bacteria to support the immune system could be important to the elderly or other people with compromised immune function.

Infections are another area with potential for fermented milks. Some infections, once thought self-limiting or readily treatable with antibiotics, are now recognized as more serious health threats. Vaginosis used to be considered just an annoyance. Now we know it is associated with low birth weight and increased risk of sexually transmitted diseases. New foodborne pathogens have emerged as prevalent and life threatening, including Shiga-like Escherichia coli strains. Multiple antibiotic resistances are a continual peril in the battle against once-treatable infections. And in non-industrialized nations, infections, such as rotavirus, claim the lives of hundreds of thousands of infants yearly. Prevention of infections before they occur is clearly the better alternative. Fermented milks harbouring probiotics may be a safe, cost-effective, "natural" loom that adds a barrier against microbial infection.

7.0 Probiotics in Human Health

Fermented milk and milk products have occupied a place of complacency in satisfying the palate and nutritional requirements of human being since the time antiquity. Fermented milk products have been well recognized to have therapeutic, anticholesterolemic, anticarcinogenic, anticariogenic properties, etc beyond their basic nutritive value. They, contributing to a variety in our gustative desire, provide important nutrients and are considered superior over non-fermented dairy products in terms of nutritional and health attributes due to the presence of several beneficial microflora.

Probiotic microflora displays numerous health benefits beyond providing basic nutrients. They cooperatively maintain a delicate balance between the GI tract and immune system. When this balance is disrupted, disease and inflammation result. The health effects attributed to the use of probiotics are numerous. Daily intake of foods containing these bacteria ($10^9$ cells/day) have been suggested to provide health benefits including improved digestion and alleviation of constipation, increased absorption of dietary minerals, reduced blood cholesterol levels and enhanced protection from tumours. The following outcomes are well documented: 1) lower frequency and duration of diarrhea associated with antibiotics
(Clostridium difficile), rotavirus infection, chemotherapy, and, to a lesser extent, traveller's diarrhea 2) stimulation of humoral and cellular immunity 3) decrease in unfavorable metabolites, e.g., ammonium and procancinogenic enzymes in the colon. Some other evidences of health effects through the use of probiotics includes: (i) reduction of Helicobacter pylori infection (ii) reduction of allergic symptoms (iii) relief from constipation (iv) relief from irritable bowel syndrome (v) beneficial effects on mineral metabolism, particularly bone density and stability (vi) cancer prevention and (vii) reduction of cholesterol and triacylglycerol plasma concentrations. Recently, the use of probiotics in management of CVDs has created an impetus to further scientific exploration in gaining better realization of the role of fermented milks containing these beneficial microorganisms in reducing the problems associated with hypercholesterolemia in human.

8.0 Probiotics and Fermented Dairy Products

Probiotic bacteria are typically, but not always, chosen from bacteria that normally inhabit the gastrointestinal system of humans, and belong to a species that is known to be safe. These bacteria are purified, grown to large numbers, concentrated to high doses and preserved. They are provided in products in one of three basic ways:

- As a culture concentrate added to a food usually a dairy product at medium levels, with little or no opportunity for culture growth
- Inoculated into a milk-based food or dietary supplement and allowed to grow to achieve high levels in a fermented food
- As concentrated and dried cells packaged as dietary supplements such as powders, capsules, or tablets

9.0 Therapeutic Potential of Fermented Milks

Interest in the health benefits of cultured dairy foods dates back to the early 1900s, when Elias Metchnikoff, in his book called The Prolongation of Life, associated intake of large quantities of Bulgarian fermented milk with a long life. Today, researchers are investigating the therapeutic role of fermented dairy products containing probiotic bacteria i.e., beneficial bacterial cultures, such as Lactobacillus and Bifidobacterium.

Health benefits of fermented dairy products harbouring beneficial cultures are being briskly investigated today at various medical and research centers around the world. Competent clinical studies are showing that regular consumption of specific fermented dairy products with probiotic bacteria can alleviate or prevent diverse intestinal disorders and even reduce the risk of cardiovascular diseases. Fermented dairy products with probiotic bacteria play a key role in human nutrition and health in balancing the intestinal microflora naturally. They have been used therapeutically to control cardiovascular diseases (CVD), lower cholesterol, modulate immunity, improve digestive processes, treat rheumatoid arthritis, prevent cancer, improve lactose, intolerance, etc.

Fermented dairy products with probiotic bacteria displays numerous health benefits beyond providing basic nutrients. They cooperatively maintain a delicate balance between the GI tract and immune system. When this balance is disrupted, disease and inflammation result. The health effects attributed to the use of fermented dairy products with probiotic bacteria are numerous. Daily intake of foods containing these bacteria (10^9 cells/day) have
been suggested to provide health benefits including improved digestion and alleviation of constipation, increased absorption of dietary minerals, reduced blood cholesterol levels and enhanced protection from tumours. The following are some of the potential health benefits associated with intake of cultured dairy foods:

9.1 Improved Tolerance to Milk

A number of studies have demonstrated that intake of yoghurt enhances lactose digestion in individuals with low intestinal levels of lactase, the enzyme necessary to digest lactose or milk’s sugar (i.e., a condition called lactase nonpersistence). The lower lactose content of some cultured dairy foods compared to milk may contribute to the beneficial effect of these foods for individuals who have intricacy digesting lactose. The beneficial effect may also be explained by the ability of the starter cultures used in the manufacture of yoghurt and other fermented dairy products with live, active cultures to produce the enzyme lactase, which digests the lactose in products. Intake of cultured dairy foods, such as yoghurt, Dahi, Lassi, etc may also improve tolerance to milk in individuals with cow’s milk allergy / sensitivity as well as help prevent allergies to other foods.

It is well-known that the presence of LAB, specifically *L. bulgaricus* and *S. thermophilus* in yogourt, improves lactose digestion. It appears that the cell walls of the bacteria have to be intact as is the case when the bacteria are alive for the effect to occur. Some possible mechanisms for the improved lactose digestion include:

- The lactase activity of the bacteria actually does the work of digesting lactose in the product once it reaches the intestine
- The slower transit time of yogourt, Dahi, etc may permit more time for the residual intestinal lactase and the yogourt or Dahi cultures to digest the lactose
- Something in the yogourt or Dahi may inhibit fermentation of lactose and thus reduce symptoms.

9.2 Management of Cardiovascular Diseases (CVD)

Coronary heart disease, the most common and serious form of CVD, is the leading cause of death in affluent societies of the developed industrialized countries today. It is responsible for 51 per cent of human deaths in the world. Fast changing lifestyle and poor dietary habits among other factors, are also making CVD the leading cause of deaths in wealthy and technologically developed societies of our country. Currently, CVDs account for around 15 per cent deaths in India. However, this figure is likely to climb to as high as 40 per cent by 2015. The prevalence of CVD in India has increased from 4 per cent in 1960 to 11 per cent in 2001. It has been estimated that one-fifth of the deaths in India are due to CHD that is inflicting at much younger age. Current projections suggest that in the next 20 years India will have the largest CVD burden in the world. With one-sixth of the world’s population in India, any advancement in containing CVD, would impact on global health immensely.

9.3 Hypocholesterolemic Effects of Fermented Dairy Products

Some strains of *L. acidophilus* can take up cholesterol in the presence of bile. Other *in vitro* research shows that cholesterol can precipitate with free bile salts in the presence of *L. acidophilus*, especially in an acid environment. Thus, it has been hypothesized that one or
both of these actions would take place \textit{in vivo} and help lower serum cholesterol in humans. Various studies with fermented milk products have shown either no effect or a reduction in cholesterol levels. In conclusion, there is not yet good evidence to confirm a cholesterol-lowering effect of fermented milk products.

### 9.4 Intestinal Health

Yoghurt with specific strains of live active cultures has been demonstrated to help maintain the normal intestinal microflora balance and repress harmful bacteria in the intestine. A particular strain of bacteria used in yoghurt, \textit{Lactobacillus} strain GG, aids in treatment and prevention of antibiotic-associated diarrhea, traveler’s diarrhea, and acute diarrhea in children. In adults, this particular strain of \textit{Lactobacillus} has been shown to stimulate bowel function by shifting the microflora and suppressing fermentation in the intestine. Yoghurt with \textit{Lactobacillus gasseri} may be helpful for older adults with “atopic gastritis,” a condition that predisposes to intestinal infections and constipation.

### 9.5 Diarrhea

Many types of diarrheal illnesses, with many different causes, interrupt intestinal function. The ability of fermented milks containing beneficial cultures to decrease the occurrence or duration of certain diarrheal illnesses is perhaps the most substantiated of the health effects of fermented milks. It has been found that \textit{"Lactobacillus is safe and effective as a treatment for children with acute infectious diarrhea."}

A number of well-designed studies have noted that fermented milk products effectively avert or treat infantile diarrhea. Effects have been noted with \textit{L. casei} and \textit{B. bifidum}. A few small studies show that LAB can reduce the incidence of antibiotic-related diarrhea. This suggests a role for LAB in immunosuppressed patients who routinely use antibiotics. A few studies of traveller’s diarrhoea have demonstrated the effectiveness of LAB in decreasing the incidence of diarrhea. Lactic acid bacteria can probably trim down diarrhea in several ways:

- Lactic acid bacteria struggle with pathogens for nutrients and space in the intestines.
- By-products of metabolism may have a direct effect against the pathogens. For example, \textit{in vitro} work shows that \textit{L. casei}, \textit{L. acidophilus} and \textit{L. bulgaricus} can all produce antimicrobial agents, such as acidophilin and bulgarican that can inhibit growth of pathogens.
- Lactic acid bacteria may be effective against diarrhea due to effects on the immune system.

### 9.6 Irritable Bowel Syndrome:

Irritable bowel syndrome (IBS) is a functional bowel disorder that can be characterized by symptoms of abdominal pain, cramps, gas, bloating, diarrhea and constipation. Surveys estimate the prevalence rate ranging from 10-20 per cent of the adult population and the condition is diagnosed three times more often in women than men. Only a few controlled studies have been conducted evaluating fermented milks and IBS.

### 9.7 Inflammatory Bowel Disease

Inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease are serious intestinal diseases that can ultimately lead to the surgical removal of the colon. The causes of these diseases are not known, but it has been hypothesized that an intolerance to the
normal flora in the gut leads to inflammation and resulting pathology. The role of gut flora in the succession of these diseases has led some researchers to study the impact of fermented products containing certain probiotic bacteria might have on maintaining the state of reduced inflammation that occurs during remission stages of the diseases. Several controlled, clinical trials have shown that high levels of certain probiotic strains can extend the disease-free remission period. Studies also have documented this effect on remission of pouchitis. Additional research in this area is progressing in Europe and the US.

9.8 Small Bowel Bacterial Overgrowth

Under certain conditions, such as production of low stomach acid or kidney dialysis, microbial populations in the small intestine can increase beyond normal levels. This is termed small bowel bacterial overgrowth. The misplaced microbes can produce by products from their growth that can be toxic. Researchers have found that feeding high levels of certain probiotic strains can control the toxic effects of these microbes. This is another example of the ability of fermented milks with probiotic strains to modulate the activity of other intestinal bacteria.

9.9 Anti-cancer Effects

The possibility that LAB in cultured dairy foods may protect against certain cancers, such as colorectal cancer, and possibly breast cancer, has been investigated. Studies indicate that specific bacterial strains of *Lactobacillus* lessen the growth of cancer cells and the activity of fecal carcinogenic enzymes implicated in the development of colon cancer. In laboratory animals, intake of yoghurt has been shown to reduce colorectal tumors. However, additional research is indispensable to confirm an anticarcinogenic effect of cultured dairy foods, such as yoghurt, *Dahi*, etc in humans.

9.10 Colon cancer

It has also been demonstrated that LAB slow the growth of experimental cancers, although the results are not long-term. It appears that LAB can reduce the levels of colon enzymes that convert procarcinogens to carcinogens. Exclusively, LAB can reduce levels of the enzymes β-glucuronidase, nitroreductase, and azoreductase. Lactic acid bacteria may also be involved in the direct reduction of procarcinogens, for example, by taking up nitrites and by reducing the levels of secondary bile salts. In most reports, these effects only occur during the period of time that the bacteria are consumed. Changes in enzyme activity in humans have been observed with *L. acidophilus* and *B. bifidum*, and LGG. Animal studies show fewer tumors in those exposed to a carcinogen, in the presence of LGG, compared to the animals exposed to the carcinogen without the benefit of LGG. However, there is not yet a clear rapport between LAB intake and cancer prevention.

9.11 Constipation

Milk products fermented with some strains of *L. acidophilus* and bifidobacteria shorten intestinal transit time. This effect may be useful for those with constipation, such as the elderly. A well controlled human study further is needed to confirm this.

9.12 Urogenital infections

Lactic acid bacteria may decrease candidal vaginal infections. This is still speculative; however it would be research worth pursuing. One small study showed that women with
recurrent vaginal candidiasis who ate 8 oz. daily of a yogourt containing \textit{L acidophilus} had fewer occurrences of vaginal candidiasis than during the control period in which they ate no yogourt.

\textbf{9.13 Enhanced Immunity}

The immune system provides the primary defense against microbial pathogens that have entered our bodies. The immune system is extremely complex, involving both cell-based and antibody-based responses to potential infectious agents. Immunodeficiency can result from certain diseases e.g., cancer, AIDS, leukemia or to a lesser extent, from more normal conditions, such as old age, pregnancy, or stress. Autoimmune diseases e.g., allergies, rheumatoid arthritis, inflammatory bowel diseases also can occur due to misdirected immune system activity.

- Lactic acid bacteria enhance immune system function at the intestinal and systemic levels. In humans, LAB have been shown to increase:
  - B-lymphocytes or B cells, which recognize foreign matter
  - Phagocytic activity, helping to destroy foreign matter
  - IgA-, IgG- and IgM-secreting cells and serum IgA levels, which would increase antibody activity
  - $\gamma$-interferon levels, which help white blood cells fight disease

\textbf{9.14 Prevention of Helicobacter pylori}

\textit{Helicobacter pylori} is a bacterium, which colonizes the stomach. Its presence is associated with gastric ulcers and gastric cancer, although its role in the etiology of these diseases is still under investigation. The effect of probiotics on \textit{Helicobacter pylori} has been studied. Most evaluations have been done either in laboratory assays or in animal models. These studies show that antibacterial substances including organic acids produced by some lactobacilli inhibit the growth and survival of this pathogen. Results in animal models exhibit that some lactobacilli inhibit \textit{H. pylori} attachment and prevent colonization. Results in humans show that milk fermented by a \textit{Lactobacillus johnsonii} strain can help control \textit{H. pylori} gastric infections, but cannot eradicate \textit{H. pylori} from the stomach.

\textbf{9.15 Kidney Stones}

A high level of oxalate in the urine is a risk factor for the development of kidney stones. Utilization of oxalate by intestinal microbes limits its absorption. A probiotic preparation that contained bacteria that were able to degrade oxalate \textit{in vitro} was shown to reduce oxalate faecal excretion in six patients. These results suggest that manipulation of the gut flora with the right probiotic bacteria may have a positive impact on gastrointestinal tract oxalate levels and may decrease oxalate absorption. These results are intriguing, but preliminary.

\textbf{9.16 Allergy}

Allergy is on the rise in industrialized nations. It is estimated that the incidence of asthma in the United States doubled between 1980 and 2000. Scientists have proposed a hypothesis known as the ‘hygiene hypotheses to explain the rise in allergic conditions, such as asthma and eczema. This hypothesis is based on observations that lower allergy incidence is associated with environments that have greater numbers of microbes, such as day care
centers, farms, or in homes with siblings or pets. Sanitary living environments and the consumption of processed foods have limited the number of microbes in the diet. The hypothesis suggests that the exposure of infants to microbes before the age of six months helps the immune system mature to be more tolerant of exposure to allergens later in life.

### 10.0 Conclusion

The potential benefits of milks containing LAB and probiotic cultures seem vast. The applications range from helping to treat acute intestinal infections to aiding in the digestion of lactose and contributing, over the longer term, to improved health and possibly reduced risk of disease. However, the long term commercial exploitation of specific probiotic products with health attribute as health promoter depends upon a number of factors, which includes clinically proven evidence of beneficial effects, accurate consumer information, effective marketing strategies and overall, the sensory quality and safety aspects of the product that can fulfills our consumer expectations. This deserves further studies in the arenas, such as appropriate strain selection, ensuring their survivability in the products and during GI transient, elucidation of mechanisms of action as well as the safety aspects of the products containing probiotic cultures. With some concrete efforts and interest of the scientists working in this field, transfer of the technologies to the food manufacturers and some marketing strategy, it may be possible in the future for our traditional cultured milks, such as those already containing live microorganisms, to be marketed in providing certain health and nutritional benefits to our consumers.

### 11.0 Suggested Readings


