Original Article

Stability of Vitamin D_3 in fortified yoghurt and yoghurt drink (Doogh)

Tina Jafari, Gholamreza Askari, Maryam Mirlohi[1], Shaghayegh Haghjooy Javanmard[2], Elham Faghihimani[3], Aziz A Fallah[4]

Departments of Community Nutrition and [1]Food Science and Technology, Food Security Research Center, School of Nutrition and Food Science, [2]Department of Physiology, Applied Physiology Research Center, [3]Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, [4]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Abstract

Background: Vitamin D deficiency and insufficiency are recognized as a worldwide problem with serious consequences. Fortification of foods with Vitamin D is a certain approach to improve serum Vitamin D status if the stability of vitamin in the foodstuffs was controlled. The purpose of this study was to examine the stability of Vitamin D_3 added to low-fat yogurt and yogurt drink "Doogh" during the products shelf-life.

Materials and Methods: Two kinds of Vitamin D_3, water- and oil-dispersible forms, suitable for food fortification, were compared to find out whether they show different stability in the products. The products were packed in opaque or translucent containers. The content of Vitamin D_3 was determined by high performance liquid chromatography method.

Results: Vitamin D was not affected by the heat treatment (pasteurization) and other processes (homogenization and fermentation). Both water- and oil-dispersible forms were stable during the shelf-life of yogurt samples packed in opaque containers. The Vitamin D_3 content of yogurt fortified with water-dispersible form and packed in translucent containers was not stable during the shelf-life and significantly reduced after 1, 2, and 3 weeks of storage compared to the day 0. The Vitamin D_3 content of samples fortified with the oil-dispersible form packed in the same container was only stable after 1-week and significantly reduced after 2 and 3 weeks of storage. The Vitamin D_3 content of Doogh packed in the opaque containers remained stable during the shelf-life while it was not stable in the samples packed in translucent containers.

Conclusion: The results suggested that both forms of Vitamin D are suitable for fortification, and opaque container is a better choice for packaging of the product.

Key Words: Doogh, fortification, Vitamin D_3, yoghurt

Address for correspondence:
Dr. Elham Faghihimani, Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: efimani@yahoo.com
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INTRODUCTION

Besides to its important role in calcium homeostasis and bone functions, Vitamin D is known to have many extra-skeletal effects like anti-inflammatory and immunomodulatory functions.[1] Body of evidence is increasing about the influence of Vitamin D in several diseases such as obesity,[2] cardiovascular disease,[3]

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autoimmune disorders,[1] metabolic syndrome,[4] some forms of cancers,[5] diabetes,[2] and microbial infections.[6] Vitamin D deficiency and insufficiency are recognized as a worldwide problem with serious consequences. It is estimated that near 1 billion people around the world suffer from various degrees of Vitamin D deficiency.[7] Studies have demonstrated that Vitamin D deficiency is not only high in North America and Northern Europe but also people in the sunniest areas of the world like Mediterranean and Middle East countries suffer from the deficiency or insufficiency.[8-10] It might be due to avoidance of exposure to the sun because of environmental, psychological, and cultural factors.[7,11]

People get Vitamin D from 4 sources: Sun exposure, natural food sources, dietary supplements, and fortified foods.[12] The major source of Vitamin D is exposure to sun when 7-dehydrocholesterol in the skin converts to pre-Vitamin D_3 under the penetration of solar ultra-violet B. However, cutaneous synthesis is affected by several factors such as season, latitude, day-time, skin pigment, widespread use of sunscreens, and aging.[10] The richest dietary sources of Vitamin D are fatty fish, fish liver oil, egg yolk, and sun-dried mushrooms, which are not commonly consumed in many diets.[13] Currently used foods are naturally poor in Vitamin D content.[14,15] It has been estimated that people cannot earn > 2 μg (80 IU)/day of Vitamin D from dietary intake while according to Institute of Medicine recommendation, adults aged 19–50 years require at least 15 μg (600 IU)/day of Vitamin D to improve bone functions.[16] Hence, oral intake of Vitamin D via fortified foods or supplements seems necessary. Subjects who use supplements currently, tend to be old, white, female, educated and usually have a good income.[17] Although Vitamin D supplementation improves serum 25-hydroxy Vitamin D (25-OHD) concentration significantly,[18] it is not a suitable way to enhance Vitamin D status of the general public as a whole. Hence, dietary intervention seems to be inevitable.

Fortified foods are defined as foods, which are enriched with one or more essential nutrients (whether or not they might have been existed in the food naturally) for the purpose of preventing or treating a demonstrated deficiency.[19] In the third decade of 20th century, rickets was eradicated by fortification of dairy products specially milk with Vitamin D.[13] Today, fortification of foods with Vitamin D has been accepted as a certain approach to improve serum Vitamin D status. Canada and United States are famous countries that fortified some foods such as dairy products, margarine and breakfast cereals mandatorily or optionally.[11] Milk is the most commonly food source for Vitamin D fortification, but it seems that consumption of milk has gradually declined during the past decades.[11,20] Moreover, considering the high prevalence of lactose deficiency, many children and adults avoid to drink it continually.[21] Hence, research are going on to introduce more sources of food that seems to be feasible for fortification with Vitamin D. The stability of Vitamin D is reported in bread,[22] orange juice,[13,23] cheddar cheese, ultra-violet light-enhanced mushrooms,[24] and edible oils.[10]

In Iran, fortification of foods with Vitamin D is not customary, and there is not a definite Vitamin D-fortified staple food that the general public can earn currently. Vitamin D-fortified milk is the most common product, which is not available every time or everywhere. Recently, Nikooyeh *et al.*[25] demonstrated that fortification of Persian Doogh (a yogurt drink consist of plain yogurt, water, and salt) with Vitamin D, improves 25(OH) D levels in type 2 diabetic patients. Yogurt is suggested as a good candidate for Vitamin D fortification in Iran because of (i) its popularity and widely consumption, (ii) its milk fat basis which is important for Vitamin D stability and absorption,[26] (iii) its calcium content, and (iv) its well tolerance by people who suffer from lactose deficiency. Moreover, individuals in any level of the economic situation seem to be able to provide it usually. Therefore, this study was aimed to determine the stability of different forms of Vitamin D_3 in yogurt and Persian yogurt drink (Doogh) packed in opaque and translucent containers.

MATERIALS AND METHODS

Experimental design

Two forms of Vitamin D_3, water- and oil-dispersible, were used for fortification of yogurt at doses of 90 and 120 IU/100 g product. Doogh was only fortified with water-dispersible form because of its watery nature, at 90 and 120 IU/100 g product. The prepared products were packed in opaque and translucent containers, and stored in a retail display case illuminated with 1000 lux fluorescent light during the shelf-life. Yogurt was stored at 4°C for 21 days while Doogh was stored at 15°C for 8 weeks. The yogurt samples were analyzed for Vitamin D stability at day 0, 7, 14, and 21 of storage while Doogh samples were analyzed at day 0, and week 2, 4, and 8 of storage. For each treatment, 3 batches were prepared, and 3 samples from each batch were analyzed.

Chemicals and reagents

All of the used chemicals and reagents were of analytical or high performance liquid chromatography (HPLC) grade and obtained from Merck (Darmstadt, Germany). Crystalline Vitamin D_3 (cholecalciferol, purity ≥98%) reference standard was obtained from Sigma-Aldrich (St. Luis, Mo, USA). Vitamin D_3 oily solution (25 mg cholecalciferol/g) and cold water-dispersible powder of Vitamin D_3 (2.5 mg cholecalciferol/g) were obtained from DSM Nutritional Products Ltd., (Basel, Switzerland). Deionized water (Milli-Q Millipore 18.2 MΩ resistivity) was applied through this study.

Stock solution of Vitamin D_3 (100 μg/ml) was prepared by dissolving the crystalline standard in acetonitrile. This solution was diluted in acetonitrile to prepare the intermediate standard solution of 10 μg/ml. Working standard solutions were prepared by diluting of intermediate standard solution in acetonitrile.

Preparation of fortified yogurt

Low-fat milk was prepared by adding 1.5% (w/v) of milk fat to the skim milk. The water-dispersible form of Vitamin D_3 was added to the low-fat milk in 2 different concentrations to prepare milks with 90 and 120 IU Vitamin D_3/100 ml. To prepare the oily form of Vitamin D_3 for fortification, the adequate amounts of this form were blended with milk fat and added to skim milks to prepare the low-fat milks (1.5% fat), which were contained 90 and 120 IU Vitamin D_3/100 ml. After homogenization at a pressure of 150 bar (APV 1000 laboratory homogenizer), the milks were pasteurized and then inoculated with a starter culture at 40–42°C for about 7 h. When the pH declined to ~4, the process was stopped and final product "low-fat yogurt" was packed and stored at 4°C.

Preparation of fortified Doogh

The yogurt was prepared from skim milk as described above. Milk fat, water, and salt were added to the product. To prepare the fortified product with water-dispersible form of Vitamin D_3, the powder was added with the other ingredients. The materials were then homogenized at a pressure of 185 bar (APV 1000 laboratory homogenizer) to prepare the final product "Doogh" with the following characteristics: Fat 1.5%, total solid content 5%, and NaCl 0.5%. The prepared samples were packed and stored at 15°C.

Vitamin D analysis

A portion of 15 g of a sample (yoghurt or Doogh) was diluted and homogenized with 20 ml of water. The homogenized sample, 35 ml of aqueous potassium hydroxide solution (35% KOH w/v), and 2 ml of ethanolic pyrogallol solution (1% w/v) were transferred into a foil covered 125 ml glass-stoppered low-actinic erlenmeyer flask, mixed gently, and flushed with nitrogen to remove oxygen. In order to saponify the mixture, the flask was capped and placed in a water bath at 70°C for 30 min with occasional stirring. The saponified sample was cooled in an ice-water bath for 15 min and extracted with ethanol and a mixture containing petroleum ether: Diethyl ether (90:10, v/v) according to the method described by Renken and Warthesen (1993). The ether extract was evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 3 ml of hexan and cleaned on a solid phase extraction (SPE) column (Silica Spe-Pak C18 Vac Cartridge; Waters Corporation, Dublin, Ireland), which was previously conditioned with 2.5 ml of hexane. The SPE column was successively washed with 2 ml of hexane, 2 ml of a mixture containing chloroform: Hexane (78:22, v/v), and eluted with 2 ml of methanol. The methanol rinsed eluate containing Vitamin D_3 was collected and evaporated to dryness under a stream of nitrogen at 40°C. The remaining residue was dissolved in 1 ml of acetonitrile. The sample was filtered through a 0.45 μm syringe filter prior to analysis.

Vitamin D_3 was quantified using an Agilent 1260 Infinity HPLC System (Agilent Corporation, USA) equipped with a quaternary pump, and auto-sampler, a vacuum degasser, and a G1315D diode-array detector set at an absorbance wavelength of 254 nm. Chromatographic separation was performed on a VYDAC® reverse-phase C18 analytical column (250 mm × 4.6 mm i.d., 5 μm particle size, 300 A° pore size; Vydac Separation Group, Hesperia, CA, USA). The HPLC mobile phase was acetonitrile: Methanol (70:30, v/v) at a flow rate of 1.5 ml/min. The injection volume of the sample or standard solutions was 100 μl. The mean recoveries of Vitamin D_3 in spiked yoghurt and Doogh samples (n = 5) were 79.9% and 83.5% for spiking level of 90 IU/g of product, and 83.7% and 88.3% for spiking level of 120 IU/g of product, respectively. The relative standard deviations were <10%. The results were corrected based on recovery scores.

Statistical analysis

To evaluate the storage stability of Vitamin D_3 in the fortified products, the multifactorial repeated measure ANOVA from SPSS software version 20 (SPSS Inc., Chicago, IL, USA) was used. The difference among means at $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Stability of Vitamin D_3 in milk

Vitamin D_3 fortified milk used for preparation of yogurt was analyzed for the content of Vitamin D_3 before and

after pasteurization. The results demonstrated that there was no significant Vitamin D_3 loss ($P > 0.05$) due to the pasteurization either for water- or oil-dispersible form (data not shown). It was stated that Vitamin D was not affected by heat treatments, that is, pasteurization and sterilization used in dairy industries.[27-29]

Multivariate tests

The results of multivariate tests showed that the content of Vitamin D_3 significantly changed ($P < 0.001$) during the products shelf-life. The interaction between time and packaging type was statistically significant ($P < 0.001$), whereas the interaction between time and Vitamin D_3 type was not statistically significant ($P > 0.05$); [Table 1]. The tests of between subject effects showed that about 50% and 80% of changes in Vitamin D_3 contents in yogurt and Doogh, respectively, was due to the packaging type (data not shown).

Stability of Vitamin D_3 in yogurt

Stability of Vitamin D_3 in fortified yogurt during 3 weeks storage at 4°C is shown in Table 2. Both water- and oil-dispersible forms were stable during the shelf-life of yogurt samples packed in opaque containers because no statistically significant differences were observed in Vitamin D_3 contents during 3 weeks of storage. The Vitamin D_3 of yogurt fortified with water-dispersible form packed in translucent containers was not stable during the shelf-life and significantly reduced after 1, 2, and 3 weeks of storage compared to the day 0. However, the oil-dispersible form packed in the same containers were only stable after 1-week and significantly reduced after 2 and 3 weeks. Kazmi *et al*.[20] found that water- or oil-dispersible forms of Vitamin D_3 were stable in yogurt during 4 weeks of storage, but they did not mention the type of the containers.

Regardless of the forms of Vitamin D_3, no significant differences were observed in the content of this vitamin in translucent containers among 1st, 2nd, and 3rd weeks of storage [Table 2]. It might be due to the fact that Vitamin D-degradation products may protect the remaining part of this vitamin from further degradation.[30]

Stability of Vitamin D_3 in Doogh

Stability of Vitamin D_3 in fortified Doogh during 8 weeks of storage at 15°C is shown in Table 3. Water-dispersible form of Vitamin D_3 was stable during the shelf-life of Doogh packed in opaque containers while it was not stable in the samples packed in translucent containers [Table 3]. Renken and Warthesen[30] reported the greatest reduction of Vitamin D due to light exposure of milk samples compared to air exposure. Furthermore, exposure to light can cause production of light-oxidized flavor and degradation of the other vitamins such as riboflavin and retinol.[30,31]

No significant difference was observed in the content of Vitamin D_3 packed in translucent containers among 2nd, 4th, and 8th weeks of storage [Table 3]. It is due to the protective effects of Vitamin D_3-degradation products on remaining Vitamin D_3.[30]

CONCLUSION

In this study, different forms of Vitamin D_3 (water- or oil-dispersible) were used for the fortification of yogurt and Persian yogurt drink "Doogh" to evaluate their stability during the products shelf-life. We also

Table 1: Interaction between the variables[a]

Effect	*P*	
	90 IU/100 g	120 IU/100 g
Yogurt		
Time	<0.001	<0.001
Time × packaging type	<0.001	<0.001
Time × vitamin D3 type	0.246	0.537
Doogh		
Time	<0.001	<0.001
Time × packaging type	0.001	<0.001

[a]Results of multivariate tests (repeated measure of ANOVA)

Table 2: Stability of vitamin D_3 in fortified low-fat yoghurt during the shelf-life

Packaging	Type of vitamin D_3	Dosage (IU/100 g)	Vitamin D content (IU/100 g)				*P*
			Day 0	Day 7	Day 14	Day 21	
Opaque	Water-dispersible	90	85.72±1.497^{ab}	86.80±1.528^{ab}	90.63±1.330^{a}	85.43±1.659^{b}	0.219
		120	117.33±1.827^{a}	116.43±2.468^{a}	119.48±2.569^{a}	115.65±2.385^{a}	0.594
	Oil-dispersible	90	83.45±1.230^{a}	85.07±1.924^{a}	82.33±2.326^{a}	80.90±1.839^{a}	0.475
		120	113.41±1.694^{a}	115.30±2.945^{a}	116.29±1.992^{a}	112.76±2.161^{a}	0.644
Translucent	Water-dispersible	90	86.23±1.862^{a}	75.01±2.175^{b}	73.40±2.109^{bc}	69.92±1.475^{c}	0.005
		120	119.13±0.875^{a}	107.25±2.333^{b}	106.24±3.766^{b}	102.53±3.663^{b}	0.009
	Oil-dispersible	90	85.54±1.631^{a}	79.33±1.719^{ab}	75.49±1.629^{b}	73.98±1.748^{b}	0.012
		120	115.25±2.094^{a}	108.72±2.479^{ab}	104.69±2.028^{b}	101.81±2.094^{b}	0.010

[a,b,c]Means±SEM in the same row with different superscript letters are significantly different ($P<0.05$). SEM: Standard error of the mean

Table 3: Stability of water-dispersible form of Vitamin D3 in fortified Doogh during the shelf-life

Packaging	Dosage (IU/100 g)	Vitamin D content (IU/100 g)				*P*
		Week 1	Week 2	Week 4	Week 8	
Opaque	90	87.02±1.523[a]	88.20±2.473[a]	84.33±1.741[a]	82.578±2.370[a]	0.397
	120	116.65±2.573[a]	118.06±1.737[a]	115.38±1.467[a]	112.92±2.040[a]	0.409
Translucent	90	88.15±1.571[a]	70.65±3.139[b]	65.62±3.432[b]	63.98±2.131[b]	<0.001
	120	117.35±1.462[a]	95.84±3.390[b]	90.56±3.303[b]	88.22±2.619[b]	<0.001

[a,b]Means±SEM in the same row with different superscript letters are significantly different ($P<0.05$). SEM: Standard error of the mean

assessed two different types of containers, opaque and translucent to find out if the packaging type could affect Vitamin D_3 content of the products. The stability of Vitamin D_3 in yogurt or Doogh packed in translucent containers was not satisfactory, while in the opaque containers was acceptable. We strongly recommend food industries to use opaque containers for storage of yogurt products or even any kinds of foodstuffs fortified with Vitamin D_3 to reduce light-related degradation of the vitamin during the products shelf-life.

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REFERENCES

1. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: Modulation of innate and autoimmunity. J Mol Med (Berl) 2010;88:441-50.
2. Reis AF, Hauache OM, Velho G. Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence. Diabetes Metab 2005;31:318-25.
3. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, *et al.* Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008;117:503-11.
4. Martini LA, Wood RJ. Vitamin D status and the metabolic syndrome. Nutr Rev 2006;64:479-86.
5. Krishnan AV, Trump DL, Johnson CS, Feldman D. The role of vitamin D in cancer prevention and treatment. Endocrinol Metab Clin North Am 2010;39:401-18.
6. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, *et al.* Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770-3.
7. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.
8. Holick MF. The role of vitamin D for bone health and fracture prevention. Curr Osteoporos Rep 2006;4:96-102.
9. Isaia G, Giorgino R, Rini GB, Bevilacqua M, Maugeri D, Adami S. Prevalence of hypovitaminosis D in elderly women in Italy: Clinical consequences and risk factors. Osteoporos Int 2003;14:577-82.
10. Yang Z, Laillou A, Smith G, Schofield D, Moench-Pfanner R. A review of vitamin D fortification: Implications for nutrition programming in Southeast Asia. Food Nutr Bull 2013;34:S81-9.
11. Wagner D, Sidhom G, Whiting SJ, Rousseau D, Vieth R. The bioavailability of vitamin D from fortified cheeses and supplements is equivalent in adults. J Nutr 2008;138:1365-71.
12. Holick MF, Chen TC. Vitamin D deficiency: A worldwide problem with health consequences. Am J Clin Nutr 2008;87:1080S-6.
13. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK, *et al.* Fortification of orange juice with vitamin D (2) or vitamin D (3) is as effective as an oral supplement in maintaining vitamin D status in adults. Am J Clin Nutr 2010;91:1621-6.
14. Lu Z, Chen TC, Zhang A, Persons KS, Kohn N, Berkowitz R, *et al.* An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? J Steroid Biochem Mol Biol 2007;103:642-4.
15. O'Donnell S, Cranney A, Horsley T, Weiler HA, Atkinson SA, Hanley DA, *et al.* Efficacy of food fortification on serum 25-hydroxyvitamin D concentrations: Systematic review. Am J Clin Nutr 2008;88:1528-34.
16. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, *et al.* The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. J Clin Endocrinol Metab 2011;96:53-8.
17. Greger JL. Dietary supplement use: Consumer characteristics and interests. J Nutr 2001;131:1339S-43.
18. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, *et al.* Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: A systematic review and meta-analysis. Am J Clin Nutr 2012;95:1357-64.
19. O'Mahony L, Stepien M, Gibney MJ, Nugent AP, Brennan L. The potential role of vitamin D enhanced foods in improving vitamin D status. Nutrients 2011;3:1023-41.
20. Kazmi SA, Vieth R, Rousseau D. Vitamin D3 fortification and quantification in processed dairy products. Int Dairy J 2007;17:753-9.
21. Vieth R. Implications for 25-hydroxyvitamin D testing of public health policies about the benefits and risks of vitamin D fortification and supplementation. Scand J Clin Lab Invest Suppl 2012;243:144-53.
22. Natri AM, Salo P, Vikstedt T, Palssa A, Huttunen M, Kärkkäinen MU, *et al.* Bread fortified with cholecalciferol increases the serum 25-hydroxyvitamin D concentration in women as effectively as a cholecalciferol supplement. J Nutr 2006;136:123-7.
23. Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: A novel approach for enhancing vitamin D nutritional health. Am J Clin Nutr 2003;77:1478-83.
24. Urbain P, Singler F, Ihorst G, Biesalski HK, Bertz H. Bioavailability of vitamin D2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: A randomized controlled trial. Eur J Clin Nutr 2011;65:965-71.
25. Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, Kalayi A, *et al.* Daily consumption of vitamin D- or vitamin D+calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: A randomized clinical trial. Am J Clin Nutr 2011;93:764-71.
26. Holmberg I, Aksnes L, Berlin T, Lindbäck B, Zemgals J, Lindeke B. Absorption of a pharmacological dose of vitamin D3 from two different lipid vehicles in man: Comparison of peanut oil and a medium chain triglyceride. Biopharm Drug Dispos 1990;11:807-15.
27. Hartman AM, Dryden LP. Vitamins in milk and milk products. In: Webb BH, Johnson AH, Alford JA, editors. Fundamentals of Dairy Chemistry. 2nd ed. New York: AVI/Van Nostrand Reinhold; 1974. p. 325-401.
28. Krauss WE, Erb JH, Washburn RG. Studies on the nutritive value of milk. II. The effect of pasteurization on some of the nutritive properties of milk. Ohio Agric Exp Stn Bull 1933;518:1-33.

29. Upreti P, Mistry VV, Warthesen JJ. Estimation and fortification of vitamin D3 in pasteurized process cheese. J Dairy Sci 2002;85:3173-81.
30. Renken SA, Warthesen JJ. Vitamin D stability in milk. J Food Sci 1993;58:552-5.
31. Gaylord AM, Warthesen JJ, Smith DE. Influence of milk fat, milk solids, and light intensity on the light stability of vitamin A and riboflavin in lowfat milk. J Dairy Sci 1986;69:2779-84.

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