

**Original Article****Influence of Time and Temperature on Stability of Added Vitamin D₃ During Cooking Procedure of Fortified Vegetable Oils**Zahra Saghafi¹, Bahareh Nikooyeh², Ali Jamali³, Marzieh Khazraie⁴, Mercedeh Mahdizadeh⁴, Azizollaah Zargaraan^{5*}

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A B S T R A C T

Background and Objectives: Previous research has established that Vitamin D₃ (Cholecalciferol) deficiency is considered to be a highly prevalent nutritional problem worldwide. Data from National Food and Nutrition Surveillance Program (2015) revealed that prevalence of vitamin D₃ deficiency in Iran is more than 70% of the population. Vegetable oils are considered to be potential candidates for fortification with vitamin D₃. Although exposure to high temperatures has been shown to cause adverse effects in vitamin D₃ content of food products, research to date has not yet determined the stability of added vitamin D₃ in vegetable oils during cooking procedures.

Materials and Methods: An 80/20 % (eighty/twenty) mixture of fortified oils/water subjected to low temperature (105°C, for 0, 60, 120 and 180 minutes) and high temperature (160°C, for 0, 5, 10, 15, 20, 30, 60 minutes) under reflux condition in order to determine the destruction rate of vitamin D₃. The vitamin D₃ concentration was determined by HPLC method with following operating conditions: apparatus, SHIMADZU10-ATVP; column, C18 column, 5 mm, 150_4mm id; mobile phase, methanol; ambient temperature; flow rate, 1.0 ml/min

Results: This study has identified that retention rate of added vitamin D₃ in corn, sunflower and canola oils during normal cooking process varies from 68.6% to 87.4%.

Conclusions: This study has shown that retention of added vitamin D₃ in various vegetable oils depends on the range of natural vitamin D₃ retention in cooking of foodstuffs. This result will be of interest to clinical researchers and policymakers concerned with the fortification of food products mainly vitamin D₃ fortification.

Keywords: Vitamin D₃, Vegetable oils, Fortification, Cooking, Stability

Introduction

Vitamin D₃, as a fat soluble vitamin and a hormone precursor, plays various critical roles in optimal health including mediating calcium and phosphorus absorption, bone health metabolism, reducing cancer risk, prevention of cardiovascular diseases and insulin resistance (1, 2). It is generally accepted that optimum intake of vitamin D₃ is an important issue for all races, ages and genders (3). Therefore, in the past decade, several researches and conferences have been

devoted to investigate the role of vitamin D₃ in human health and diseases prevention (4).

The regular sources of vitamin D₃ are solar ultraviolet-B irradiation, as the main source, and oily fish and fish liver oil which are not so common in many regular diets (5). Moreover, vitamin D₃ can be taken from artificial sources such as supplements and fortified foods since unfortified foods do not contain sufficient amounts of vitamin D₃ (6, 7).

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Food fortification is a potential way to improve intake of vitamin D₃ in the population (8). In order to select appropriate vehicle food for vitamin D₃ fortification, several factors should be considered such as availability, price and stability during cooking procedures (9). In addition, it should be noted that the amount of added vitamin D₃ should be high enough to ensure adequacy and preventing the risk of over vitamin D₃ intake (10).

All around the world, the variety of fortified staple food with vitamin D₃ such as milk in Finland and USA, margarine in the UK, cereal grain in USA and the UK are offered mandatorily or voluntarily, while according to the estimation, 60% of vitamin D₃ intake is from fortified foods in US population diet (4). Interestingly, two recent studies have shown that the bioavailability of vitamin D₃ in fortified food was almost similar to that of vitamin D₃ supplements (11, 12).

About one billion people suffer from vitamin D₃ deficiency all around the world. Recent studies have proven that some tropical countries including Turkey, India, Iran, Saudi Arabia and China suffer from vitamin D₃ deficiency, approximately 30 to 93 % (13), while, according to the report of Iranian Food and Nutrition Surveillance program, over 70% of the Iranian people, aged between 12-65 have suffered from hypovitaminosis D₃, even during summer (14). Therefore, for adequate intake of vitamin D₃, fortified staple food can act as a valuable vehicle, especially for those populations exposed to little sunlight or not being able to afford vitamin D₃ supplements (15).

Yang *et al.* (2013) suggested that it can be useful to evaluate edible oils as a potential vehicle for vitamin D due to the marginal consumption of milk and milk product in south Asia since vegetable oils are suitable for fortification with vitamins A, D₃, and E based on their nature and easy distribution in oil. For instance, fortification of vegetable oils with vitamin D₃ has resulted in 7% to 9% of the Institute of Medicine Estimated Average Requirement (IOM EAR) of vitamin D₃ for an individual woman, and 4% to 5% of the IOM EAR for a child under 5 years old based on their daily vegetable oil consumption with fortification level of 7.5 to 10 µg/100 g of vitamin D₃ in Indonesia (16).

Results of Household Budget Survey in Urban Areas of Iran illustrate that oil consumption per capita is approximately 29.18 g/d, therefore it seems that edible oil can be a good potential staple food (17).

However, there are some concerns on stability of vitamin D₃ in vegetable oils with household applications, due to their shelf life and high temperature of cooking procedures. Therefore, given the evidence coming from the research, this paper aims to determine the effect of two cooking conditions (high and low temperature) on vitamin D₃ content of fortified sunflower, canola and corn oil and to investigate the stability of vitamin D₃ in household cooking procedures.

Materials and Methods

Materials

Refined, bleached, deodorized vegetable oil samples: canola, sunflower and corn oils, were generously provided by Kouros Food Industry (Tehran, Iran). Detailed information about vegetable oil characteristics before and after the fortification process is briefed in Table 1. All chemicals used in experiments were purchased from Merck Company (Darmstadt, Germany). Vitamin D₃ was purchased from DSM (peroxide value: max. 2.0 mEq/kg, Acid value: max. 1.0 mg KOH/g, vitamin D₃ content: 0.90-1.10 MIU/g).

Methods

Fortification of vegetable oils: Standard solution of vitamin D₃ with the concentration of 1 MIU/gr was used in order to fortify oil samples with the concentration of 1.05 µgr vitamin D₃/14 gr oil sample under 10 minutes stirred at room temperature. The destruction rate of added vitamin D₃ in fortified oils was determined during two different cooking types known as the low (105°C) and high temperature (160°C) procedures.

Description of cooking processes: Cooking of fortified oils and water with the proportion of 20% fortified oil: 80% tap water were performed at two different conditions: lower temperature (105°C) and higher temperature (160°C) under reflux conditions. In 105° C, vitamin D₃ concentration was determined at 0, 60, 120 and 180 minutes while in 160°C, it was determined in intervals of 0, 5, 10, 15, 20, 30, 60 minutes of cooking procedures.

Determination of vitamin D₃ concentration by HPLC method: The vitamin D₃ concentration was determined by HPLC method in mentioned intervals with following operating conditions: apparatus, SHIMADZU10-ATVP; column, C18 column, 5 mm, 150_4mm id (Beijing Analysis Instrument Company);

mobile phase, methanol; ambient temperature; flow rate, 1.0 ml/min (18).

Oxidative stability: The induction period of incubation of fortified oil samples was conducted using a Metrohm Rancimat instrument model 743 (Herisau, Switzerland) with 3 ± 0.2 g of fortified oil under 2.5 mL s^{-1} air flow rate and 110°C temperature condition (19).

Free fatty acid and peroxide value: Peroxide value (PV) was investigated according to AOCS Ca 5a-40 method while free fatty acid of fortified oil samples was determined using AOCS Cd 8-53 method. All tests were performed in triplicate.

Results

Table 1 illustrates information about four quality characteristics of sunflower, canola and corn oils before and after fortification with vitamin D₃. It is apparent from Table 1 that fortification of mentioned

oils with vitamin D₃ causes some changes in physicochemical attributes of oils. However, all of the physicochemical properties were in standard range. Table 2 presents information about the effect of time on destruction of vitamin D₃ in low temperature cooking procedure for sunflower, canola and corn oil. The results showed that in low temperature cooking condition, which is 105°C for 180 minutes, destruction rate was 16.9, 16.7 and 16.3% for sunflower, canola and corn oil, respectively. Table 3 also shows destruction of vitamin D₃ in high temperature cooking conditions. What stands out in the Table 3 is the amount of destruction of the vitamin D₃ in higher temperatures (160°C) being about twice more than the low temperature cooking process. As shown in Table 3, maximum destruction of vitamin D₃ in higher temperature (160°C) process was reported 31.4, 30.3 and 30.8% for sunflower, canola and corn oil, respectively.

Table 1. Mean \pm Physicochemical properties of unfortified and fortified vegetable oils with vitamin D₃

Properties	Unfortified sunflower oil	Fortified sunflower oil	Unfortified corn oil	Fortified corn oil	Unfortified canola oil	Fortified canola oil
PV(meq/kg)	0.77 ± 0.01^b	1.43 ± 0.1^a	0.32 ± 0.02^a	0.30 ± 0.01^a	0.28 ± 0.01^a	0.26 ± 0.01^a
FFA (%)	0.034 ± 0.00^a	0.034 ± 0.04^a	0.066 ± 0.012^a	0.056 ± 0.02^b	0.036 ± 0.01^a	0.037 ± 0.04^a
IP ₁₁₀ (h)	13.03 ± 0.1^a	12.63 ± 0.2^a	16 ± 0.2^a	16 ± 0.1^a	13 ± 0.5^a	13 ± 0.06^a
Anisidine value	3.02 ± 0.02^a	3.45 ± 0.03^a	4.76 ± 0.06^a	4.45 ± 0.4^a	1.67 ± 0.02^a	1.75 ± 0.05^a

Numbers with different superscript letters for each oil sample (fortified/unfortified) are significantly different (Duncan post hoc test, $P < 0.05$).

Table 2. Effect of time on destruction proportion of vitamin D₃ in cooking procedure

Time (min)	Temperature ($^\circ\text{C}$)	Sunflower oil		Canola oil		Corn oil	
		Vitamin D ₃ concentration	Destruction proportion	Vitamin D ₃ concentration	Destruction proportion	Vitamin D ₃ concentration	Destruction proportion
0	25	1.05 ± 0.01^a	0.0%	1.02 ± 0.02^a	0.0%	1.04 ± 0.01^a	0.0%
60	105	0.901 ± 0.05^b	14.2%	0.879 ± 0.03^b	13.8%	0.895 ± 0.11^b	13.9%
120	105	0.884 ± 0.05^c	15.8%	0.862 ± 0.04^c	15.5%	0.880 ± 0.03^c	15.4%
180	105	0.873 ± 0.01^d	16.9%	0.850 ± 0.1^d	16.7%	0.871 ± 0.02^c	16.3%

Mean \pm SD (n = 3). Values with different superscript letters within the same column are significantly different (Duncan post hoc test, $P < 0.05$). The concentration of vitamin D₃ is based on μg vitamin D₃/g oil

Table 3. Effect of time on destruction proportion of vitamin D₃ in frying procedure

Time (min)	Temperature ($^\circ\text{C}$)	Sunflower oil		Canola oil		Corn oil	
		Vitamin D ₃ concentration	Destruction proportion	Vitamin D ₃ concentration	Destruction proportion	Vitamin D ₃ concentration	Destruction proportion
0	25	1.050 ± 0.02^a	0.0%	1.02 ± 0.05^a	0.0%	1.04 ± 0.01^a	0.0%
5	160	0.908 ± 0.02^b	13.5%	0.891 ± 0.03^b	12.6%	0.902 ± 0.01^b	13.3%
10	160	0.791 ± 0.01^c	24.7%	0.765 ± 0.04^c	25.0%	0.800 ± 0.02^c	23.1%
15	160	0.772 ± 0.01^{cd}	26.5%	0.744 ± 0.04^d	27.1%	0.768 ± 0.03^{cd}	26.2%
20	160	0.768 ± 0.06^{cd}	26.9%	0.731 ± 0.03^c	28.3%	0.752 ± 0.02^{cd}	27.7%
30	160	0.766 ± 0.03^{de}	27.0%	0.720 ± 0.01^f	29.4%	0.733 ± 0.02^{cd}	29.5%
60	160	0.720 ± 0.05^e	31.4%	0.711 ± 0.01^g	30.3%	0.720 ± 0.04^d	30.8%

Mean \pm SD (n = 3). Values with different superscript letters within the same column are significantly different (Duncan post hoc test, $P < 0.05$). The concentration of vitamin D₃ is based on μg vitamin D₃/g oil

Discussion

There are relatively few studies in the area of thermal deterioration of vitamin D₃ in foodstuffs (20-25). Previous researches have established that pure vitamin D₃ is susceptible to degradation and easily decomposed by heat (26). Table 4 shows the results obtained from previous studies. In a general view, rate of vitamin D retention in normal cooking processes of foodstuffs like frying, boiling and grilling is dependent on the severity and duration of heat exposure. In some cases, for example, oven-cooking of pork, increased the final amount of vitamin D₃

although not statistically significant. As can be seen from Table 2, retention of vitamin D₃ in normal cooking conditions ranges between 35% for cooked beef and 100% for pork. Range of vitamin D₃ retention in low temperature cooking conditions in the present study was estimated between 86.2% (canola, 5 min, 160 °C) and 83.1% for (sunflower, 60 min, 160 °C). We also found that in the high temperature cooking process, range of deterioration was between 87.4% (canola, 5 min, 160 °C) and 68.6% for (sunflower, 60 min, 160 °C).

Table 4. Effect of temperature on retention of vitamin D in normal cooking of foodstuffs

Food product	Heating conditions	Retention (%)	Reference
Beef	N/A	35-42(%) Retention	(27)
Atlantic Mackrel	Drying: 60 minutes at 30°C cooking: 50°C at 45 min final cooking period 180 min at 80°C.	1.7% loss but not statistically significant	(28, 29)
Pork	Oven: 250 °C, 20 min 150 °C, till meat core reaches 80 °C	Cooking increases vitamin D content, but not statistically significant (0.15 µg-0.18 µg/100g)	(20)
Fish	172 or 200°C for 20 min,	83% (Rainbow trout) to 93% (vendace) retention	(24)
Fish	Pan frying for 6 min at 180 °C	85-90 % retention	(25)
egg yolk	boiling in water for 10 min,	94-99% retention	(24)
Boiled and scrambled egg	Boiled egg: 10 min in boiling water, scrambled eggs: stirred for 30 s and heated for 3 min in a pan	82% - 88%	(24)
mushroom ergocalciferol (D2)	frying in pan for 5 min	86% (<i>C. cibarius</i>) to 99% (<i>tubaeformis</i>) retention	(24)
Margarine	fried in a pan for 3 min, heated for 40 min 175 200 °C for 60	82% and 45% respectively	(24)
Cakes	Baking conditions: 60 min in 175 °C	64% retention	(24)
Bread	200 °C	Wheat bread, oven, 30 min: 89% Wheat bread, oven, 60 min: 85% Rye bread, oven, 60 min: 73%	(24)

Results of the present study seem to be consistent with other research which found that heat treatment causes some decomposition in vitamin D content of cooked foodstuffs. Our results may support the hypothesis that destruction of added vitamin D in vegetable oils under heat treatment falls in the range of its natural form in foodstuffs. It is generally accepted that fortification of vitamin D in staple foods is a cost effective intervention to overcome vitamin D₃ deficiency (22, 23). Estimation of final amount of the micronutrients after various processes which is implemented on foodstuffs plays a crucial role in designing fortification programs. Therefore, results of the present study has important implications for developing new policies in the area of fortification of vegetable oils with vitamin D.

Previous studies showed that mandatory fortification of staple food with vitamin D plays a critical role in providing sufficient levels of this vitamin in many countries. Data from several studies suggest that even in mandatory fortification of staple foods with vitamin D, because of food choice problems and dietary habits, some groups are under the risk of vitamin D deficiency (30). The evidence from this study suggests that due to regular consumption of vegetable oils and its popularity in all groups in various populations, fortification of these oils could improve the intake of vitamin D₃ in countries which are have high vitamin D deficiency.

It is unfortunate that our study did not include investigation of the effect of storage time on stability and physicochemical properties of fortified vegetable oils. In order to enlighten all of the aspects of fortification on of vegetable oils with vitamin D₃, a further study could assess the effects of storage in various conditions on stability of added vitamin D₃ in vegetable oils. Furthermore, more research using controlled trials is needed to determine effectiveness of fortification of vegetable oils with vitamin D₃ in management of vitamin D₃ deficiency and to estimate sufficient value of vitamin D₃ to obtain meaningful changes in serum vitamin D₃ levels.

Conclusion

The purpose of the current study was to determine the influence of time and temperature on stability of vitamin D₃ during cooking procedures of fortified vegetable oils. This study has identified that retention rate of added vitamin D₃ in sunflower, canola oil

under normal cooking temperatures varies from 68.6% to 87.4%. These results suggest that edible oils including sunflower, canola and corn oils could be regarded as a suitable vehicle for the fortification with vitamin D₃.

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References

1. Rajakumar K. Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. *Pediatrics*. 2003;112(2):e132-e5.
2. Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *The American journal of clinical nutrition*. 2004;79(5):820-5.
3. Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. *The Journal of Clinical Endocrinology & Metabolism*. 2001;86(3):1212-21.
4. Allen RE, Dangour AD, Tedstone AE, Chalabi Z. Does fortification of staple foods improve vitamin D intakes and status of groups at risk of deficiency? A United Kingdom modeling study. *The American journal of clinical nutrition*. 2015;102(2):338-44.
5. 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. *The American journal of clinical nutrition*. 2008;88(6):1528-34.
6. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. *Altern Med Rev*. 2005;10(2):94-111.
7. Moulas AN. Fortification of foods with vitamin D. *Journal of Biotechnology*. 2017;256:S13.
8. Cashman K, Kiely M. Recommended dietary intakes for vitamin D: where do they come from, what do they achieve and how can we meet them? *Journal of human nutrition and dietetics*. 2014;27(5):434-42.
9. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. *The American journal of clinical nutrition*. 2004;80(6):1710S-6S.
10. Black LJ, Seamans KM, Cashman KD, Kiely M. An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *The Journal of nutrition*. 2012;142(6):1102-8.

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. *The Journal of nutrition*. 2008;138(7):1365-71.
12. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK, et al. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults-. *The American journal of clinical nutrition*. 2010;91(6):1621-6.
13. Heshmat R, Mohammad K, Majdzadeh S, Forouzanfar M, Bahrani A, Ranjbar Omrani G. Vitamin D deficiency in Iran: A multi-center study among different urban areas. *Iran J Public Health*. 2008;37(suppl).
14. National Nutrition FTRI. National Food and Nutrition Surveillance Program. 2015.
15. Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, et al. The national diet & nutrition survey: adults aged 19 to 64 years. 2003.
16. 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 and nutrition bulletin*. 2013;34(2_suppl1):S81-S9.
17. Iran CBo. Household expenditure report 2014. Tehran: 2014.
18. ISIRI. Foodstuffs - Determination of vitamin D by high performance liquid chromatography-Measurement of cholecalciferol (D3) or ergocalciferol (D2). 1398.
19. Naeli MH, Farmani J, Zargaraan A. Rheological and Physicochemical Modification of trans-Free Blends of Palm Stearin and Soybean Oil by Chemical Interesterification. *Journal of Food Process Engineering*. 2016.
20. Saghafi Z, Naeli MH, Tabibiazar M, Zargaraan A. Zero-Trans Cake Shortening: Formulation and Characterization of Physicochemical, Rheological, and Textural Properties. *Journal of the American Oil Chemists' Society*. 2018;95(2):171-83.
21. Bennink M, Ono K. Vitamin B12, E and D content of raw and cooked beef. *Journal of Food Science*. 1982;47(6):1786-92.
22. Vatanparast H, Calvo MS, Green TJ, Whiting SJ. Despite mandatory fortification of staple foods, vitamin D intakes of Canadian children and adults are inadequate. *The Journal of steroid biochemistry and molecular biology*. 2010;121(1-2):301-3.
23. Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global perspective of current status. *The Journal of nutrition*. 2005;135(2):310-6.
24. Freire WB, Waters WF, Rivas-Mariño G, Nguyen T, Rivas P. A qualitative study of consumer perceptions and use of traffic light food labelling in Ecuador. *Public health nutrition*. 2017;20(5):805-13.
25. Orozco F, Ochoa D, Muquinche M, Padro M, Melby CL. Awareness, Comprehension, and Use of Newly-Mandated Nutrition Labels Among Mestiza and Indigenous Ecuadorian Women in the Central Andes Region of Ecuador. *Food and nutrition bulletin*. 2017;38(1):37-48.
26. Chen PS, Raymond Terepka A, Lane K, Marsh A. Studies of the stability and extractability of vitamin D. *Analytical Biochemistry*. 1965 1965/03/01;10(3):421-34.
27. Bennink MR, Ono K. Vitamin B12, E and D Content of Raw and Cooked Beef. *Journal of Food Science*. 1982;47(6):1786-92.
28. Aminullah Bhuiyan AKM, Ratnayake WMN, Ackman RG. Nutritional Composition of Raw and Smoked Atlantic Mackerel (*Scomber scombrus*): Oil- and Water-Soluble Vitamins. *Journal of Food Composition and Analysis*. 1993 1993/06/01;6(2):172-84.
29. Bhuiyan A, Ratnayake WMN, Ackman RG. Effect of Smoking on the Proximate Composition of Atlantic Mackerel (*Scomber scombrus*). *Journal of Food Science*. 1986;51(2):327-9.
30. Calvo MS, Whiting SJ, Barton CN. Symposium: vitamin D insufficiency: a significant risk factor in chronic diseases and potential disease-specific biomarkers of vitamin D sufficiency. *J Nutr*. 2005;135:301-37.