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REVIEW ARTICLE

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Fortification of Dairy Products: A Review

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ABSTRACT

Food fortification 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. This is an effective way to combat the micronutrient deficiency and thus to alleviate "hidden hunger". Currently food fortification encompasses a broader concept, and might be done for several reasons. The objectives may be: to maintain the nutritional quality of foods, keeping nutrient levels adequate, to correct or prevent specific nutritional deficiencies in the population at large or in groups at risk of certain deficiencies (i.e., the elderly, vegetarians, pregnant women etc);

to increase the added nutritional value of a product (commercial view); and to provide certain technological functions in food processing.
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 (Bonner et al., 1999). The focus of the international community has so far been on the three most prevalent deficiencies: Vitamin A, iodine, calcium and iron. 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 (Nestle, 2013).
Key words: Dairy products, Food fortification and Nutritional value.

INTRODUCTION

Milk in its natural form is almost unique as a balanced source of man's dietary need. The various steps in processing and storage have a measurable impact on some specific nutrients. Modern consumers are increasingly interested in their personal health, and expect the food that they eat to be healthy or even capable of preventing illness apart from being flavorful. Consumption of fermented milks has increased significantly around the world and nowadays various popular ingredients of functional significance are being incorporated into cultured dairy products to enhance their market value (Deeth and Tamime, 1981). Since consumption of functional foods containing nutraceuticals is being highly encouraged, thus fermented milks produced with incorporation of these ingredients with specific health benefits could be of potential interest.

Fermentation is a chemical process in which enzymes breakdown 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 (Yousef and Carlstrom, 2003).

Yogurt is 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, thiamine, vitamin B_{12}, folate, niacin, magnesium and zinc. Since lactose in milk is 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 (J. O'connell and Fox, 2001). 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 (Preedy *et al.,* 2013).

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 (Walstra and Wouters, 2010). 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 (Akın, 2006).

The consumption of fermented dairy products containing probiotic bacteria would decrease cholesterol absorption (Anderson and Gilliland, 1999). 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. Sphingo lipids 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 (Sandholm and Saarela, 2003). Fermented dairy products contain adequate levels of certain live and active cultures, namely probiotic 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 (Miller *et al*., 2006). 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 Sphingo lipids, conjugated linoleic acid and butyric acid may play a role as anti-cancer agents (Sanders *et al*., 2007). The positive effect of probiotic on food allergies in children is another promising area of study. Recent studies in infants have revealed that probiotic not only alter the response to potentially harmful antigens (substances that induce allergies), but also reduce their allergenic potential (Isolauri and Walker, 2004). 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 (McGill *et al*., 2008).

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 (Akın, 2006). Furthermore, evaluation of vitamins is more difficult since processes like heat treatment, incubation time, temperature and storage conditions change the vitamins content in yogurt [Rao and Shahani, 1987).

Under ambient conditions the water soluble vitamin C and vitamins of the B complex group such as thiamine, riboflavin, vitamin B_6, niacin, pantothenic acid, folic acid, biotin and vitamin B_{12} 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 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 B_6 and vitamin B_{12} are less stable. While in the case of fat soluble vitamins 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 (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 form, 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 the fat soluble vitamins have to be microencapsulated.

Fortification with minerals

Fermented dairy products are a good source of protein and Ca (USDA, 1982), while dairy products are poor in iron and some other minerals (Blanc 1981). Fortification of dairy products with Fe would help nutritional deficiencies. Iron-fortified yogurt has a relatively high iron bioavailability (Woestyne *et al.,* 1991). 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 (Zhang and Mahoney, 1990). 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 (Jackson & Lee, 1991).Oxidation of fat occurred in yogurt and milk which were fortified with ferrous sulfate, ammonium and ferric (Hegenauer *et al.,* 1979), reduced the absorption of this element in the fortified milk (Hegenauer *et al.,* 1979). Fat oxidation in chocolate milk and similar products was not promoted by fortification with a ferric polyphosphate-whey protein complex (Douglas *et al*., 1981). 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 (Sadler *et al.,* 1973). Several researches indicated that the lipid oxidation process evaluated by TBA test was reduced using capsulated iron, compared to encapsulated iron fortified yogurt. During three weeks of experiment, no change was found in micro capsulated 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 (Kim *et al.,* 2003). Yogurt fortification with iron can be an important and effective strategy to control iron deficiency anaemia, 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 fortificant 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 anaemia 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 diarrhoea, articular pain, hormonal disturbance, heart disorder and osteoporosis (Schumann, 2001).

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, Ocak 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 (Ocak & Rajendram, 2013). 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 [Deeth and Tamime, 1981). 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 (Gerhart and Schottenheimer, 2013). 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 (Gerhart and Schottenheimer, 2013).

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 (Lunn and Buttriss, 2007). 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 (Larrauri, 1999). Consumption of products containing high fiber may prevent or decrease hypertension, hypercholesterolemia, obesity (Dam and Seidell, 2007), gastrointestinal disorders (Elia and Cummings, 2007), coronary heart disease (Mann, 2007), diabetes (Anderson *et al.,* 2004) and cancer (Bingham *et al*., 2003). Fortifying yogurt or dairy products with fiber is of increasing interest to create functional foods with health benefits and improve their functionality. 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 (Fernandez *et al*., 1998). In one experiment, pectin and raspberry concentrate was incorporated in commercial stirred yogurt samples, increasing the consistency and it was found that yogurt with pectin was more shear stable in comparison with yogurt with raspberry concentrate (Ramaswamy and Basak, 1992). In another study, seven types of insoluble dietary fibers from five different sources (soy, rice, oat, corn and sugar beet) were used to fortify sweetened plain yogurt. Fiber addition caused acceleration in the acidification rate of the experimental group yogurts, and most of the fortified yogurts also showed increases in their apparent viscosity. However, soy and sugar beet fibers caused a significant decrease in viscosity due to partial synersis. In general, fiber addition led to lower overall flavor and texture scores as a grainy flavor and a gritty texture were intense in all fiber-fortified yogurts, except in those made with oat fiber, which gave the best results [69]. Similarly, b-glucan was used to prepare low fat yoghurt and as the amount of b-glucan increased a corresponding increase in yogurt consistency and firmness as well as a decrease in synersis was reported. Furthermore, the micrographs revealed a denser structure with trapped water, similar to a full-fat yogurt [Tudorica *et al*., 2002). Recently, Palacios *et al.* (2005) prepared yogurt systems from whole milk, with Calcium (50 mg of calcium/100 mL of yogurt) and three levels of fiber from two wheat-bran sources. In comparison with a plain yogurt, the presence of fiber and calcium augmented the consistency, diminished the synersis and the pH was higher.

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, phenolic 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 (Ostertag *et al.,* 2010). The major dietary source of phenolic compounds is fruit (Record *et al*., 2001). It has been suggested that fruit juices (Coisson *et al*., 2005), powders (Wallace *et al*., 2008) 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 (Blando *et al*., 2004).

Plant callus/cell cultures were shown to possess a promising potential for the production of mainly anthocyanin and other phenolic in grapes (Hiroyuki *et al.,* 2002), carrots (Glabgen *et al.,* 1992) and cherries (Blando *et al*., 2004). These *in vitro* cultures exhibit several advantages over fresh fruit extracts such as possibility of continuous production of natural compounds (Blando *et al.,* 2004), large scale production depending on specific needs (Smith & Pepin , 1999), lower cost and opportunity of manipulating the direction of anthocyanins or other phenolic biosynthesis (Curtin *et al.,* 2003).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 (J. O'connell & Fox, 2001). The amount of phenolic in dairy products is extremely restricted, which may be because of cattle feed containing high level of phenolic, 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 (O'connell & Fox, 2001). In another study, yogurt was enriched with acidified ethanol extracts of four different grape varieties and grape callus which were regarded as functional ingredients (Karaaslan *et al*., 2011).

Milk is well known source of folates. Food composition tables and review papers based on microbiological assay report folate values for cow's milk in range 5 - 7 mg/100g. Most HPLC studies indicate 5-methyltetrahydrofolate (5-MTHF) as the major form of folates in milk. (Forssen *et al.* 2000). Heat treatment such as pasteurisation and UHT processing are known to reduce the folate content (Wigertz *et al.* 1996). However, milk processing can lead to a positive effect when milk fermentation is applied. The fermented milk products are reported to contain higher amounts of folate. This high level is result of the production of additional folates by the bacteria. However many bacteria synthesize this cofactor by themselves from a simple precursors, but some auxotrophic bacteria, including many lactic acid bacteria, have a strict growth requirement for folic acid. The production and consumption of folates by applied microorganisms will be probably the most important factor determining the folate level in fermented milk products. In general, *Lactobacillus* strains did not produce folates with exception of *Lactobacillus plantarum*. *Streptococcus thermophilus* is reported to produce folates, however large differences were observed in production ability of individual strains. Some other lactic acid bacteria - Lactococcus lactis, *Leuconostoc lactis, Bifidobacterium longum were* recognized as a folate producers. Some strains of Propionibacteria, well known vitamin B12 producer, are able to increase folate level as well. The contents of folate in fruit and berries range from a few µg to approx. 100 µg/100 g. The highest concentrations of about 50–100 µg/100 g are found in frozen concentrated orange and grapefruit juices and in strawberries (Witthöft *et al*. 1999). Holasová *et al.,* 2005 evaluated the possibility of the increase of the natural folate content in fermented milk products by the fermentation process and by the addition of fruit component . Among the rest of the components tested, the strawberry component proved to be the best source of folate with the content of 9.11 µg 5-MTHF/100 g. The The folate content in the fermented milk product may be increased in this way by 4.8 µg/100 g, with 69% originating from the fermentation and 31% from the fruit component addition.

Fortification with Isoflavones

These are functional ingredients of a more recent interest (Mason ., 2001), even though their commercial source, soy beans have been consumed for over 5000 yrs. Isoflavones are part of the diphenol compounds, called "phytoestrogens," which are structurally and functionally similar to estradiols, the human estrogens, but much less potent. Because of this similarity, isoflavones were suggested to have preventive effects for many kinds of hormone-dependent diseases (Uzzan & Labuza, 2004). Isoflavones occur naturally in plants and mostly in soybeans. In nature, isoflavones usually occur as glycosides and, once deconjugated by the intestinal micro flora, the isoflavones can be absorbed into the blood. At present, their possible protective action(s) against various cancers (Setchell, 1998), osteoporosis and menopausal symptoms and high levels of blood cholesterol are under investigation and, although the epidemiological evidence seems convincing, no recommended daily intake has been published (Mason, 2001).

Fortification with phytosterols

Plant sterols compromise a group of compounds which is the focus of research at the moment. They decrease cholesterol absorption and may thus protect against atherosclerosis (Hendricks *et al.,* 1999).Furthermore, they may have beneficial effects against colon cancer (Awad *et al.,* 1997). 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 (Ostlund, 2002). By placing the fat globules in the intestinal cavity, phytosterols prevents absorption of cholesterol in the small intestine (Quilez *et al.,* 2003). These compounds improve type II diabetes, reduce the risk of stomach cancer, inhibit the growth of tumours and enhance inflammatory diseases and arteriosclerosis (Awad and Fink, 2000). It has been proven that, compared to cereals, margarine and phytosterols-enriched dairy products (yogurt and milk) are more effective in lowering cholesterol (Jong *et al*., 2008). Jones *et al*. (2002) indeed showed that the inclusion (1.7 g/day) of phytosterols into the diet of hypercholesterolemic men had the effect of lowering blood cholesterol. Oil-based products enriched with plant stanol esters can lower low-density lipoprotein (LDL) cholesterol concentrations by 10-14% and Mensink *et al*. (2002) concluded that low-fat yoghurt enriched with plant stanol esters lowers LDL cholesterol to the same extent as oil-based products within 1-week. Recently, Awaisheh *et al*. (2005) prepared yoghurts from modified milk base containing three important nutraceuticals, namely omega-3-fatty acids, isoflavones and phytosterols. The cultures employed to make the yoghurts were single probiotic strains of *Lactobacillus gasseri* or *Bifidobacterium infantis* and, to achieve a short production time, a two-stage fermentation procedure was used with *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* providing the rapid acidification. The nutraceuticals appeared to have no adverse effect on flavour and storage trials at 50C showed that the viability of the probiotic cultures was retained over 15 days.

Fortification with *w*-3 - Fatty Acids

Milk fat composition in dairy products can be altered by reducing the ratio of saturated to unsaturated fatty acids and increasing the contents of fatty acids that are more desirable for human nutrition, such as the w-3 polyunsaturated fatty acids (PUFAs). The importance of w- 3 - fatty acids like alpha-linolenic has been widely publicized because they are precursors of important long-chain fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthesized in the human body. Yet they are vital for the normal functioning and development of the brain, and are believed to reduce plaque formation in the arteries (Milner & Alison, 1999). They are also claimed to exert cancer inhibition, anti-allergy effects and improvement in learning ability (Wolfram G, 2003). The DHA-fortified drinks are targeted at school children in Japan. Increased levels of healthy fatty acids in dairy products can be efficiently achieved by the use of selected bacteria during fermented milk manufacture (Kim & Liu, 2002) or the substitution of milk fat by oils with high levels of PUFAs. However, replacement of milk fat by oils with high levels of PUFAs yielded yoghurts with less firmness and higher synersis (Barrantes *et al*., 1996). A different possibility for increasing w-3 PUFA content in milk is to include fish and vegetable oils or marine algae in animal diets (Dave *et al*., 2002). In one study, modified milk where fat had been replaced by oils enriched in w-3 polyunsaturated fatty acids was used for the manufacture of a set-type fermented product and no effect was found on yoghurt flavor however, product texture was adversely affected (Diana *et al*., 2004). In another experiment yogurt rich in poly- and mono- unsaturated fatty acids were prepared by replacing milk fat. Fortification with oils did not have any effect on microbial growth however texture and flavor were adversely affected (Barrantes *et al.,* 1994).

CONCLUSION

Fortification ensures a safest method by which manufacturers can deliver health promoting, nutritionally dense food products. Fermented dairy products are the most consumed healthy and nutritious food around the world. Therefore, it offers an appropriate potential to convey nutritious ingredients to human diet. Fortification of fermented dairy products is considered as an emerging technology as it considers the issues of the role of fermented dairy products in quality of life and in reduction of the risk of chronic diseases. The risks associated with fortification are minimal except if good manufacturing practices are not followed. 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 fortification of fermented dairy products.

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