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Scientific and technical aspects of yogurt fortification: A review

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Abstract

Food fortification is one of the most important processes for improvement of the nutrients quality and quantity in food. It can be a very cost effective public health intervention. Due to the high consumption rate of dairy products such as yogurt, fortification of these products will effectively reduce or prevent diseases associated with nutritional deficiencies. The aim of this investigation is to study the technical aspects involved in production of different types of fortified yogurts and their role in disease prevention and correction of deficiencies. In this paper, firstly, fortification is defined and the main reasons behind carrying out this process are presented and then yogurt production process and a variety of minerals, vitamins, and functional ingredients which are used in the process are briefly discussed.

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1. Introduction

Considering the importance of food safety and quality, more attention is being paid to the health of consumers [1]. However, due to nutrients deficiencies in human societies especially in certain periods of life, embracing, importing, and consumption of fortified foods is increasing [2]. In general, adding one or more essential nutrients to a food and increasing their concentration in that particular food to levels higher than normal is known as fortification and is aimed at preventing and correcting deficiencies in one or more nutrients in the society or specific population groups [3]. Nutrition scientists have mentioned that fortification of food products using natural resources (fruits, cereal, etc.) is one of the best ways to improve the overall nutrient intake of food with minimal side effects [4]. However, compliance notes on the production of fortified foods with the aim of safeguarding

consumers' health and lack of toxicity resulting from the use of this material seem necessary.

Fermentation is a method that has been used for thousands of years to provide longer shelf life for perishable foods and to increase the flavor and odor of final food products [5]. It is known that fermented foods have been made since Neolithic times. The most famous examples of fermented foods are wine, bread, and cheese. Also, in middle Asian regions, yogurt and other fermented milk products (such as kefir and kumys), traditional alcoholic beverages, vinegar and pickles are common [6]. Fermentation is a chemical process in which enzymes break down organic substances into smaller compounds. As the result of fermentation, more digestible, stable and flavored foods with enhanced nutritional value are produced. Fermentation is carried out by molds, yeast or bacteria. During the growth of these microorganisms, fermented foods are produced incidentally [7]. Yogurt is a fermented milk produced by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. The production of yogurt as a fermented milk product has been started in the Middle East and spread all over the world. Compared to milk, yogurt is more nutritious and is an excellent source of protein, calcium, phosphorus, riboflavin, thiamin, vitamin B₁₂, folate, niacin, magnesium and zinc. Since lactose in milk is

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converted to lactic acid during fermentation and due to the presence of lactose fermenting bacteria in yogurt, lactose intolerant people can consume yogurt without any adverse effect. Moreover, consumption of fermented milk products causes a slight reduction in stomach pH which reduces the risk of pathogen transit and the effects of low gastric juice secretion problem [8]. Since fermented milk products are among highly-consumed food in the world, they have been used to deliver nutritional components into human diet. Furthermore, fortification of these products such as yogurt, is a good way to improve nutrient intake in daily food products [2].

2. Yogurt consumption and health effects

Obviously, the nutritional value of any material depends on its components. Because of the presence of precious compounds in milk, yogurt is of great importance. Regarding the chemical composition of milk and yogurt, no changes will occur during fermentation. However, the fermentation process causes a beneficial effect on yogurt [9]. Table 1 shows the components of full-fat milk, non-fat milk, and the corresponding yogurts [10].

Milk is a physiological liquid containing bioactive and nutrients components which have beneficial effects on the newborn infant's growth and the digestive system. It may also improve the symbiotic micro flora and the development of lymphoid tissues. Several bioactive compounds are present in milk, notably in fermented milk products, which are of great importance and include certain specific proteins, vitamins, bioactive peptides, organic acids and oligosaccharides [10].

The consumption of fermented dairy products containing probiotic bacteria would decrease cholesterol absorption [11]. Beneficial effects of dairy foods on the body fat and body mass may be caused by whey proteins, medium-chain fatty acids, and the high level of calcium and other minerals. Milk components containing proteins, peptides, probiotic lactic acid bacteria, calcium, and other minerals have a noteworthy effect on the reduction of blood pressure. There are several components in milk fat with functional properties. Sphingolipids and their active metabolites may exert antimicrobial influences either directly or upon digestion. A review of literature revealed that the consumption of recommended level of milk and fermented dairy products, as part of a healthy diet, can result in reducing the risk of many diseases [12].

Fermented dairy products contain adequate levels of certain live and active cultures, namely probiotics that aid to improve the balance of “beneficial” versus “undesirable” bacteria in the intestinal tract. Several researches on fermented dairy products showed their effect on the immune system, as well as promoting intestinal health. The impact of consuming fermented products such as yogurt on improving the immunity function in body against carcinogens and harmful toxins is being studied. In order to find out the effect of milk and dairy products intake in reducing the risk of diseases, clinical investigations have been done on diseases such as GI system diseases, cardiovascular system diseases, musculoskeletal system diseases, urogenital system diseases, immune system diseases, allergy, nervous system diseases, cognitive system diseases, weight control, obesity, aging and dental health [92].

Table 1
Nutritional composition of full-fat milk, non-fat milk and correspondent yogurts (100 g) [10,16].

Components	Unit	Full-fat milk	Full-fat yogurt	Non-fat milk	Non-fat yogurt
Energy	Kcal	68	70	35	39
Protein	G	3.3	3.8	3.5	4.4
Fat	G	3.8	3.8	0.1	0.1
Lactic acid	G	0	0.8	0	1.0
Potassium	mg	157	157	150	187
Calcium	mg	120	120	123	143
Phosphorus	mg	92	92	97	109
Magnesium	mg	12	12	14	14
Sodium	mg	48	48	53	57
Iodine	mg	0.46	0.46	0.45	0.44
Chlorine	mg	102	102	100	121
Retinol	μg	52	28	1	8
Carotene	μg	21	21	Tr*	5
Vitamin B ₁	μg	30	60	40	50
Vitamin B ₂	μg	170	270	170	250
Vitamin B ₆	μg	60	100	60	90
Vitamin B ₁₂	μg	0.4	0.2	0.4	0.2
Vitamin C	mg	1	1	1	1
Vitamin D	μg	0.03	0.04	Tr*	0.01
Vitamin E	μg	90	50	Tr*	10
Folic acid	μg	6	18	5	17
Nicotinic acid	μg	100	200	100	100
Pantothenic acid	μg	350	500	320	450
Biotin	μg	1.9	2.6	1.9	2.9
Choline	mg	12.1	–	4.8	0.6

Tr*: trace.

Such products as yogurt play a potential role in decreasing intestinal disorders and chronic diseases. According to a recent study, several non-nutrient components in yogurt such as sphingolipids, conjugated linoleic acid and butyric acid may play a role as anti-cancer agents [13]. The positive effect of probiotics on food allergies in children is another promising area of study. Recent studies in infants have revealed that probiotics not only alter the response to potentially harmful antigens (substances that induce allergies), but also reduce their allergenic potential [14]. Fermented dairy products are a good instance of functional foods. Many scientific studies confirm yogurt and other fermented dairy products' functional role which are increasingly advancing the immune system and preventing diseases. Fermented dairy products are good, and in some cases excellent, sources of nutrients namely calcium, protein and potassium [15].

3. Fortification with vitamins

Vitamins are compounds which play a role as cofactors in the body. Fermented milk products such as yogurt can be thought as vitamin sources. However, due to the ability of some starters to synthesize vitamin B that is necessary for their growth, there are different vitamin contents in yogurts. Thus, yogurt and fermented milk products which are produced by strains, may have different vitamins according to the starter that is used [10].

Furthermore, evaluation of vitamins is more difficult since processes like heat treatment, incubation time, temperature and storage conditions change the vitamins content in yogurt [17].

The approximate vitamin content of full fat and nonfat yogurt, and comparison of whole milk and skimmed milk are shown in Table 1 [16].

Vitamin D is vital for appropriate skeletal development which plays a fundamental role in regulating serum calcium and phosphorus concentrations in the body. Due to the photosynthesis of vitamin D in the skin after exposure to solar UV radiation, it is not included in the category of essential nutrient of the body [18]. On the other hand, at latitudes above 40° N or below 40° S and for several months of the year, no photosynthesized vitamin D is produced in the skin; thus, in order to prevent deficiency, supplementation of vitamin D is required [19,20]. Furthermore, the amount of vitamin D produced in the skin as a result of exposure to the sun is limited by application of sunscreen [21].

Diseases such as childhood rickets, osteoporosis and osteomalacia are influenced by vitamin D deficiency. It has also been shown that the increase in the risk of developing cancers, osteoporotic fractures, and autoimmune diseases have a direct relationship with vitamin D deficiency [22].

Since vitamin D is a hormone, its receptor, which belongs to the family of steroid/thyroid hormone nuclear receptors, mediate its genomic mechanism of action. Antiproliferative, differentiative and apoptotic effects of this vitamin were observed on prostate cancer cells in vitro [23–25]. Concerning the epidemiological studies, vitamin D has negative effects on breast [26] and colon cancers [27]. Also, vitamin D deficiency might result in type I diabetes, hypertension, multiple sclerosis and some other cancers [28]. Several researches have been conducted on the

stability of vitamin D in milk and other dairy products [29–33], which all have claimed that vitamin D is stable during processing and storage. There are not adequate amount of vitamins A and C in low fat dairy products which are not fortified. Usually, low fat milk and other dairy products are enriched with vitamin A but not with vitamin C. Fortification of dairy products with vitamins A and C leads to improvement in their nutritive quality and consequently, increases their acceptability [34,35].

Vitamin A is toxic in high amounts. But, the provitamins such as carotene are not toxic. It is reported that doses such as 40,000–50,000 and 25,000 IUs are toxic for adults and children respectively [2]. So, it is recommended to use β -carotene for fortification of dairy products.

Health risks begin to increase, by the intake of more than 100 $\mu\text{g/day}$ of vitamin D. Also, very high doses of vitamin D (more than 250 $\mu\text{g/day}$) are known to cause tissue and kidney damages [2]. Although, Hanson and Metzger reported no adverse effects at level of 250 $\mu\text{g/day}$ of vitamin D, during 5 months of consumption [33].

4. Yogurt fortification with iron

Yogurt is a good source of protein and Ca [36], while dairy products are poor in iron and some other minerals [37]. Fortification of dairy products with Fe would help nutritional deficiencies. Iron-fortified yogurt has a relatively high iron bioavailability [38]. However, before doing any process such as fortification, the effects of added iron to yogurt must be assayed. The parameters including oxidation of fat, taste, shelf life and microbial physiology are important, and the sensory quality and overall acceptance of a fortified yogurt must be ascertained [39–41]. Properties of fortified dairy products are influenced by the type of mineral source and the amount of component which is added to the product. Two principal off-flavors have been created with fortified yogurt: oxidized flavor and metallic flavor, which are due to the catalytic role of iron and the presence of iron salts, respectively [42].

Oxidation of fat occurred in yogurt and milk which were fortified with ferrous sulfate, ammonium and ferric [43,44], reduced the absorption of this element in the fortified milk [45].

Fat oxidation in chocolate milk and similar products was not promoted by fortification with a ferric polyphosphate–whey protein complex [46]. Chocolate milk was fortified by iron and had acceptable flavor properties. But other products with ferric chloride or ferrous gluconate were not acceptable. Such oxidation has been effective on sensory characteristics and thiobarbituric acid (TBA) values, which were high in the fortified milk. Although ferric ammonium citrate increases the oxidation in milk, it is not observed in solid dairy product such as cottage cheese [39]. Several researches indicated that the lipid oxidation process evaluated by TBA test was reduced using capsulated iron, compared to uncapsulated iron fortified yogurt. During three weeks of experiment, no change was found in microcapsulated iron and vitamin C in the fortified yogurt in terms of sensory parameters and acceptance. Therefore, these researches showed that microcapsules of iron and vitamin C are effective means of fortification, and can be used

to fortify dairy product without any changes in sensory aspects [47].

Yogurt fortification with iron can be an important and effective strategy to control iron deficiency anemia, but adding iron to yogurt still remains a problem. Compared to ferrous sulfate, iron compounds which are water insoluble, are less absorbed. Thus, concerns about their benefit as yogurt fortificants has been increased in the past, especially because the target is young. For many reasons ferric pyrophosphate is one of those compounds that have been widely assayed in many products difficult to fortify such as cereals, salt, rice, infant formulas, and even dairy products. Nonetheless, these data about ferric pyrophosphate will be important in the development of food-fortification strategies to fight anemia and iron deficiency in highly vulnerable populations. Chronic overload of iron would cause some adverse effects including, cirrhosis, hepatitis, liver cancer, intestinal irritation, vomiting and diarrhea, articular pain, hormonal disturbance, heart disorder and osteoporosis [89–91].

5. Fortification with calcium

Osteoporosis is a very common disease that affects not only elder women but also elder men and has been related to increased bone fracture risk. Due to the fact that calcium and vitamin D are very important in reducing the risk of fracture, several supplementation researches have examined their effects on bone mass and bone metabolism indices. Still, there are only a few clinical trials examining the effect of these nutrients when supplemented to susceptible population groups via fortified dairy products.

The sensorial properties of fortified yogurt should not be influenced by using high concentration of minerals. In this regard, Ocaik and Rajendram have reported that calcium must be used in micronized type to prevent the adverse impact on the sensorial properties [48]. Thus a possible way to enhance the level of minerals in the dairy products is achieved by micronization of the minerals which is mainly due to the fact that ultrafine particles ease dispersion, improve mouth feel, acceptance and texture of dairy products [48]. The premium option in calcium fortified yogurt and dairy products is the application of micronized tricalcium citrate, which can give rise to good technological properties and nutritional value [16]. Indeed, in contrast to calcium, fortification with Mg and Zn is not important in developed societies. The tricalcium citrate can be used in yogurts and other dairy products at concentrations of more than 1 g/L calcium [49].

In yogurts and other dairy products, a liquid mineral suspension can be used and the addition of hydrocolloid or starch would result in the stabilization of these suspensions by reducing sedimentation of minerals. All around the world, especially in Europe, where health claims on products are regulated by the new EFSA (European Food Safety Authority) health claim regulation, Mg and Zn offer various options for new fortified product concepts. By raising the awareness of these minerals and their various beneficial effects on human health, they should gain importance in dairy products as well as calcium and other nutritional ingredients. As technological problem will increase with higher fortification levels of mineral, trimagnesium and

zinc citrate will be able to prove their superior application in dairy products [49].

6. Fortification with fiber

There is no fiber in yogurt and dairy products. Fiber is a component of the cell wall of fruits, grains, seeds and vegetables [50,51]. Fiber of various sources is added to dairy products because of its water-holding capacity and its ability to increase the production yield, reduce the lipid retention, improve textural properties and structure, and reduce caloric content by acting as a bulking agent [52].

Consumption of products containing high fiber may prevent or decrease hypertension, hypercholesterolemia, obesity [53], gastrointestinal disorders [54], coronary heart disease [55,56], diabetes [57,58], and cancer [59]. Fortifying yogurt or dairy products with fiber is of increasing interest to create functional foods with health benefits and improve their functionality. Fortifying yogurt with dietary fiber would complement its healthy properties. The maximum acceptable amount of date fiber in fortified yogurt with potential beneficial health effects is 3%. Many researchers evaluated the effect of dietary fiber on dairy products and yogurt quality. The addition of 1.32% oat fiber improved the body and texture of unsweetened yogurt and decreased the overall flavor quality [60].

7. Fortification with fruits and vegetables

Plants produce a vast amount of secondary metabolites in order to better adapt to the environmental conditions, and protect themselves from microbial attacks and resist both biotic and abiotic stresses. Of these compounds, phenolics have received significant attention in recent years due to their antioxidant, anti-inflammatory, anti-mutagenic and anti-clotting power which has been correlated with a declined risk of cardiovascular diseases and cancer development [61–63]. The major dietary source of phenolic compounds is fruit [64]. It has been suggested that fruit juices [65], powders [66] and extracts have the potential to be used as functional ingredients in the food industry including dairy sector. But, seasonal production of some fruits and vegetables, economic restrictions, and high requirement of fruits in the fresh market, forced researchers to look for alternative strategies for the bio-production of natural compounds similar to anthocyanin and phenolic acids [67].

Plant callus/cell cultures were shown to possess a promising potential for the production of mainly anthocyanin and other phenolic in grapes [68], carrots [69] and cherries [67]. These *in vitro* cultures exhibit several advantages over fresh fruit extracts such as possibility of continuous production of natural compounds [67], large scale production depending on specific needs [70], lower cost and opportunity of manipulating the direction of anthocyanin's or other phenolic biosynthesis [71,72].

One of the well-known fermented dairy products is yogurt; despite its nutritional characteristics and importance in human diet, it is not being considered as a major source of phenolic compounds [8]. The amount of phenolics in dairy products

is extremely restricted, which may be because of cattle feed containing high level of phenolics, contamination of food production equipment with sanitizing agents, and bacterial decomposition of proteins in milk. Hence, plant-based additives had been applied to improve the phenolic content of yogurt [8]. In another study, yogurt was enriched with acidified ethanol extracts of four different grape varieties and grape callus which were regarded as functional ingredients [73].

8. Fortification with seed oil containing unsaturated fatty acids and phytosterols

From the perspective of nutrition, unsaturated fatty acids are a nutritional hot topic in oil quality evolution. Compared to animal fat, vegetable oils contain a fatty oil rich in unsaturated fatty acids, mainly linoleic and oleic, which can improve the amount of cholesterol in the food stream.

Plant sterols comprise a group of compounds which is the focus of research at the moment. They decrease cholesterol absorption and may thus protect against atherosclerosis [74,75]. Furthermore, they may have beneficial effects against colon cancer [76,77]. To produce functional foods containing elevated levels of plant sterols is the aim of many food companies. On

the other hand, for evaluation of their effects on human health at their natural levels, reliable data on plant sterol concentrations in various plant-based foods are needed.

Phytosterols is a white powder insoluble in water and has a melting point of 100–215 °C. Unlike drugs which are basically intestinal, cholesterol is not absorbed intestinally [78]. By placing the fat globules in the intestinal cavity, phytosterol prevents absorption of cholesterol in the small intestine [79]. These compounds improve type II diabetes, reduce the risk of stomach cancer, inhibit the growth of tumors and enhance inflammatory diseases and arteriosclerosis [80]. The plant sterols, soy protein and isoflavones in reducing blood cholesterol is meaningful effects.

It seems that due to their strong lipophilic properties, margarine, yogurt, salad dressing, cheese and butter are suitable carriers for phytosterol. It has been proven that, compared to cereals, margarine and phytosterol-enriched dairy products (yogurt and milk) are more effective in lowering cholesterol [81–83].

9. The amount of nutrients needed by different age groups

In selecting micronutrients for society's different age groups, considering its trophic status and food requirements is of great

Table 2
Nutrient compounds proposed for fortified foods (amounts per 100 g food) [84–88].

Micronutrient	Units	BF infant ^a	MAM child ^b	NBF infant ^c	Older & PL ^d	Composite ^e
Biotin	mg	2.9	4.9	–	–	–
Choline	mg	91.8	83.8	–	–	–
Folic acid	mg	83	131.6	156	273	95
Niacin	mg	6.1	6.8	7.3	8.2	9.74
Pantothenic acid	mg	0.7	1.1	3.3	2.7	3.53
Riboflavin	mg	0.36	0.68	0.73	0.64	0.967
Thiamin	mg	0.36	0.38	0.55	0.64	0.746
Vitamin A	mg	500	714.4	734	377	154
Vitamin B6	mg	0.44	0.68	0.55	0.87	0.752
Vitamin B12	mg	0.52	0.98	1.27	1.29	1.5
Vitamin C	mg	140–280	37.6	55	28	40
Vitamin D3	mg	2–4	4.1	9.2	8.1	25
Vitamin E	mg	10	8.3	5.2	4.7	10.88
Vitamin K	mg	–	15.0	–	–	33
Calcium	mg	200–400	315.8	734	698	353
Copper	mg	400–800	334.6	–	–	390
Iodine	mg	180	75.2	164	113	230
Iron	mg	14	6.8	17.1	11.6	15.5
Magnesium	mg	80–120	112.8	99	111	94
Manganese	mg	1200	451.2	–	–	790
Phosphorus	mg	150–200	338.4	504	606	513
Potassium	mg	–	601.6	2654	2699	707
Selenium	mg	20	20.7	18.3	18.4	20
Sodium	mg	–	206.8	219	327	239
Zinc	mg	8.3	7.5	15.4	8	6.85

This table provides target vitamin and mineral levels recommended in the literature for various vulnerable groups among populations of food aid beneficiaries, including breast-fed (BF) and non-breast-fed (NBF) infants, children suffering from moderate acute malnutrition (MAM), older children and adults—including pregnant and lactating women (older & PL), and a composite group comprises infants, young children, and pregnant and lactating women

^a Breast-fed infants, 6–23 months of age.

^b Children suffering from moderate acute malnutrition. Recommendation is for age with highest nutrient density requirement. Data presented on per 1000 kcal basis has been converted to “per 100 g FBF” basis, using kcal density of USAID CSB.

^c Non-breast-fed infants. Recommendation is for age with highest nutrient density requirement.

^d Older children and adults, including pregnant and lactating women. Recommendation is for group with highest nutrient density requirement.

^e Composite vulnerable group, comprises infants, young children, and pregnant and lactating women.

importance. Unfortunately, infants and children are more prone to the deficiency crisis of these materials. In addition to infants and children, other vulnerable groups including, children with malnutrition and adolescents, patients (especially those suffering from HIV/AIDs or Tuberculosis), pregnant and lactating women and people suffering from malnutrition in all ages are of importance. It is obvious that some of these groups have completely different food requirements.

Several groups, who were expected to be users of any potentially redesigned product-line of FBF, have different micro and macronutrient requirements which have been reported in the existing literature. They include some suggestions for breast and non-breast-fed infants, young children, malnourished children, older children and adults such as pregnant and lactating women. Table 2 presents a summary of these authors' recommendations.

10. Conclusion

Yogurt is the most consumed healthy and nutritious food around the world. Therefore, it offers an appropriate potential to convey nutritious ingredients to human diet. Research shows that most people in developing or underdeveloped countries suffer from micronutrient deficiency and enriched food products can dramatically reduce the nutritional diseases. In this study, a variety of different nutritious components and the manner of using them in dairy products were discussed and the impact of enriched food on preventing or treating disease was shown. This paper shows that food enrichment can prevent or treat most of diseases, especially in young children and it has a significant impact on improving the health of the community.

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