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Fortification of Food and Beverages with Phytonutrients

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Abstract With the emergence of fortified foods there is a worldwide increase in health awareness and interest in herbal alternatives. The change in outlook of consumers has encouraged the food and beverage industry to adopt fortification techniques to enhance the appeal of their products. Beverages and common foods like soups, yogurt, sauces, mayonnaise, jams, jellies, marmalades, cheese, margarine, bread and biscuits can be fortified with enriched herbal extracts. This article reviews some of the trends in food fortification and its significance in the prevention of diseases.

Keywords Food Fortification, Functional Foods, Phytonutrients

1. Introduction

Life styles of people all over the world have changed in the last century due to rise in income, increased leisure time and reduced physical activity. The new life styles have considerable impact on health. Consequently, there is a global rise in the incidence of diseases like obesity, cardiovascular diseases, diabetes mellitus and rheumatoid arthritis. The medical world is therefore, looking for better strategies to contain this trend. As a parallel development there is a worldwide increase in health awareness and interest in herbal alternatives. Elderly people all over the world are nowadays more concerned about the quality of the food and beverages they consume. Many of them periodically monitor biomarkers like LDL, blood glucose, C-reactive protein etc in an effort to reduce the risk of diseases. Functional foods have therefore, come up as an effective means for prevention of diseases. This change in outlook of consumers has encouraged the food and beverage industry to apply modern manufacturing technology in food fortification[1].Weight reduction, reduction of cholesterol, promotion of bone health, energy, enhancement of disease resistance through immune system and improvement of digestive functions are the major health concerns that influence the purchase of functional foods [2] . Functional foods are foods or food ingredients that provide a health benefit beyond their nutritive value.

The concept of functional foods was born in Japan. As a result of the systematic and large-scale research on chemical compounds derived from natural products, the concept of Foods for Specific Health Use (FOSHU) was born in 1991 [3]. These foods are intended to improve the lives of people and the manufacturer is permitted to display on the container, the specific health claims. Functional foods are also known as nutraceuticals, designer foods, farmafoods, pharmafoods, medifoods, vitafoods, dietary supplements, fortified foods or foodaceuticals [4]. These are *foods marketed with the message of a benefit to health* [5], or *foods or isolated food ingredients that deliver specific non-nutritive physiological effects that may enhance health* [6].

Fortification is achieved by adding the nutrient (fortificant or additive) to the food in question, which serves as a vehicle for carrying this nutrient. The early examples of food fortification involved iodine, vitamins and minerals. Fortification of foods is now achieved using numerous beneficial compounds derived from natural products.

2. Demonstration of Functional Effects

The functional nature of fortified foods needs to be proven on the basis of objective parameters. This can be achieved by studying the ability of the functional food in modulating target functions in the body, as these target functions are directly related to improved state of health or reduced risk of developing a disease. Well-defined biochemical, physiological or behavioural markers are used in the assessment of the modulatory effect. Markers may represent an event of interest or correlated events. For example, anthropometry, body fat mass, total body water, procollagen propeptide excretion and urinary creatinine excretion can be the possible markers for assessing the effects on growth and body composition. Similarly, tests of behaviour, cognitive function and visual acuity can be the markers for psychomotor and cognitive development [7].

3. Some Classes of Foods that can be Fortified

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Advances in food technology have made it possible to manufacture many ready-to-eat foods. Even in a country like India, where food habits have been guided by tradition, several ready-to-serve foods have appeared in recent years [8]. Many of them can be fortified with phytonutrients. The following account can serve as a guide to innovative food formulators.

3.1. Beverages

Unfermented and fermented beverages have been in use in different cultures since ancient times. Archaeological oncology research has demonstrated that remnants of an alcoholic beverage from ancient Egypt contained many plant-derived compounds with lung- and colon cancer-fighti ng activity. Residue from an ancient Egyptian wine was obtained from an urn dating to 3150 B.C. found in the tomb of pharaoh Scorpion I of dynasty 0. Using sophisticated analytical tools it is now established that the substance was grape wine, with such other ingredients as savory (*Satureja* spp), wormwood (*Artemisia annua*), tansy (*Tanacetum* spp.) balm, (*Melissa* spp.) etc. It is inferred that this medicinal wine was perhaps intended to be dispensed as a drug[9].

Beverages are easily consumed along with meals and so they are good vehicles for delivery of nutritional supplements into the body [10]. The first fortified beverage to appear in the world market was Aqua Libra, launched in England in the 1980s. According to the label of the product it contains juices of fruits like apple, grapes, passion fruit and melon, carbonated spring water, malic acid, flavours and extracts of sesame seed, sunflower seed and tarragon [11]. Over the years many such products have appeared in market and they address health problems like ageing, stress and weakness. Otsuka Pharmaceuticals produced FibeMini containing dietary fibres, minerals and vitamins. This is perhaps Japan's best-selling soft drink. The beverage giant Coca Cola has lately introduced another fibre-rich drink named Fibi. The Japanese company Ootsuka introduced Pocari Sweet Stevia containing stevioside from *Stevia rebaudiana* as the sweetener [12]. The leaves of *Moringa oleifera* have been processed into an innovative drink named Zija™, which according the manufacturers "overflows with antioxidants, minerals, omega oils, vital proteins and cell-ready nutrients" [13].

The segment of fortified beverages includes four sub-segments like sports drinks, enriched beverages, nutraceutical drinks and energy drinks [14]. While formulating these products, the food technologist takes into consideration many factors like compatibility of ingredients, pH of the medium, nature of flavourings used and their stability during production and on storage [15].

3.1.1. Herbal Extracts in Beverages

Herbal extracts, especially the infusion type, can be incorporated into soft drinks, mineral water-based drinks and energy drinks. On account of their mind-calming and soporific properties *Panax ginseng*, *Echinacea purpurea*, *Chamomilla recuttiana*, *Passiflora incarnata* and *Valeriana officinalis* are the herbs used in European mineral water-based drinks. On the other hand, herbs commonly used in energy drinks are: guarana (*Paullinia cupana*), kola nut (*Cola acuminata*, *Cola itida*, *Cola vera*), coffee (*Coffea Arabica, Coffea canephora*), tea (*Camellia sinensis*), mate (*Ilex paraguariensis*) and cocoa beans (*Theobroma cacao*) [14].

A Swedish beverage manufacturer launched in 2001 *Nexcite*, claimed to be an all-natural blend of proven herbal aphrodisiacs. The product contains extracts of damiana (*Turnera diffusa*), guarana (*Paullinia cupana*), mate (*Ilex paraguariensis*), schizandra (*Schisandra chinensis*) and ginseng (*Panax ginseng*). This cobalt blue beverage is claimed to be a powerful aphrodisiac [16].

The fruit of *Euterpe oleracea*, known as the açai berry is a nutritious food consumed by the indigenous people of the Amazon. A functional juice beverage named MonaVie Active®, having good safety profile was recently launched in the USA [17]. It has açai berry as the predominant ingredient, along with lesser amounts of 18 fruits and berries in descending order of dominance [18]. MonaVie Active® has significant antioxidant and anti-inflammatory activities [19,20]. An open-label clinical pilot study involving 14 subjects demonstrated that consumption of this beverage resulted in significant pain reduction [21].

Dartsch et al (2010) [22] investigated the health benefits of some functional drinks designed to improve the body's performance and health. Using cell -based assays they investigated the potential of the products eQ Brain, eQ Beauty and Let's Get Red. These drinks were prepared from ingredients like natural green tea flavour, acerola flavour, dragon fruit flavour, rooibos extract, cinnamon extract, grapeseed extract etc. The drinks caused a dose-dependant stimulation of the metabolic activity of cultured connective tissue fibroblasts. They were also able to inactivate exogenous and endogenous free oxygen radicals.

Attempts have been made to develop non-alcoholic beverages using combinations of maize powder, mango fruit powder and soy flour. Beverage fortified with mango and maize had low titratable acidity and ranked highest in sensory evaluation [23].The study also demonstrated the feasibility of developing good quality beverages from sour water that is generally discarded as waste from manufacture of the popular Nigerian fermented food product *ogi* [24].

Pomegranate seeds are by-products from pomegranate juice industry. The seed oil has nutritional and medicinal properties. Mohagheghi et al (2011)[25] formulated a stable pomegranate-seed-oil-in-water emulsion that could be used as the base formula for a new functional beverage.

Dahl *et al* (2005)[26] conducted a double-blind, three-week study testing an inulin-fortified beverage against a starch-thickened one. The test beverage was well accepted by the subjects and increased stool output. Temelli *et al* (2004)[27] developed an orange-flavoured beverage containing β-glucan extracted from barley. Trained panelists observed fruity orange aroma and sufficient intensity of sweetness. Its shelf stability was also satisfactory.

Cocoa beans are known to have the highest content of flavan-3-ols [28,29], and epidemiological studies show an inverse relationship between consumption of flavonoid-rich diet and risk of hypertension. Impaired endothelial vasodilator function is an important factor that contributes to the development of cardiovascular diseases [30]. It is well known that obesity and hypertension are associated with impaired vasodilatation [31,32]. Short –term intake of cocoa polyphenols is reported to lower blood pressure and improve endothelium-dependant vasodilatation [33-36]. It is believed that this beneficial effect of cocoa polyphenols is brought about by an increase in nitric oxide synthase activity in endothelial cells leading to an increase in nitric oxide concentration [37-42].

Martin *et al* (2010)[43] reported that daily consumption of 40 g of dark chocolate for 14 days could reduce urinary excretion of the stress hormone cortisol and catecholamines in 30 stressed human volunteers. The therapy also normalized stress-related differences in energy metabolism and gut microbial activities.

Tomas-Barberan *et al* (2007)[44] and Cienfuegos-Jovellanos *et al* (2009)[45] have shown that flavonoids from cocoa beans can be incorporated into beverages with enhanced bioavailability in humans.

3.2. Soups

The word *soup* is derived from the French *soupe* which means “a broth”. Soups are liquid food prepared by boiling vegetables and or meat in water. Soups form an important part of European cuisine from ancient days. Soup as a commercial product became popular ever since canning technology was invented in 1795 by Nicolas Appert [46]. Dr. John T. Dorrance (1873-1930), a chemist working for Campbell Soup Company is the inventor of condensed soup, which is cooked with minimum water, forming a kind of thick stock that can be diluted with hot water and consumed [47]. The manufacture of soup powders was pioneered by Karl Heinrich Knorr in 1873 [48]. Lately frozen soups have also become popular [49].

Chinese medicine recommends the drinking of soups to prevent or treat diseases. Chinese medicinal herbs are grouped into four categories viz., tonifying, eliminating, harmonizing and fortifying. Many soups are prepared by combining these herbs and meat. Medicinal soups are very popular in China [50]. The recipe one such soup- Six Herb Regulating Soup- is described below.

Cornelian cherry fruit (3/4 ounce), alisma (1/2 ounce), treated rehmannia (3/4 ounce), *Poria cocos* sclerotium (3/4 ounce), white peony root (1/2 ounce) and wild yam (3/4 ounce) are put in a ceramic pot, brought to a boil over high heat and left to simmer for thirty minutes until the mixture is reduced to two cups. The filtered soup, when consumed twice a day is said to improve physical and mental stamina [51].

Soup powder is a convenient form on account of longer shelf life and ease of transportation. The common ingredients are starch, spices like ginger, garlic and onion, milk powder, salt, monosodium glutamate (MSG), ascorbic acid, sugar, dehydrated vegetables and or meat [52]. Although MSG is considered to be a safe flavour additive [53], it is known to have some shortcomings [54]. Recently Abeysinghe and Illeperuma (2006)[55] reported the development of a vegetable soup powder devoid of MSG, but having appreciable flavour, on account of the colourant lycopene present in tomatoes.

Soup powders are usually prepared by blending dehydrated vegetables or meat with the other ingredients [55,56]. They can also be prepared by grinding the fried vegetables followed by blending with other ingredients and spray drying [57].

Soups do have detectable physiological effects as was shown by Midoh and Noguchi (2009)[58]. They reported that 2-week of consumption of chicken soup could reduce tension-anxiety score and increase peripheral blood flow in healthy human volunteers. The antioxidant activity of soups have also been reported [59]. Thus it is evident that soups can be transformed into fortified food by replacing conventional ingredients with those having proven health benefits.

Nagatsuka *et al* (2006)[60] reported that gelatin gel *Nikogori* soup prepared from chicken wing meat has high peroxyl and hydroxyl radical scavenging activities, as evidenced by chemiluminescence and electron spin resonance methods. Addition of soy sauce enhances the hydroxyl radical scavenging activity.

Drinking of the Mediterranean soup *gazpacho* prepared from tomato, pepper and cucumber significantly increases plasma concentrations of vitamin C[61]. Similarly, the consumption of quercetin-rich onion soup inhibited some aspects of collagen-induced platelet aggregation. This observation from a double-blind, randomized, cross-over, pilot study substantiates the epidemiological data, suggesting that individuals who preferentially consume large amounts of quercetin-containing foods have a reduced risk of succumbing to thrombosis and cardiovascular diseases [62]. In the same way, *Corchorus olitorius*, a leafy vegetable popular in Asia and Africa and used in soups, has significant antioxidant activity as judged by DPPH method [63].

A recent study reports that once a day consumption of *Chaihu Shihuang* soup of China for seven days, reduces significantly TNF-alpha and interleukin-6 in patients suffering from severe acute pancreatitis [64].

3.3. Yogurt

Yogurt is a modern version of curd. It is prepared by bacterial fermentation of milk. Lactic acid formed from lactose in milk curdles the milk proteins and the resultant is a soft, white coloured product [65].The word *yogurt* is derived from the Turkish word *yoğurt*, meaning “dense” or “thick”. Armenian immigrants brought yogurt to America and commercial manufacture started in 1929.

The composition and possible ways to modify appearance, quality and texture of yogurt are described in length by Sodini and Tang ((2006)[66]. The organic ingredients defined

by F.D.A. for inclusion in yogurt are organic fruits, organic cane sugar juice, citric acid, vegetable colours, flavours, ascorbic acid, low methoxyl pectin, high methoxyl pectin, sodium citrate and locust bean gum [67].

Successful fortification of yogurt has been reported by several workers. The preparation of yogurt fortified with whey powder has been reported by Dave and Shah (1998)[68] and Bhullar *et al* (2002)[69]. Attempts have also been made to achieve fortification with calcium, iron, fibre, mango and soy [70-74]. However, fortification of yogurt with herbal extracts can be attempted by imaginative food technologists.

There has been much interest in fortifying yogurt with soya products and phytosterols. Kitawaki *et al* (2009)[75] studied in rats the cholesterol-lowering effects of lactic acid fermented soymilk, in which part of the soy milk was replaced by soy yogurt. The results indicate that soy yogurt in helpful in preventing hepatic lipid accumulation in rats. Based on a study carried out in rabbits, Cavallini *et al* (2009)[76] suggest that soy yogurt could be consumed to reduce the risk of cardiovascular disease as it improves the lipid profile. Their study also shows that isoflavone supplementation enhances the antiatherogenic effect of soy yogurt. Results of two clinical studies suggest that phytosterol-enriched yogurt has the ability to lower commonly measured lipid parameters [77,78].

In addition to the hypercholesterolaemic effect, yogurts made with plant-derived lactic acid bacteria have the ability to increase defecation frequency in patients suffering from constipation [79]. Yogurts prepared from cow or goat milk are also effective in offering protection against experimental, acute liver injury in mice [80].

Many studies have been conducted on the physiological effects of yogurt. Ataie-Jafari *et al* (2009)[81] reported that yogurt containing the probiotic bacterial strains *Lactobacillus acidophilus* and *Bifidobacterium lactis* lowered the serum cholesterol of hypercholesterolaemic human volunteers.

Experimental studies in mice offer evidence for the anti-inflammatory effect of yogurt in inflammatory bowel disease. The effect is believed to be mediated by an increase in the number of IgA+ cells, a decrease in CD8+ population and enhancement of apoptosis of infiltrative cells in the large intestine [82].

Though many health benefits are attributed to yogurt, the alleged cholesterol-increasing property of milk fat is an impediment to its wider use. Therefore, several studies have been reported on the improvement of the physical, textural and rheological properties of reduced-fat yogurts by incorporating fat-replacers and manipulating the parameters of manufacture. Whey protein concentrate can be used in the manufacture of non-fat yogurt with satisfactory physical and organoleptic properties [83]. Singh and Kim (2009)[84] reported that starch lipid composite could be used as a fat replacer and stabilizer in yogurts.

Inulin is a polysaccharide isolated from the roots of *Chicorium intybus* and is used as a fat replacer in water-based foods. Because of the rheological properties inulin in aqueous medium gives a fat-like mouth feel and texture [85]. Inulin has been successfully incorporated into yogurts [86-89]. It is found to be an efficacious functional ingredient having a prebiotic effect [90]. In a clinical study Perrigue et al (2009) [91]observed that yogurt beverages containing inulin could significantly suppress appetite and promote satiety.

Fortification of yogurt has been attempted with a view to prevent weight gain by controlling appetite. Llluch *et al* (2010)[92] investigated short-term appetite-reducing effects of yogurt fortified with protein and guar gum. From two randomized cross-over studies the authors concluded that consumption of the fortified yogurt significantly reduced short-term appetite.

3.4. Sauces

These are liquid or semisolid preparations served on food. Occasionally they serve as ingredients of other food as well. According to the famous chefs Carême and Soyer, sauces are to cookery what grammar is to language and melody is to music. They add flavour and visual appeal to the basic food. Sauces are said to have originated in France of the Middle Ages, as testified by a cookery book by Montardier-Gilde, published in 1394 [93].

The ingredients of a representative sauce are natural thickeners like xanthan or guar gums, butter, cane sugar, glucose syrup, fruit juice, tomato concentrate, citric acid and salt. White sauces contain milk [94,95].

Some of the sauces are proven to have remarkable biological effects. The best one in this regard is Japanese style fermented soy sauce (*shoyu*). An anti-hypertensive compound having the ability to inhibit angiotensin I-converting enzyme has been detected in soy sauce. The responsible compound is nicotianamine derived from soybeans. Soy sauce exhibits significant anti-carcinogenic effects and acts against bacteria like *Staphylococcus aureus*, *Shigella flexneri*, *Vibrio cholerae*, *Salmonella enteritidis*, non-pathogenic *Escherichia coli* and pathogenic *Escherichia coli*. Three tartaric isoflavones present in fermented soy sauce have the ability to inhibit the enzyme histidine carboxylase, that produces histamine, the mediator of inflammation, allergy and gastric secretion. Interestingly, though soy bean and wheat, the major ingredients of soy sauce are allergenic, soy sauce does not contain any allergens. This has been confirmed by enzyme-linked immunosorbent assay[96]. Addition of soy sauce slightly enhances the hydroxyl radical scavenging activity chicken jelly soup [60].

Oke *et al* (2010))[97] evaluated the effects of external supplementation of soy lecithin on physico-chemical properties of sauces. The levels of protein, soluble solids and ash content were significantly increased by the addition of lecithin. The bulk viscosity and yellow colour of the sauce were also enhanced.

Sauces are at times used along with salads and they are called salad dressings. Studies show that these low- fat food are ideal carriers of n-3 fatty acids [98], and plant sterols [99].

Abumweiss *et al* (2008)[100] carried out a meta-analysis of 59 eligible randomized clinical trials published from 1992 to 2006. They observed that reductions in LDL were greater when the plant sterols were incorporated into foods like salad dressings. These fortified dressings are well tolerated and do not show any adverse effects even at a daily intake of 9g/day for eight weeks [101].

The usefulness of the extract of *Eleutherine americana* crude extract as an antibacterial agent in salad dressings was investigated by Ifesan *et al* (2009)[102]. The extract reduced *Staphylococcus aureus* population significantly, exerted strong antioxidant action, and had good retention of organoleptic properties and acceptability.

Mellies *et al* (1985)[103] have reported that sucrose polyester can effectively replace dietary fat in salad dressings. It can provide lubricity and organoleptic benefits devoid of the high caloric content that is characteristic of digestible fats.

3.5. Mayonnaise

Mayonnaise is a creamy, pale yellow, flavoured sauce made of oil, water, vinegar and egg yolk. This unique emulsion is prepared by adding the oil slowly to the egg yolk, accompanied by vigorous whisking. It is served in sandwiches and salads [104]. At the industrial level, mayonnaise is manufactured in stainless steel Dixie mixers and Charlotte colloid mills [105].

A few attempts have been made recently to develop low fat mayonnaise. Mun *et al* (2009)[106] developed an acceptable low-fat mayonnaise using 5.6 wt% of 4-alphaGTase-treated starch and 0.1 wt% of xanthan gum. β-glucan prepared from spent brewer's yeast was used by Worrasinchai *et al* (2006) as a fat replacer in mayonnaise. Sensory evaluation and stability studies showed that mayonnaise substituted with not more than 50% β-glucan was acceptable to consumers[107].

Su *et al* (2010)[108] developed a low fat mayonnaise with polysaccharide gums as functional ingredients. Xanthan gum, citrus fibre and guar gum were used in the development of the product. Using these polysaccharides the authors could reduce the fat content in the mayonnaise to 50% while maintaining ideal rheological properties. The innovative product had acceptable organoleptic properties as well.

Cholesterol-lowering property of a fortified mayonnaise was reported by Saito *et al* (2006)[109]. Various quantities of plant sterol ester were dissolved in 15 g of a diacylglycerol-containing mayonnaise. 15 g of the product was administered to human volunteers every day for 4 weeks. At the conclusion of this randomized, placebo-controlled, double-blind, parallel study, total serum cholesterol was found to be decreased significantly by ingestion of the fortified mayonnaise containing at least 0.4 g/day of the phytosterols.

3.6. Jams, Jellies and Marmalades

These are sweet preparations made by concentrating fruits to nearly 70% total suspended solids by adding sugar and heating the mixture. The high content of sugar and the moisture reduce the chances of microbial spoilage. Jams are pectin gels, jelly is jam-like, but transparent. Marmalades are jellies prepared from citrus fruits like oranges and lemons. However, shredded peels of the fruits are suspended in them [110].

Fortification of jams and jellies has been attempted in recent years. Qasi *et al* (2003) claimed in their patent application, invention of a nutraceutical jam with proven memory enhancement, antidepressant, adaptogenic and immunomodulatory properties[111]. Similarly, Toves (2004) reported the fortification of jams with soluble dietary fibres[112].

A novel way to fortify apple fruits was attempted by Lavelli *et al* (2010). They fortified apple puree with green tea extract equivalent to that present in a cup of green tea and freeze dried the material. After one month of storage, the green tea-fortified product retained almost the entire monomeric flavan-3-ols and total procyanidins. Green tea fortification was found to increase the antioxidant potential of the fruit puree 3-6 fold. The authors remark that the novel fortification strategy adopted by them would be advantageous for two reasons. From an economic point of view this would facilitate the appearance of more dehydrated apple products available in the market. Secondly, on nutritional grounds, the novel product can offer consumers a simple opportunity for regular consumption of green tea as a health-enhancing dietary aid [113].

Consumption of fruit juice powders has obvious health benefits. Jin *et al* (2010) administered encapsulated fruit powders to 117 human volunteers for two months in a randomized, double-blind, placebo-controlled clinical study. The fruit powders were derived from acerola, cherry, apple, cranberry, orange, peach, papaya, pineapple, bilberry, blackberry, black currant, blueberry, Concord grape, elderberry, raspberry and red currant. Analysis of blood samples showed that markers of inflammation, like monocyte chemotactic protein-1, macrophage inflammatory protein 1-β and RANTES (Regulated upon Activation , Normal T cell Expressed and Secreted) were significantly reduced and superoxide dismutase and micronutrients (β-carotene, vitamin C, tocopherol) were increased[114]. Fruit juice concentrate powders can be used in food fortification, as they reduce inflammatory load in healthy humans.

3.7. Cheese

Cheese is a protein and fat-rich product made from milk of cows, buffalo, goats and sheep. Milk is coagulated with the addition of the enzyme rennet. The curdled portion is collected and pressed into desired forms [115]. The various steps in the production of cheese are described by Fox *et al* (2000)[116]. Cheese is a good medium for carrying oil-soluble natural colourants and phytochemicals. Carotenoids and bixin are extensively used in this regard.

Lutein is known to be a dietary factor that can prevent the appearance of age-related macular degeneration[117,118]. Considering this aspect Jones *et al* (2005) incorporated into

Cheddar cheese, various amounts of lutein isolated from corn and assessed its stability during storage. Significant quantity of lutein was recovered and no lutein disintegration products were detected. While there was difference in the colour on account of the colour of lutein, the pH remained unchanged and products were free from pathogenic microbes. The authors suggest that cheese is a good medium for the delivery of lutein[119].

Martini *et al* (2009) fortified 50% reduced fat Cheddar cheese with the omega 3-fatty acids docosahexanoic acid and eicosapentanoic acid. Though fishy smell was perceivable in the products, the off-flavour disappeared after three months. This study shows that 50% reduced fat Cheddar cheese aged for three months can be fortified with omega 3-fatty acids, without generation of off-flavours[120].

3.8. Margarine

Margarine resembles butter in appearance, consistency and composition. It is used as a substitute for butter. Margarine was invented in 1869 by the French chemist Meges-Mouries, shortly before the Franco-Prussian War. Meges-Mouries was awarded a prize of 50,000 francs for his innovation made of processed bovine fat mixed with a paste of cow's udders. Milk was added to this mixture and churned to give a butter-like appearance [121].

Margarine at present is made of a blend of vegetable oils and other ingredients. The final product is used as a spread or for baking purposes. According to U.S. Standards of Identity, margarine should have 80% fat. The second component is water and cow's milk was used in its place in the original formula. The third ingredient is an emulsifier like lecithin or mono / diglycerides to prevent phase separation. Preservatives like sodium benzoate, benzoic acid or potassium sorbate are added to act as antimicrobials. Butter-like flavour is imparted by diacetyl and rancidity is prevented by antioxidants [121].

Corn oil is used as the major oil, as it is a polyunsaturated fat and thereby beneficial for health. However, due to growing concerns about harmful effects of the trans-fatty acids in margarines and spreads, other vegetable oils having medicinal value are also being used in the manufacture of margarine and spreads. Important among them are soybean oil, sunflower oil, safflower oil, canola oil and rice bran oil [122,123]. Recently, El-Haddad et al (2011) formulated functional chocolate spread replacing butter fat with red palm olein. This substitution was found to increase significantly the content of tocopherols, tocotrienol and carotenes[124]. Herbal extracts can take the place of emulsifiers, antioxidants, antimicrobials and colourants. Such innovations can popularize the consumption of analogue foods like fortified spreads.

3.9. Bread

Many different kinds of breads are nowadays available with different shapes, sizes, textures, crusts, colours, elasticity, eating qualities and flavours. Bread making has a long history. Neolithic communities (New Stone Age) prepared the first bread, which was a kind of flat bread. It was the people of Sumeria, in southern Mesopotamia, who baked the first leavened bread. The Sumerians passed on their style of bread making to the Egyptians, around 3000 B.C. The Egyptians refined the process and started adding yeast to the flour [125,126]. Industrial production of bread was pioneered by Otto Frederick Rohwedder (1880-1960). In 1912 he invented a machine that sliced and wrapped the bread [127].

Bread dough is a versatile matrix and therefore, attempts have been made to fortify flour with powders of herbs. Considerable success has been achieved in fortification with ingredients like amaranth[128], sorghum flour [129], buckwheat [130], okra (*Abelmoschus esculentus*) seed [131], potato[132] and chempedak (*Artocarpus integer*) seed flour [133].

Fortification of bread with phytochemicals was attempted in recent years and the topic has been reviewed by Hayta and Őzuğur (2011)[134]. Green tea extract was incorporated into bread (50, 100 and 150 mg/100g of flour). There was no detectable loss of tea catechins in bread during storage at room temperature for four days [135].

Peng et al (2010) fortified bread with grape seed extract (GSE), which contains catechins and proanthocyanidins having strong antioxidant and free radical scavenging activity. The GSE- fortified bread had stronger antioxidant activity than blank bread. Antioxidant property of the bread increased with increasing level of GSE. Nevertheless, thermal processing caused a decline in the antioxidant activity by 30-40-%[136].

Lemon flavonoids also seem to be useful for fortifying bread. Lemon flavonoid extract prepared from lemon peel contains 30% eriocitrin, which is a potent antioxidant. Sixty-five per cent of eriocitrin was retained in the bread and 78% of antioxidant activity remained after the baking process. Sensory tests showed that up to 0.50% lemon flavonoid could be added and larger amounts impart a bitter taste to the bread[137].

Abd El-Megeid et al (2009) investigated the protective effect of 2% and 4% green tea-fortified bread on renal failure induced by excessive dietary arginine. Feeding of green tea-fortified bread could ameliorate the pathological effects on kidney enzymes, uric acid, urea nitrogen and creatinine[138]. Clifton et al (2004) studied the effect of phytosterol - enriched bread in mildly hypercholestrolaemic men and women. On consumption of this bread plasma sitosterol content increased by 23% and campesterol by 52%. There was significant reduction in serum LDL cholesterol level[139].

Extracts of green tea and grape seed offer unique flavour and powerful antioxidant action. However, there are some basic issues to be addressed. The most important are the stability of active compounds and their interaction with other components of the food matrix [140].

Roller milling of wheat produces a fraction known as wheat bran which contains the outer layers (pericarp) in addition to the hyaline and aleuronic layers of the grain. Bran has high content of fibre, vitamins and minerals than the endosperm. These qualities attribute interesting nutritional

properties to it [141,142]. In addition to this bran has ability to prevent the appearance of diseases [143-145]. On account of these interesting features, bran is also a good candidate for fortification of breads [146-149].

3.10. Biscuits

Biscuits are small, crisp and flat baked products made from wheat flour, starch, powdered sugar, glucose syrup, emulsifiers, food colour and flavours. Originally spelled *bisket*, the word *biscuit* is derived from the Old French *bescuit* and Medieval Latin *biscoctum*, both meaning "baked twice" [150], alluding to the now-discontinued process of baking first at high temperature, followed by drying in a cooler oven. In conventional biscuits, the ingredients are mixed and the dough is cut into pieces of desired size. The dough pieces are baked carefully at 180°C for about 8 minutes [151]. On account of the high sugar and fat content, biscuits are sometimes viewed as unhealthy foods. However, biscuits are an ideal medium for carrying many nutritionally beneficial ingredients. Manley (2003) suggests several ways in which biscuits can be fortified[152].

Several studies have been carried out on the substitution of wheat flour with other flours. Rababah *et al* (2006) attempted fortification of biscuits using chickpea flour, broad bean flour and soy protein. Their study shows that high-protein biscuits can be produced using these ingredients[153]. Studies conducted in India show that 30% soy flour can be used in the baking of biscuits enhancing their nutritive value and without any loss of physical characteristics [154]. Salama *et al* (1997) incorporated the rootlets of malt sprouts into biscuits. Biscuits that contained 15% roots were acceptable. However their sensory qualities were affected[155].

Addition of red palm oil as an alternative fortificant for addressing vitamin A deficiency has been proposed by van Stuijvenberg *et al* (2001), on the basis of a randomized, controlled clinical study[156].

Boobier *et al* (2006) converted a traditional biscuit to a functional food by adding B_{12}, folic acid, vitamin C and prebiotic fibre. Consumption of these fortified biscuits by human volunteers reduced the blood levels of homocysteine and glucose, suggesting their ability to reduce risk factors associated with heart diseases like myocardial infarction, stroke, and venous thrombosis [157-159].

Replacement of sugar and fat in biscuits have also been a subject of investigation [160]. Gallagher *et al* (2003)[161] reported that sugar in biscuits can be reduced by 20-30% with the incorporation of the oligofructose Raftilose. Sucralose is a sugar substitute currently being used in bakery products in Europe. It is heat stable and no loss occurs during baking processes[160]. Savitha *et al* (2008) studied the replacement of 30% sugar with 0.5% sucralose and of different levels of maltodextrin on dough rheology and quality of biscuits. The results show that sucralose and maltodextrin can replace sugar in biscuits[162].

Sharif *et al* (2005) observed that superior quality cookies can be produced by replacing normal shortening in the recipe with rice bran oil[163]. Rice bran oil is a good source of polyunsaturated fatty acids and lowers blood cholesterol better than sunflower, corn or safflower oils[164].

There is a growing demand for biscuits for diabetics. This can be met by replacing sugar with polyols like mannitol or polydextrose and including in the recipe, dietary fibres which have proven hypolipidaemic or anti-diabetic property. Jenkins *et al* (2008) extracted glucomannan, a glucose-mannose polysaccharide from the tuberous roots of *Amorphophallus konjac* and incorporated it into the dough of biscuits. Consumption of these biscuits reduced the glycemic index significantly by 74% in healthy human volunteers and by 63% in participants with diabetes mellitus. This study suggests that biscuits fortified with other viscous and starchy foods can replace high glycemic index snack foods[165].

Many varieties of starches are used as ingredients in food products. Starches can escape digestion if they are embedded in a matrix that renders them inaccessible to digestive enzymes. Some others like starch from potato, green bananas and maize also resist digestion on account of their structural properties. Foods formulated with such resistant starches are effective in lowering the glycemic index of the food, despite formulation with high glycemic index ingredients. This was proven in a recent clinical study [166]. As resistant starch is known to reduce food intake, it can be also considered in food fortification aimed at weight management [167].

4. Concluding Remarks

Food technologists and nutritionists are evincing greater interest in the fortification of food and beverages with natural ingredients. A few interesting functional foods have also made their appearance recently. The stem bark of magnolia has been used in the treatment of cough, diarrhoea and allergic rhinitis in traditional medicine of China, Japan and Korea. Recent research indicates that magnolol and honokiol in the bark have strong anti-inflammatory and antimicrobial activities. They are active against an array of organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *Porphyromonas gingivalis*, *Epidermophyton floccosum*, *Aspergillus niger*, *Cryptococcus neoformans* and *Candida albicans* [168]. Considering these activities the gum giant Wrigley has been granted novel food approval in the European Union for extract of *Magnolia officinalis* to be used in chewing gums and mints with perceived breath-freshening benefits [169]. Chewing gum is also intended to be a dosage form to deliver the PYY hormone reported to promote feeling of fullness and thus act as a weight loss agent [170].

Several problems need to be addressed for achieving successful fortification of foods. Physico-chemical properties like pH, water or oil content, proteins and fibres can influence the stability of the nutrient added. Fortification can alter the sensory characteristics of the food and some fortificants can change the colour and flavour of the product. Ingredients of the fortified food can at times interact with

each other. The processes used in the manufacture of the fortified food should be designed in such a way that they do not cause denaturation or loss of the nutrients. The packaging of the fortified food can affect the stability of the nutrients. Colour of the product may change, haziness or sedimentation can develop in liquid preparations or ingredients like vitamin C or β-carotene may undergo oxidation[171].

The components of functional foods can enhance vital functions in the body and these beneficial effects can be assessed by identification, characterization, measurement and validation of relevant markers. Functional food science is predicted to contribute to human health in the coming decades, by reducing the risk of diseases [4].

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