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Review

Fortification aspects of vitamin D in dairy products: A review study

Malihe Zahedirad [a], Sepideh Asadzadeh [b], Bahareh Nikooyeh [a], Tirang R. Neyestani [a], Nasim Khorshidian [c], Mojtaba Yousefi [c], Amir Mohammad Mortazavian [d,*]

[a] Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[b] Student Research Committee, Department of Nutrition Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[c] Food Safety Research Center (Salt), Semnan University of Medical Sciences, Semnan, Iran

[d] Department of Food Science and Technology, Faculty of Nutrition Sciences and Food Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

There is a higher demand for calorie-reduced foods. In the dairy industry, fat is separated to produce low-calorie products and fat-soluble vitamins such as A and D are also removed along with them. There are different factors leading to a significant decrease in people's sun exposure, which has a substantial role in isomerisation of 7-dehydrocholesterol to vitamin D. It seems logical to add vitamin D to dairy products and potentially restore their nutritional value or fortify them to improve vitamin D intakes. The main biological function of vitamin D is to control the absorption, transport and deposition of calcium and, to a lesser extent, phosphorus, which are important in bone mineralisation. Dairy products are the ideal candidates for vitamin D fortification. This article reviews the vitamin D fortification of dairy products including the concepts, fortification methods, sensory properties of final products and storage stability.

Contents

* Corresponding author. Tel.: + 98 21 22360657.
E-mail address: mortazvn@sbmu.ac.ir (A.M. Mortazavian).

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1. Introduction

Vitamin D is an essential fat soluble vitamin that plays critical skeletal and non-skeletal roles such as prevention of chronic diseases including diabetes, cardiovascular diseases (CVDs), autoimmune disorders, and cancer (Pludowski et al., 2013). Sufficient amounts of this vitamin can be endogenously synthesised in the skin directly exposed to ultraviolet radiation (UVR; at wavelengths of 290–315 nm) from sunlight or can be obtained from the diet or supplements (Zahedi-Rad, Nikooyeh, Kalayi, Shariatzadeh, & Neyestani, 2015a).

Nowadays, there is an emerging demand for low energy, low-fat foods with minimum amount of saturated fats in both developed and developing countries (Huth & Park, 2012; Khorshidian, Yousefi, Shadnoush, & Mortazavian, 2018; Yousefi, Khorshidian, & Hosseini, 2018). In industrial processes for fat-free dairy products, the fat in whole milk is removed to produce low-calorie milk products and vitamin D is removed along with fat separation. Considering the diverse functions of vitamin D and its inadequate intake in many parts of the world makes its restoration or fortification very important. In this article, fortification aspects of vitamin D in dairy products are discussed.

2. Vitamin D metabolism, functions, reference values and deficiency

2.1. Metabolism

Vitamin D is synthesised in the skin or obtained from food (vitamin D_3). Exposure to ultraviolet radiation converts 7-dehydrocholestrol to pre-vitamin D_3 in the skin. For full activation, both endogenous and exogenous (dietary) pre-vitamin D must undergo two steps of hydroxylation in the liver and kidney to form 25-hydroxyvitamin D (25-OHD) (the major circulating isoform) and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) (the most active form) (Holick, 2003; Thacher & Clarke, 2011).

2.2. Functions

1,25-$(OH)_2D_3$ enhances calcium and phosphorous absorption necessary for bone mineralisation (calcaemic functions). Apart from its skeletal actions, vitamin D has some non-calcaemic functions such as the regulating of the cell growth and differentiation, muscle health, cardiovascular health, antimicrobial properties (Neyestani, 2013), regulation of the blood lipid profile (Heravifard et al., 2013) and antioxidative properties (Nikooyeh et al., 2013). Moreover, vitamin D insufficiency is associated with type 1 and 2 diabetes, hypertension, multiple sclerosis, other autoimmune diseases and some types of cancers (Holick, 2004b). Fig. 1 shows vitamin D biosynthesis and functions in the body.

2.3. Daily recommended intakes

The 2011 report on dietary requirement for vitamin D from Institute of Medicine (IOM) suggest that 25-OHD serum levels of 16 ng mL^{-1} (40 nmol L^{-1}) meet approximately half of the population needs and levels of at least 20 ng mL^{-1} (50 nmol L^{-1}) cover at least 97.5% of the population needs [according to the recommended dietary allowance (RDA): average daily level of intake sufficient to meet the nutrient requirements of nearly all (97–98%) healthy people].

The adequate intake [AI; estimates of nutrient intake by a group (or groups) of apparently healthy people who are assumed to be maintaining an adequate nutritional state] in infancy is estimated to be 400 IU d^{-1}. After age 1, the RDA is estimated to be 600 IU d^{-1} for all life-stage groups except men and women aged 71 and older (for whom the RDA is 800 IU d^{-1}; Ross et al., 2011). The revised vitamin D upper intake levels (ULs: the highest daily intake of the nutrient that is likely to pose no risk) for ages 9 and older are 4000 IU d^{-1}, but are lower for infants (1–3 years: 2500 IU d^{-1}) and young children (4–8 years: 3000 IU d^{-1}; Ross et al., 2011).

2.4. Deficiency

Vitamin D status is determined by the levels of serum 25-OHD. Although there is no consensus on optimum levels of this compounds, vitamin D deficiency is defined as 25-OHD levels of less than 20 ng mL^{-1} (50 nmol L^{-1}), while levels of 25-OHD between 21 and 29 ng mL^{-1} (52 and 72 nmol L^{-1}) indicate a relative insufficiency of vitamin D. Finally, levels of 30 ng mL^{-1} or greater are considered adequate levels (Holick, 2008). Nowadays, there are significant rates of vitamin D deficiency/insufficiency, particularly during the winter, in different populations all over the world; one billion people around the world are vitamin D deficient or insufficient (Mithal et al., 2009; Naeem, 2010).

Our ape ancestors probably had adequate vitamin D status, unlike us because of urbanisation, indoor working and environmental condition (air pollution) that cause a remarkable decrease in duration and intensity of the sun exposure and endogenous synthesis of vitamin D. Geographical (high latitude) and cultural (type of clothing, veil) status in many regions worsen these conditions (Holick, 2004a). The epidemiologic evidences linking sun exposure and skin cancer lead to more sun protection creams application and less sun exposure time. These conditions resulted in a lower vitamin D status of the population (Holick, 2004b).

People at risk of vitamin D deficiency are exclusively breastfed infants without vitamin D supplementation, children, pregnant women, old people, homebound people and patients using immunosuppressive drugs. People who live in industrial or polluted cities, and higher latitude; dark skin ones, smokers and women with high body mass index (BMI) will be in an increased risk of vitamin D deficiency (Zahedi-Rad, Nikooyeh, & Neyestani, 2015b).

In Iran, the first National Investigation for Micronutrient Status (NIMS) revealed the high prevalence of vitamin D deficiency in various age and sex subgroups. In Tehran, 95% and 89% of healthy school girls and boys, respectively, and 81.3% of adults had hypovitaminosis D (Hashemipour et al., 2004; Neyestani et al., 2012). Similar data have been obtained in other countries. In studies conducted in India, hypovitaminosis D was reported in 96% of neonates, 91% of healthy school girls, 78% of healthy hospital staff, and 84% of pregnant women (Puri et al., 2008; Sachan et al., 2005). In another survey related to vitamin D status in geriatric patients in France, 92.4% of patients were vitamin D

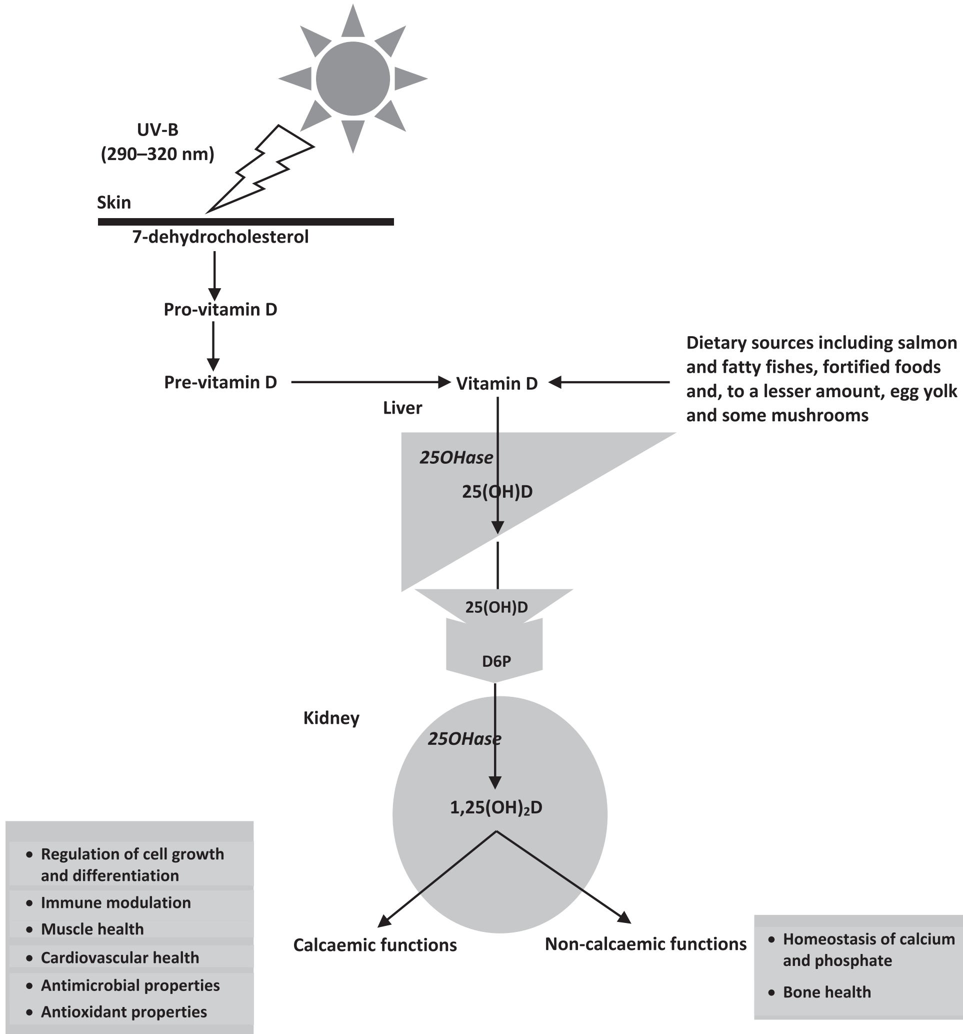

Fig. 1. Vitamin D_3 biosynthesis and functions (Neyestani, 2013).

insufficient, 68.3% had deficiency and 33.7% had severe deficiency (Annweiler et al., 2017). In a study conducted in Netherlands, 8% of the old men and 14.4% of the women were in deficient status, 44.7% of men and 56.1% of women were in insufficient status (Snijder et al., 2005). Vitamin D deficiency was recorded in 75% of young adults and 80% of pregnant women in Toronto and Melbourne, respectively (Gozdzik et al., 2010; Grover & Morley, 2001). In Mexico, Chile and Brazil, vitamin D insufficiency was observed in 67%, 50% and 42% of post-menopausal women, respectively (Lips et al., 2006). In such situations, these deficiencies and insufficiencies should be managed properly and the design and utilisation of suitable strategies are required for improvement of the vitamin D status and intake to meet the dietary vitamin D recommendations.

3. Strategies to promote an adequate vitamin D status of the community

The simplest way of vitamin D intake is the direct exposure to sunlight, but ultraviolet energy is insufficient in winter days, especially in northern latitudes (Webb, DeCosta, & Holick, 1989). Promotion of a healthier lifestyle and weight loss may help in mobilising vitamin D and its metabolites from the adipose tissue have been suggested as the approaches for improvement of vitamin D status, but were not effective attempts. Increasing consumption of naturally containing vitamin D foods was not helpful as well (Pilz et al., 2018). There are limited dietary sources of vitamin D, including cod liver oil and fatty fish such as salmon, which are not commonly used in the diet (Calvo, Whiting, & Barton, 2004) especially in Iran (Kalantari et al., 2005). The best dietary source of this vitamin is oily fish, but it should be consumed at least 3–4 times per week to reach the daily recommended intake, which seems impossible (de Lourdes Samaniego-Vaesken, Alonso-Aperte, & Varela-Moreiras, 2012; Holick & Chen, 2008).

On the other hand, the major source of this vitamin is not food, since limited food sources naturally contain vitamin D or their vitamin is removed during processing. Intake of vitamin D supplements is limited because supplementation is not practiced at the population level (O'Donnell et al., 2008). In many national supplementation programs, lack of supplies and low acceptance rate are the main barriers of success (Allen, de Benoist, Dary, & Hurrell, 2006). However, fortification of foods with vitamin D can be an effective approach and many countries have implemented either mandatory or voluntarily food fortification to eradicate vitamin D deficiency epidemic (Calvo et al., 2004).

According to dietary patterns and fortification policies, foods that have the highest contribution to vitamin D dietary intakes vary from country to country. Studies in different countries (Canada, Finland, France, Ireland, Norway, Spain, Sweden, United Kingdom and United States) show that the main food products used for fortification with vitamin D include cereal-based foods, bread, milk and milk products, soy milk, margarine and fat spreads, egg, fish, vegetable oils and fruit juices (Burgaz, Åkesson, Öster, Michaëlsson, & Wolk, 2007; Calvo & Whiting, 2003; Gahruie, Eskandari, Mesbahi, & Hanifpour, 2015; González-Rodríguez, Estaire, Peñas-Ruiz, Ortega, & Valornut, 2013; Gupta, 2014; Huybrechts et al., 2011; Johnson et al., 2005; Lamberg-Allardt et al., 2001; O'Mahony, Stepien, Gibney, Nugent, & Brennan, 2011; Spiro & Buttriss, 2014; Whiting, Green, & Calvo, 2007).

4. Evaluation of the efficacy of vitamin D-fortified foods

There are a limited number of studies regarding the effective role of food fortification with vitamin D on the improvement of the vitamin D status. Most studies that investigated vitamin D-fortified milk efficacy have been limited to serum 25-OHD level or bone mineral density changes for relatively short time (Calvo et al., 2004). Cross-sectional studies reported that current US and Canadian fortification programs were not effective in preventing vitamin D deficiency, especially among vulnerable populations during winter (Tangpricha, Pearce, Chen, & Holick, 2002; Vieth, 2001).

Shakur, Lou, and L'Abbe (2014) studied milk, yoghurt and cheese fortification with 6.75 μg per serving (270 IU) in Canada and showed that the vitamin D intake would double and dietary inadequacy drop from >80% to < 50% in all groups. This study concluded that dairy product fortification with vitamin D will safely increase vitamin D intake without any toxification signs.

Jayaratne, Hughes, Ibiebele, van den Akker, and van der Pols (2013) studied fortified milk and breakfast cereal effects on Australian adults' vitamin D intake. The study found that the mean vitamin D intake was 4.4 ± 4 μg d^{-1} and it was below the adequate intake for most age and sex groups. Dairy products were on the second level (43%) of contribution to vitamin D intake. This study suggested that if all the milk and breakfast cereals were fortified with vitamin D, mean intake of this vitamin would increase in all age and sex groups from 3.6 ± 2.4 μg d^{-1} to 6.3 ± 3.2 μg d^{-1}.

In Finland, vitamin D deficiency was common during winter (Lamberg-Allardt et al., 2001; Välimäki et al., 2004). The Ministry of Social Affairs and Health therefore recommended adding vitamin D to liquid milks (0.5 mg dL^{-1}), butter and margarines (10 μg 100 g^{-1}) from February 2003. They also studied vitamin D fortification effect on 25-OHD concentrations of Finnish young men. The study showed that after fortification, mean serum 25-OHD concentrations were increased were increased to 40 nmol L^{-1} in winter and vitamin D insufficiency decreased by 50% (Laaksi et al., 2006). Lehtonen-Veromaa et al. (2008) studied Finnish adolescent girls and showed that mean dietary vitamin D intake of 7.5 μg was seen in 91.5% of this demograph in 2000 and 83.8% in 2004. The study revealed no significant changes in serum 25-OHD concentrations during the follow-up period. The prevalence of 25-OHD < 50 nmol L^{-1} was 60.6% in 2000 and 65.5% in 2004. The national vitamin D fortification policy improved the vitamin D status of young Finnish men, but was inadequate for adolescent girls, which might be due to low consumption of fortified foods. Bonjour et al. (2009) studied 71 healthy postmenopausal French women consuming fortified [vitamin D (2.5 μg d^{-1}) and calcium] soft plain cheese. Results showed that fortified cheese increased protein intake and decreased bone resorption markers (Bonjour, Benoit, Rousseau, & Souberbielle, 2012). Bonjour, Benoit, Payen, and Kraenzlin (2013) also conducted a study in elderly institutionalised women consuming fortified yoghurt and observed that daily consumption of two serving (125 g) fortified (10 μg d^{-1} vitamin D and 800 mg d^{-1} calcium) yoghurt increased mean serum 25-OHD levels (25.3 ± 1.8 versus 5.2 ± 2.5 nmol L^{-1}) in the intervention group compared with the control group. Moreover, bone resorption was decreased as a result of consuming vitamin D and calcium fortified dairy products.

Manios Moschonis, and Lyritis (2011) studied postmenopausal women in Greece and showed that consumption of vitamin D3 fortified dairy products (7.5 μg d^{-1} for 12 months and 22.5 μg d^{-1} for last 18 months) maintained the serum 25-OHD level of the intervention group in the months of winter as high as in those of summer. After 30 months intervention, vitamin D deficiency prevalence was higher in the control group than intervention group (60% versus 25%). It was concluded that this level of vitamin D_3 fortification will decrease prevalence of vitamin D deficiency, but it cannot prevent it. Kozłowska-Wojciechowska, Jastrzebska, Naruszewicz, and & Foltynska (2003) studied butter replacement with vitamin D fortified margarine in Polish young men. The study showed that after 4 weeks of consumption of 30 g d^{-1} margarine, 25-OHD concentration was increased by 32.4% and calcium absorption was improved.

Neyestani et al. (2014) studied Iranian school children in Tehran to compare the efficacy of vitamin D plus calcium fortified milk or orange juice (both contain 100 IU vitamin D and 500 mg calcium per 200 mL package) and supplement (contained 200 IU of vitamin D and 500 mg of calcium) consumption for 12 weeks. The study showed that consumption of either supplement, fortified orange juice or fortified milk resulted in an increase of 20.8 nmol L^{-1}, 9.9 nmol L^{-1} or 6.9 nmol L^{-1}, respectively, in the levels of serum 25-OHD compared with control groups. It was shown that consuming 200 IU d^{-1} vitamin D via supplement or 100 IU d^{-1} through fortified products was not effective in the protection of children from hypovitaminosis D during cold seasons. Nikooyeh et al. (2011) investigated the effect of consuming Doogh (the typical Iranian drink based on fermented milk) fortified with vitamin D (500 IU 250 mL^{-1}) or Doogh fortified with vitamin D and calcium (500 IU 250 mL^{-1} and 500 mg 250 mL^{-1}, respectively) for 12 weeks on diabetic patients in Tehran and showed that serum 25-OHD concentrations increased significantly in the intervention group. This study suggested that Doogh was an effective vehicle for vitamin D fortification.

Jafari et al. (2015) reported a significant increase in serum 25-OHD and decrease in parathyroid hormone in postmenopausal women with type 2 diabetes consuming vitamin D fortified yoghurt (2000 IU 100 g^{-1} day^{-1}) or plain yoghurt for 12 weeks. Other positive effects were the improvement of glycaemic markers (except HbA1C), anthropometric indexes, inflammation, and bone turnover markers in the intervention group. In another study on diabetic persons who consumed either plain Doogh (170 mg calcium) or vitamin D3-fortified Doogh (170 mg calcium and 500 IU 250 mL^{-1} vit D) twice a day for 12 weeks, a significant improvement in fasting glucose and other measured indexes in fortified group was observed. Ameliorating vitamin D status was accompanied by improving glycaemic status, lipid profile and endothelial biomarkers in type 2 diabetic subjects. This study suggested that both direct and indirect effects of vitamin D ameliorate the endothelial biomarkers (Shab-Bidar et al., 2011).

5. Dairy products fortified with vitamin D

Natural vitamin D concentrations in whole milk ranges from 0.34 to 0.84 IU g^{-1} of fat and unfortified milk is not a significant source of this vitamin (Murphy et al., 2001). A significant part of natural vitamin D in raw milk is lost during sterilisation or pasteurisation and by removing milk cream in the fat standardisation step (separation process) (Banville, Vuillemard, & Lacroix, 2000). Fortified milk and dairy products are one of the most frequently used sources of vitamin D, because of high consumption, popularity and health benefits (Calvo et al., 2004). Different dairy products

Table 1
Dairy products fortified with vitamin D.[a]

Type of product	Product specification	Form of vitamin D added	Amount of vitamin D added	Vitamin stability in product	Effect on sensory properties of product	References
Bovine whole milk	3.25% fat	–	400 IU L^{-1}			MacDonald (2010)
Natural whole milk	–	–	From 0.34 to 0.84 IU g^{-1} fat			Murphy et al. (2001).
Milk	–	–	400–600 IU qt^{-1}			Murphy et al. (2001); Patterson et al. (2010)
Milk		–	0.5 μg 100 mL^{-1}			Hirvonen et al. (2007)
Fluid milk		–	10 mg (400 IU) or 9.6 mg (385 IU) L^{-1}			Calvo and Whiting (2003)
Fluid milk	3.25% fat (whole), 2% fat, 1% fat	–				Murphy et al. (2001)
HTST-processed milk and UHT-processed chocolate milk	2% fat	100 IU $serving^{-1}$ treatment (1.4 g vit D3 98.59 g^{-1} distilled water) 250 IU $serving^{-1}$ treatment (3.52 g of vit D3 96.48 g^{-1} distilled water)	250 IU $serving^{-1}$ 100 IU $serving^{-1}$	In milk: tolerate sterilisation no loss of vit D reported	No significant sensory characteristics change No composition change	Hanson and Metzger (2010)
Cheddar cheese	Low pH high salt longer storage further thermal processing	Commercial emulsion of vit D Encapsulated vit D; powdered form; oil forms The addition of a commercial water-soluble emulsion of vit D (Vitex D) and homogenisation of crystalline liposoluble vit D in a portion of cream used	400 IU L^{-1}	Stable during both short-term and long-term storage Stable in cheeses about 3–5 months of ripening Entrapped in liposomes cause high recovery		Banville et al. (2000); Ganesan et al. (2011); Kazmi et al. (2007); Wagner et al. (2008)
Processed cheese		Commercial water-soluble emulsion	100 IU $serving^{-1}$ or 28 g based on manufacture	Heated for 5 min in 232 °C: 25–30% lost in pasteurised Process Finally no loss reported during processing and storage at room or refrigeration about 9 mo period at 21–29 °C and 4–6 °C		Banville et al. (2000); Upreti et al. (2002)
Low-fat cheese		Microencapsulation by liposomes is recommended	100 IU g^{-1}	Vit D_3 did not degrade during processing, over 1 year of ripening (3–8 °C), or after thermal treatment at 232 °C for 5 min. Vit D_3 recovery in the fortified Cheddar and low-fat cheeses were, respectively, 91 and 55% of the vit D_3 added to the milk used to make each cheese.	Not alter the chemical composition or flavour and yield	Banville et al. (2000); Wagner et al. (2008)
Lab-scale Cheddar cheese	Unfortified, pasteurised and homogenised milk	Two methods suggested: pre-dissolved crystalline vit D3; emulsified vit D3.	100000 IU mL^{-1}	Loss into whey: 7–9% (w/w). Emulsified form more stable than the crystalline form for over three months		Kazmi et al. (2007)
Pasteurised processed cheese		–		No loss reported over 9 months storage		Kazmi et al. (2007); Upreti et al. (2002)
Hard cheese		–	28000 IU cholecalciferol, equivalent to 4000 IU (100 mg d^{-1})			Wagner et al. (2012)
Low-fat strawberry yoghurt		–	100 IU $serving^{-1}$ (0.80 g vit D 99.2 g^{-1} distilled water) 250 IU $serving^{-1}$ (2.0 g vit D 98 g^{-1} distilled water)	No detectable change during the 42 d storage period at 4 °C (Kazmi et al, 2007)	No differences in the composition and sensory properties.	Hanson and Metzger (2010)
Set-style yoghurt	Unfortified pasteurised homogenised milk	Crystalline emulsified formed	50,000 IU kg^{-1}	Stable for four weeks of storage		Kazmi et al. (2007)
Yoghurts		–	0.5 μg 100 mL^{-1}			Hirvonen et al. (2007)
Doogh		Free form	500 IU in 250 mL			Nikooyeh et al. (2011)

(*continued on next page*)

Table 1 (*continued*)

Type of product	Product specification	Form of vitamin D added	Amount of vitamin D added	Vitamin stability in product	Effect on sensory properties of product	References
					There was no significant change of flavour	
Ice cream		Pre-dissolved crystalline vit D3 Emulsified	Oil emulsion: 100,000 IU kg^{-1}. For crystalline form: 50,000 IU kg^{-1}	No loss		Kazmi et al. (2007)
Butter milk		–	0.5 μg 100 mL^{-1}			Hirvonen et al. (2007)
Household margarines		–	10 μg 100 g^{-1}			Hirvonen et al. (2007)
Margarine		–	Recommended levels 5 mg d^{-1} (200 IU d^{-1}). 10 mg 100 g^{-1} for margarine or spreads.			
Non-fat dry milk		–	10,000 IU lb^{-1} of the product Final 2,000 IU of vit D per pound of product	Retention of vit D was 100%	Without off-flavours	Thomas et al. (1965)

[a] The form of vitamin D was D_3, except Thomas et al. (1965).

have been fortified with vitamin D such as fluid milk, cheese and fermented milks. Table 1 indicates the dairy products fortified with vitamin D. There are regulations supervising vitamin D fortification to prevent its over or under fortification, which are discussed below.

6. Regulations related to vitamin D fortification in dairy products

Vitamin D-fortified milk is defined as milk in which a determined amount of vitamin D (approved by Milk Ordinance and Code) has been added (Public Health Service, 1940). Although there was no defined level of actual vitamin D fortification, the regulation emphasised the monitoring of the levels of vitamin D in the final products by bioassay methods and in a certified laboratory. According to the American Medical Council of Food and Nutrition, vitamin D fortification level is considered as 400 IU qt^{-1} (1 q = 946.4 mL). Vitamin D added to milk based on the Pasteurised Milk Ordinance (PMO) concern in 1978 and the Code of Federal Regulations (CFR) must be 400 IU qt^{-1} or 25% daily value (DV) (100 IU) per a standard 8 oz serving (Murphy et al., 2001). In the United States and Canada, 10 μg (400 IU) or 9.6 μg (385 IU) vitamin D is added per quart or per litre, respectively (Calvo & Whiting, 2003).

Before 1992, it was noted that the acceptable limits for vitamin D fortification must be in the range of ±20% of the label claim. Currently, according to FDA, the acceptable range of the label claim for vitamin D concentrations in fortified milks is 100–150%. Therefore, the final acceptable range is 400–600 IU qt^{-1} (Murphy et al., 2001). New vitamin D standards were established in 1990 to prevent vitamin D over-fortification and to control the label claims (Murphy et al., 2001). Vitamin D over-fortification leads to toxicity, soft tissue damage and renal failure (Koul et al., 2011). On the other hand, under-fortification leads to rickets in children and osteomalacia or osteoporosis in old age. Canadian Food Inspection Agency (CFIA) simplified this problem by saying that all the fortified milks must have vitamin D in a range of 31.7–51.6 IU 100 mL^{-1} (Faulkner, Hussein, Foran, & Szijarto, 2000).

New established regulations include revision of the vitamin D acceptable range in the final product, revision of dairy product authentication standards, and performance of laboratory certification. Adequate capacities are necessary for suitable monitoring of vitamin D concentration. In 1995, newly revised PMO obligated industries to send a representative sample of their dairy product for testing in an FDA certified laboratory by an FDA accepted method (Murphy et al., 2001). The Milk Safety Branch of FDA performs a certification program to certify laboratories if they meet quality control standards and technicians when they test spiked samples correctly. They suggested that all the fortified milk must have vitamin D fortified labels because they are important in human nutrition (Murphy et al., 2001).

Another important issue in vitamin D fortification is the storage of the vitamin preparation. The vitamin preparations are water-based or oil-based; these two kinds of vitamin preparation must be stored in different conditions. The best storage conditions for water- and oil-based vitamins are refrigeration and ambient temperature, respectively. Vitamins stored in unsuitable conditions will degrade before their expiration date and undergo phase separation. Studies showed that 54% of companies keep all vitamin D preparations at ambient temperature and 46% of them keep preparations refrigerated (Hicks, Hansen, & Rushing, 1996).

In some countries such as Germany, vitamin D fortification is forbidden due to the risk of toxicity occurred by exceeding the upper intake levels (UIL) (MacDonald, 2010). In France, fortification can be done if the producer follows the Regulation 1925/2006/EC (European Commission, 2006). Regulation (EC) No 1925/2006 (OJ

L404, p26, 30/12/2006) is provided by the European Parliament on the addition of vitamins and minerals and of certain other substances to foods. Various products such as dairy products, breakfast cereals and vegetable oils are fortified with vitamin D in France. Vitamin D-fortified foods and advertisement is supervised by Regulation 1924/2006/EC (Dhaussy, 2014).

7. Methods related to the vitamin D fortification in dairy products

Different methods have been used for the fortification of dairy products with vitamin D such as animal feed supplementation, direct irradiation and direct addition of vitamin D concentrate to milk (direct way). The direct way is more reliable, effective and well accepted by industries (MacDonald, 2010; Murphy et al., 2001).

Murphy and Newcomer (2001) suggested specific points in vitamin D fortification that should be emphasised: (i) vitamin D concentrates must be added prior to pasteurisation process, (ii) in continuous pasteurisation systems (high temperature, short time: HTST), continuously metered vitamin addition must be used and the metering pump must be connected to HTST control panel to shut down during diverted flow and product recycle mode to ensure that vitamin D is added only in forward flow; (iii) the amount of vitamin used must be recorded and checked with the amount of fortified product to ensure that an accurate amount of concentrate is used; (iv) fortified products must be tested by a FDA certified laboratory.

Vitamin D could be added to products by (i) metered injection or (ii) batch additions (Hicks et al., 1996). The producer can use both of these methods for addition of vitamin D into milk, and if they are used correctly, both have good recovery (Hicks et al., 1996). The point of adding vitamin D concentrate and the method used for addition are related to the kind of vitamin concentrate. Oil-based vitamin concentrates must be added after product standardisation. Water-based vitamin concentrates must be added prior to fat separation (Murphy & Newcomer, 2001). Adding vitamin concentrates after separation/standardisation and before homogenisation will help dispersion and will stabilise the vitamins. Vitamin D is fat-soluble and has the potential to become more concentrated in the fat portion of the milk (Murphy & Newcomer, 2001; Patterson et al., 2010).

For metered addition of vitamins, the following are necessary: (i) sanitary metering positive displacement pumps, (ii) food grade tubing, (iii) quick release, cleanable check valves and (iv) calibrated vitamin reservoirs. It is important to note that once a metering system is fully installed, the pump should be calibrated using the entire delivery system (tubing, check valves, reservoir, etc.). Regardless of whether the system has microprocessor controls or manual settings, all calibrations should be checked using a certified graduate cylinder and a stop watch (Murphy & Newcomer, 2001).

In batch fortification, it is necessary to record the amount of processed milk, the type and the amount of vitamin D concentrate added, the mixing time before pasteurisation and any relevant information (Murphy & Newcomer, 2001). Fortification inconsistencies may lead to variable vitamin D concentration. Standardisation could decrease these variations. Further research is required to ensure optimal fortification practices for each dairy product.

Cream homogenisation and liposome encapsulation are other methods for vitamin D fortification, which are done by direct homogenisation of vitamin D emulsion into milk, using oil- and water-based preparation or a food-grade emulsification base. The best carrier for vitamin D is oil-based preparations (Kazmi, Vieth, & Rousseau, 2007; Upreti, Mistry, & Warthesen, 2002; Wagner et al., 2008).

8. Stability of fortified vitamin D in dairy products

Stability is one of the most important issues in dairy products fortification. The vitamin added must remain active until the end of storage time as the label concentrations claim. Vitamin D_3 has been widely accepted for fortification of dairy products (Greenbaum, 1973; Upreti et al., 2002); results have shown that vitamin D_3 is more stable than other forms of vitamin D during processing and storage in dairy products.

Some researchers reported that vitamin D_3 was susceptible to oxidation, light and acid; others reported its stability to light, heat and oxidation. Some studies reported its instability to irradiation and acid, but stability to oxidation and alkali (Kazmi et al., 2007). Banville et al. (2000) showed that cheese ripening conditions (vacuum packaging and low temperature in dark storage) protect vitamin D_3. However, studies have provided contradictory results and it appears that vitamin D stability is strongly dependent on the matrix as well as the environmental conditions, especially during storage (Kazmi et al., 2007).

Vitamin D was stable in ordinary thermal applications of sterilisation at 115.6 °C for 15 min or pasteurisation at 62.8 °C for 30 min; at higher temperatures such as 232.2–260 °C, Vitamin D is unstable (Weckel, 1941). Inconsistencies between claimed and measured values in vitamin D content may be associated with chemical breakdown and/or processing errors. The stability of vitamin D in different dairy products is discussed below.

8.1. Fluid milk

Because of high consumption, liquid milk is regarded as an important carrier for vitamin D fortification (Fulgoni et al., 2007; Koushki, Koohy-Kamaly, Azizkhani, & Hadinia, 2016; Moore, Murphy, Keast, & Holick, 2004; Patterson et al., 2010). In this way, milk can be considered as one of the dietary sources of vitamin D. In the 1990s, it was stated that for 70% of the milk of Canada and the United States, vitamin D was 80% below the label claims and 14% of the skim milk had no vitamin D (Holick, Shao, Liu, & Chen, 1992; Kazmi et al., 2007).

Determination of vitamin D_3 in 104 fluid milk samples purchased from retail markets in Canada showed 54% under-fortification and 4% over-fortification with significant variation in the vitamin D_3 among milks from different brands. Furthermore, skim milk was fortified with four types of vitamin D_3 formulations and their stability to light exposure of 2000 Lux light showed a loss in the range of 37–71% after 22 days of storage at 4 °C (Liu, 2013).

Tanner et al. (1988) analysed skim-, 2% fat-, and whole milk as well as high fat- and half-and-half whipping cream. In skim and 2% fat milk samples, vitamin D was less than the label claim. It was pointed out that vitamin D added to the raw milk before processing may result in over-fortification of high-fat milk and under-fortification of low-fat milk (Patterson et al., 2010; Tanner et al., 1988).

In one study, vitamin D-fortified milk samples (non-fat milk, milk with 1% fat, milk with 2% fat, whole milk and chocolate milk with 1% fat) were collected from 24 supermarkets in the United States; although the vitamin D_3 level was not different between the different kinds of milk, it was variable from non-detectable in one sample to 800 IU qt^{-1} in another (Patterson et al., 2010). Jakobsen and Saxholt (2009) showed that the type of product and sampling season affected the vitamin D level in the milk. Comparing the effect of fat content on vitamin D in semi-fat milk (1.5% fat), whole milk (3.5% fat), cream (9.13% fat and 38% fat) and butter (80% fat) showed that the vitamin D level was significantly related to the fat content. The determination of vitamin D in milk available in the

Arabic United Emirates showed a recovery of 89–105% of added vitamin D to milk (Laleye, Wasesa, & Rao, 2009).

Several studies have demonstrated good stability of vitamin D_3 in fluid milk during storage. However, its stability might be significantly affected by type of packaging, temperature–time combinations of heat treatments, especially in the presence of oxygen, light exposure during storage as well as the fat content of the milk. In a study by Murphy et al. (2001), vitamin D_3 levels were analysed in four types of milk including whole fat, 2% fat, 1% fat milk and non-fat milk and it was observed that only 47.7% of 648 samples were within the acceptable range for vitamin D_3. In another study, high-temperature short-time (HTST)-processed milk (2% fat) and UHT-processed chocolate milk (2% fat) were fortified with 100 and 250 IU per serving vitamin D_3 and stored for 21 and 60 days, respectively. No loss of vitamin D_3 was reported during the process and shelf life. Thus, it was possible to increase the fortification level without loss of vitamin (Hanson & Metzger, 2010).

8.2. Cheeses

Consumption of fluid milk products has gradually declined in the last 20 years (Tanner et al., 1988), and lactose intolerance is a common problem for the elderly (Ryan, Eleazer, & Egbert, 1995). This heightens interest in achieving vitamin D fortification in other dairy products, especially cheese. Cheese is a dairy product that is widely consumed in various forms across different populations. It is a universal product and its production is expected to grow in the future due to an increase in the purchasing power of the populations and developments of new cheese varieties (Banville et al., 2000). Cheese such as Cheddar has only trace amounts of lactose and is a best source of calcium for peoples with lactose intolerance and the best choice for those who do not drink milk. Furthermore, the cheese per capita consumption has increased since the 1980s and, in the future, this increase is expected to be continued (Wagner, Sidhom, Whiting, Rousseau, & Vieth, 2012).

Generally, vitamin D_3 appears to be stable in cheese during both short-term (Banville et al., 2000) and long-term storage (Ganesan, Brothersen, & McMahon, 2011; Kazmi et al., 2007; Wagner et al., 2008). Cheese milk fat enhances vitamin D stability and absorption (Wagner et al., 2012). Recent fortification research focused on vitamin D_3 added to the cheese. Cheese can be relatively easily fortified with vitamin D_3 to provide up to 100% daily value in the diet as its fat content is higher than liquid milk, which helps inclusion of fat-soluble vitamins (Ganesan et al., 2011). Thus, it has been defined as an ideal vehicle for vitamin D in most research.

Cheddar cheese is one of the most popular cheeses in North America. It is rich in vitamins A and B (Ganesan et al., 2011; Kazmi et al., 2007; Upreti et al., 2002; Wagner et al., 2008); its fortification with vitamin D_3 would increase its acceptance as a nutritious food that is rich in protein, vitamins, and minerals. Studies have shown that about 85–90% of vitamin D_3 added into Cheddar cheese is effectively retained (Kazmi et al., 2007; Wagner et al., 2008). However, there are studies in which this vitamin stability ranged between 40 and 50% (Banville et al., 2000). Differences in stability are likely due to the different forms of vitamin D used for fortification, matrix differences (e.g., pH, salt content and microflora), further thermal processing, and storage time. The ability of bacteria to metabolise vitamin D_3 is not determined, but it may contribute to a reduction (or increment) of fortified vitamin D_3 levels (Banville et al., 2000).

In one study (Ganesan et al., 2011), Cheddar cheese was fortified with vitamin D_3 (150 IU per serving) in powdered, oil or emulsified form, with or without homogenisation. When this fortified milk was used for cheese making, 80% of vitamin D_3 was retained independent of homogenisation or of the form of vitamin D_3. In another phase of the study, Cheddar cheese was fortified with 200 and 400 IU per serving with emulsified vitamin D_3 to evaluate vitamin D_3 stability and cheese flavour change. It was shown that vitamin D_3 was stable for 9 months and flavour did not change and was accepted by the consumers (Ganesan et al., 2011). In contrast, some studies found that vitamin D_3 levels were reduced during aging.

In a study by Banville et al. (2000), fortification of Cheddar cheese with vitamin D_3 using three different addition methods (a commercial water-soluble emulsion of vitamin D, Vitex D); homogenisation of crystalline liposoluble vitamin D in a portion of cream used for cheesemilk standardisation; and addition of water-soluble vitamin D entrapped in multilamellar liposomes) at a final concentration of 400 IU L^{-1}, showed variation in stability. This study showed that the vitamin D_3 recovery was higher in the case of liposome-entrapped vitamin D_3 (61.5%) than for vitamin D_3 homogenised in cream (40.5%) and for Vitex D_3 (42.7%). Vitamin D_3 concentration was stable for 3–5 months of ripening depending on the addition method, but decreased afterwards, especially in cheeses containing liposome-encapsulated vitamin D_3. After 7 months of ripening, vitamin D_3 concentration showed a decrease of 40, 11 and 16% for cheeses containing liposomes, vitamin D_3 in cream and Vitex D_3, respectively. This reduction may be due to the destruction of vitamin D_3 during Cheddar cheese manufacturing, possibly because the fermentation by lactic acid bacteria, acidification, and oxidation. Vitamin D_3 fortification causes no change in milk or whey composition during Cheddar making. Fortification had no effect on Cheddar cheese flavour. After three months of ripening, the vitamin D_3 recovery in Cheddar cheese and low-fat Cheddar cheese was 91 and 55%, respectively (Banville et al., 2000). Remaining vitamin D_3 was extracted in whey; these results were similar to that of Kazmi et al. (2007). It was shown that vitamin D_3 was not destroyed during cheese making, because all of the added vitamin D_3 was recovered. Vitamin D_3 retention in Cheddar cheese was better than in low-fat Cheddar cheese because of 5 fold higher fat content in this type of cheese (Banville et al., 2000).

Three different preparations of vitamin D_3 for cheese fortification: a commercially available emulsion of vitamin D_3, an encapsulated vitamin D_3 preparation in powdered form and oil forms were compared in the study by Ganesan et al. (2011). Each preparation contained 400 IU L^{-1} vitamin D_3, which was added to cheese milk by two methods: (i) mixing directly in the cheese milk; or (ii) homogenising the preparation in 10% of the cheese milk. Mixing the powder directly in the cheese milk was the best method, but a proportion of vitamin D_3 added to the cheese milk was lost in the whey. When vitamin D_3 preparation was homogenised in the milk before adding to cheese vat, the retention of vitamin D_3 in cheese became consistently more than 95% (Ganesan et al., 2011). Experiments with powder and emulsion forms showed that the emulsion form was more stable than the powder form when they were added before renneting (Banville et al., 2000; Ganesan et al., 2011).

For manufacturing Cheddar-like cheese, bench top-scale methodology and pre-dissolved crystalline vitamin D_3 and emulsified vitamin D_3 were used by Kazmi et al. (2007). The result illustrated that the loss of vitamin D_3 into whey was 7–9% (w/w). The stability of the vitamin in the cheese was dependent on different forms of vitamin D_3 used. The emulsified form was more stable than the crystalline form during three months of ripening.

According to some previous studies, fortification of natural cheeses such as Cheddar cheese with vitamin D_3 to consistent levels is difficult (Banville et al., 2000). Processed cheese is therefore a better alternative, because it can be easily fortified and the fortified vitamin D_3 is distributed evenly in the mass. For processed cheese, fortification with commercial water- or fat-dispersible forms of vitamin D_3 could be used at a level of 100 IU per serving or 28 g based on manufacturing protocol (Upreti et al., 2002). There were no differences between water and fat-dispersible forms of vitamin D_3 in fortified cheeses. One of the major problems was 25–30% vitamin loss in pasteurised processed cheeses, when heated for 5 min at 232 °C. The lower recovery after heat treatment is due to the vitamin destruction or its binding to protein or entrapment. It should be mentioned that one of the special applications of pasteurised processed cheese is in ready-to-eat foods; these foods are baked at high temperatures to melt the cheese. This vitamin could uniformly distribute in the structure of cheese because of the vigorous mixing. Finally, there was no loss of the vitamin D_3 during processing and storage at room or refrigeration temperatures (21–29 °C and 4–6 °C) during about 9 months period (Upreti et al., 2002). When low-fat cheese was fortified with vitamin D_3, which was more stable and potent compared with vitamin D_2, results showed that vitamin D3 was recovered in the cheese and the whey (Wagner et al., 2008). A high amount of vitamin D3 (45% of the vitamin D_3 added to the milk) was lost in the whey, because it partitioned into the fat (0.11%, w/w) that is present in the whey. It was illustrated that the protective fat matrix for vitamin D3 is lost during production of fortified low-fat cheese (Wagner et al., 2008).

Some studies suggested that vitamin D_3 may be stabilised by binding to whey proteins, especially β-lactoglobulin (Fenelon & Guinee, 1999; Wagner et al., 2008, 2012). Other studies indicate that vitamin D_3 is stable in fortified low-fat cheeses during processing, even after high temperature heating of the cheeses to 232 °C for 5 min and over 1 year ripening. Therefore, a manufactured low-fat cheese was realised as a suitable product for vitamin D_3 fortification (Wagner et al., 2008).

8.3. Fermented milks

In the United States, per capita consumption of fluid white milk decreased continuously in the last 50 years, as well as vitamin D intake (Hanson & Metzger, 2010). The idea of fermented milk fortified with vitamin D is growing; fermented milks and their products could be used as another vehicle for supplying consumers' vitamin D. This fortification can compensate reduction of milk consumption (Hanson & Metzger, 2010). Yoghurt is the most common fermented milk product consumed in the world (Remeufa, Mohammed, Sodini, & Tissierb, 2003). Fortification techniques used for fluid milk can also be used for flavoured milk and yoghurt (Hanson & Metzger, 2010; Heydari et al., 2018). Generally, vitamin D is stable in different fermented milks during processing and storage (Kazmi et al., 2007; Renken & Warthesen, 1993; Upreti et al., 2002; Wagner et al., 2008). Yoghurt fermentation did not affect vitamin D and it was stable during 4 °C storage (Kazmi et al., 2007).

In research by Kazmi et al. (2007), incorporation of 50,000 IU kg^{-1} vitamin D_3 in two forms (crystalline and emulsified) into yoghurt was evaluated with respect to recovery and stability. The result showed that there were no detectable recovery differences between these two forms of vitamin D_3. The level of vitamin D_3 recovery relative to that initially added (50,000 IU kg^{-1}) was $96.6 \pm 0.5\%$ and $97.8 \pm 0.5\%$ for the crystalline and emulsified forms, respectively. The vitamin D content remained stable during milk acidification and yoghurt storage at 4 °C. Acid production had small effect (small loss of ~3%) on vitamin D_3 stability in yoghurt. The study showed that vitamin D_3 was stable in yoghurt stored for four weeks, so this product can be used as vitamin D-fortified product (Kazmi et al., 2007). In an Iranian study, vitamin D_3 and calcium were added to the Doogh (typical Iranian drink based on fermented milk) at level of 500 IU vitamin D_3 in 250 mL of the final product. Measuring vitamin D_3 concentration during the study period (12 weeks) showed that vitamin D_3 was stable in Doogh. Sensory evaluation tests showed that vitamin D fortification did not significantly change fresh and stored product flavour (Nikooyeh et al., 2011).

8.4. Other dairy products

Other dairy products that can be fortified with vitamin D include ice cream, butter and margarines. Ice cream can be a good choice because it is popular among people who do not drink milk. There are limited data concerning ice cream feasibility as a vehicle for vitamin D_3 fortification. There was no significant loss of vitamin D_3 during the ice cream manufacturing process (Kazmi et al., 2007). There was no vitamin D_3 decomposition after 4 weeks storage at −25 °C. Vitamin D_3 retention was 98–100%. This result was interesting because vitamin D_3 is sensitive to both air and light, each of which can destroy this vitamin (Renken & Warthesen, 1993), and 50% of ice cream volume is air. It can be explained by the low temperature of the process and storage dramatically slowing down any reaction related to vitamin D breakdown.

Butter, margarine, dairy fat spreads and butter milk are other dairy products that can be fortified with vitamin D. In Finland, all retail milk and margarine are fortified with vitamin D (e.g., 0.5 μg 100 g^{-1} of milk and 10 μg 100 g^{-1} to margarines) (Hirvonen, Sinkko, Valsta, Hannila, & Pietinen, 2007).

Non-fat dried milk is another choice for vitamin D fortification (Thomas, Coulter, & Kudale, 1965). Confirmatory data have reported that the level of vitamin D fortification can be as high as 10,000 IU of vitamin D_2 per pound of the product, without encountering unpleasant off-flavours in the freshly made product. However, there was significant flavour deterioration (stale flavour) during storage for one month at 100 °F (~38 °C) and three months at 70 °F (~21 °C), associated with the interaction between lactose and protein. In all batches, the retention of vitamin D was 100% (Thomas et al., 1965). The study showed that measured losses during pasteurisation, high pressure evaporation and drying process were not significant (Indyk, Littlejohn, & Woollard, 1996).

9. Effects of vitamin D fortification on sensory characteristics of dairy products

A limited number of studies have documented the effect of vitamin D fortification on cheese flavour (Wagner et al., 2008). Banville et al. (2000) discovered that flavour perception of vitamin D_3-fortified Cheddar cheese was similar to that of unfortified cheese through 2 months of aging. Ganesan et al. (2011) found that vitamin D fortification was not related to flavour development and other sensory profile parameters. There were no significant sensory differences (especially flavour attributes) among vitamin D_3-fortified products of low-fat strawberry yoghurt, HTST-processed with 2% fat, UHT-processed with 2% fat, chocolate milk, ice cream, and margarine compared with their controls, irrespective of fortification levels (e.g., 100 or 250 IU) (Ganesan et al., 2011; Hartman, Dryden, Webb, Johnson, & Alford, 1974; Kazmi et al., 2007; Renken & Warthesen, 1993). It is reported that yoghurt fortification with vitamin D_3 might decrease the textural quality of final product, probably due to minor changes occurring in fermentation characteristics or the effects of stirring before packaging (Hanson & Metzger, 2010).

School children's acceptance of vitamin D_3 and calcium fortified milk was assessed in Tehran (Neyestani et al., 2012). This study evaluated 212 school children and showed that in 44.4% of students, the taste of fortified milk was scored as bad, but in compliance study, 85% of children were satisfied with the taste of fortified milk. Only less than half of the students agreed with fortification program. The study showed that calcium and vitamin D_3 fortification results in unpleasant changes of milk taste and is a challenge in fortification program (Neyestani et al., 2012). Low-sugar, calcium/vitamin D_3-fortified orange juice may be a suitable replacement for children who have milk intolerance. The high acceptability of orange juice among children makes it a good candidate for further fortifications (Neyestani et al., 2014).

10. Role of packaging in stability of vitamin D in dairy products

Vitamins in milk and dairy products are sensitive to light oxidation and this process can cause significant losses of vitamin A, B_2, C, E and D (Mestdagh, De Meulenaer, De Clippeleer, Devlieghere, & Huyghebaert, 2005; Saffert, Pieper, & Jetten, 2008). Studies showed that vitamin D in milk is normally stable to oxygen (especially at low temperatures) in the absence of light, but the main damage occurs in light and oxygen exposure. This can be explained by oxidation reactions in which light acts as catalyst (Banville et al., 2000). Therefore, photooxidation control is a key influence in dairy product quality and packaging technology can play a vital role. So, preserving vitamins in the product matrix from oxygen and light during storage period is important. With improper packaging techniques, considerable amounts of molecular oxygen may enter into the matrix. Thus, it is necessary to select suitable materials (type, diameter, portion and opacity) as well as suitable packaging machines for packaging (Mestdagh et al., 2005).

There are few studies in which vitamin D stability in dairy products packed with different packaging materials with a different transmittance of light is investigated. In one study, the changes in vitamin A, B_2 and D_3 contents of UHT whole milk packed in PET bottles with different pigmentation levels during 12-weeks storage period was investigated (Saffert et al., 2008). The impact of light on vitamin A, B_2 and D_3 stability was established, based on comparison between control samples that were stored in dark place and light-exposed samples. The results of the research showed that the pigmented PET bottles were not efficient to exclude light transmittance when the product was stored under light. The losses of vitamin D_3 in clear PET bottles were obvious for the wavelengths more than 320 nm (critical point), in which the concentrations of vitamin A and D_3 reduced at least by half (Saffert et al., 2008). Therefore, only the suitable pigmentation (colour, lightness and concentration) associated with each vitamin (or vitamin mix) could be efficient in maintaining the stability of vitamins in dairy products exposed to light.

It was reported that vitamin D was stable over 7 days storage of milk packaged in plastic and glass bottles in 4–7 °C in refrigerator (Kaushik, Sachdeva, Arora, & Gupta, 2015). Storing the milk in polyethylene packages for 7 days in a refrigerator decreased vitamin D levels and it was concluded that vitamin D is absorbed by polyethylene. Evaluating vitamin D loss in three different light intensities showed that during 32 h storage, loss was observed in polyethylene package, but there was no loss in glass packages (Kaushik et al., 2015). The effect of light transmittance in packaging on vitamin D stability in UHT whole milk was studied by Saffert et al. (2008). They stored milk at three different light intensities (790, 1590, 3080 lux) in polyethylene terephthalate (PET) bottles with different light transmittance at 23 °C for 12 weeks. The study showed that vitamin D loss in clear PET bottles was independent of the light intensity. In pigmented PET bottles, only the increase in light was found to be of relevance. Vitamin D was stable in samples stored in the dark (Saffert et al., 2008). In another study, Saffert, Pieper, and Jetten (2009), stored UHT low-fat milk under light with an intensity of 700 lux in PET bottles for 12 weeks at 23 °C. The study showed that in clear PET bottles, a 66% reduction in initial content of vitamin D was observed. In all pigmented PET bottles, the vitamin retention was only slightly higher and the vitamin D losses ranged between 63 and 95% depending on the pigmentation level. Vitamin concentration was stable in control samples stored in a dark place.

11. Conclusions

Vitamin D is one of the essential nutrients maintaining blood calcium and phosphorus concentrations within a narrow physiological range by increasing their absorption from the intestine and enhancing their renal reabsorption. Given the undesirable vitamin D status in different populations, fortification of food with vitamin is inevitable. Dairy products are one of the best choices for vitamin D fortification because they are healthy, highly consumed and the main source of calcium, vitamin D is stable in most of them and its addition does not significantly affect the sensory characteristics of products. Different studies indicate that diverse types of dairy products such as fluid milk (HTST-processed milk, UHT-processed) in plain or flavoured form, plain and flavoured yoghurts as well as yoghurt drinks, different kinds of cheeses (e.g., Cheddar and processed) and to a lesser extent, ice cream, butter, dairy fat spreads and margarine can be suitable for fortifying with vitamin D. Vitamin D might be added into these products in emulsified, crystalline or encapsulated (e.g., liposome) forms. Generally, vitamin D in dairy products can be stable during production, storage and the shelf life period. Future research regarding the fortification of dairy products with vitamin D might be focused on the formulation of new products, stabilisation of this vitamin in products with long shelf life, inventing new methods of industrial fortification from stability and convenience standpoints as well as development of suitable packaging systems.

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