

**Original Article****Influence of Time and Temperature on Stability of Added Vitamin D<sub>3</sub> During Cooking Procedure of Fortified Vegetable Oils**Zahra Saghafi<sup>1</sup>, Bahareh Nikooyeh<sup>2</sup>, Ali Jamali<sup>3</sup>, Marzieh Khazraie<sup>4</sup>, Mercedeh Mahdizadeh<sup>4</sup>, Azizollaah Zargaraan<sup>5\*</sup>

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**A B S T R A C T**

**Background and Objectives:** Previous research has established that Vitamin D<sub>3</sub> (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 D<sub>3</sub> deficiency in Iran is more than 70% of the population. Vegetable oils are considered to be potential candidates for fortification with vitamin D<sub>3</sub>. Although exposure to high temperatures has been shown to cause adverse effects in vitamin D<sub>3</sub> content of food products, research to date has not yet determined the stability of added vitamin D<sub>3</sub> 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 D<sub>3</sub>. The vitamin D<sub>3</sub> 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 D<sub>3</sub> 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 D<sub>3</sub> in various vegetable oils depends on the range of natural vitamin D<sub>3</sub> 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 D<sub>3</sub> fortification.

**Keywords:** Vitamin D<sub>3</sub>, Vegetable oils, Fortification, Cooking, Stability

**Introduction**

Vitamin D<sub>3</sub>, 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 D<sub>3</sub> 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 D<sub>3</sub> in human health and diseases prevention (4).

The regular sources of vitamin D<sub>3</sub> 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 D<sub>3</sub> can be taken from artificial sources such as supplements and fortified foods since unfortified foods do not contain sufficient amounts of vitamin D<sub>3</sub> (6, 7).

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Food fortification is a potential way to improve intake of vitamin D<sub>3</sub> in the population (8). In order to select appropriate vehicle food for vitamin D<sub>3</sub> 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 D<sub>3</sub> should be high enough to ensure adequacy and preventing the risk of over vitamin D<sub>3</sub> intake (10).

All around the world, the variety of fortified staple food with vitamin D<sub>3</sub> 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 D<sub>3</sub> intake is from fortified foods in US population diet (4). Interestingly, two recent studies have shown that the bioavailability of vitamin D<sub>3</sub> in fortified food was almost similar to that of vitamin D<sub>3</sub> supplements (11, 12).

About one billion people suffer from vitamin D<sub>3</sub> deficiency all around the world. Recent studies have proven that some tropical countries including Turkey, India, Iran, Saudi Arabia and China suffer from vitamin D<sub>3</sub> 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 D<sub>3</sub>, even during summer (14). Therefore, for adequate intake of vitamin D<sub>3</sub>, fortified staple food can act as a valuable vehicle, especially for those populations exposed to little sunlight or not being able to afford vitamin D<sub>3</sub> 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, D<sub>3</sub>, and E based on their nature and easy distribution in oil. For instance, fortification of vegetable oils with vitamin D<sub>3</sub> has resulted in 7% to 9% of the Institute of Medicine Estimated Average Requirement (IOM EAR) of vitamin D<sub>3</sub> 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 D<sub>3</sub> 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 D<sub>3</sub> 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 D<sub>3</sub> content of fortified sunflower, canola and corn oil and to investigate the stability of vitamin D<sub>3</sub> 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 D<sub>3</sub> was purchased from DSM (peroxide value: max. 2.0 mEq/kg, Acid value: max. 1.0 mg KOH/g, vitamin D<sub>3</sub> content: 0.90-1.10 MIU/g).

### Methods

**Fortification of vegetable oils:** Standard solution of vitamin D<sub>3</sub> with the concentration of 1 MIU/gr was used in order to fortify oil samples with the concentration of 1.05 µgr vitamin D<sub>3</sub>/14 gr oil sample under 10 minutes stirred at room temperature. The destruction rate of added vitamin D<sub>3</sub> 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 D<sub>3</sub> 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 D<sub>3</sub> concentration by HPLC method:** The vitamin D<sub>3</sub> 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  $3 \pm 0.2$  g of fortified oil under  $2.5 \text{ mL s}^{-1}$  air flow rate and  $110^\circ\text{C}$  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 D<sub>3</sub>. It is apparent from Table 1 that fortification of mentioned

oils with vitamin D<sub>3</sub> 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 D<sub>3</sub> in low temperature cooking procedure for sunflower, canola and corn oil. The results showed that in low temperature cooking condition, which is  $105^\circ\text{C}$  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 D<sub>3</sub> in high temperature cooking conditions. What stands out in the Table 3 is the amount of destruction of the vitamin D<sub>3</sub> in higher temperatures ( $160^\circ\text{C}$ ) being about twice more than the low temperature cooking process. As shown in Table 3, maximum destruction of vitamin D<sub>3</sub> in higher temperature ( $160^\circ\text{C}$ ) 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 D<sub>3</sub>

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

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 D<sub>3</sub> in cooking procedure

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

**Table 3.** Effect of time on destruction proportion of vitamin D<sub>3</sub> in frying procedure

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

## Discussion

There are relatively few studies in the area of thermal deterioration of vitamin D<sub>3</sub> in foodstuffs (20-25). Previous researches have established that pure vitamin D<sub>3</sub> 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 D<sub>3</sub>

although not statistically significant. As can be seen from Table 2, retention of vitamin D<sub>3</sub> in normal cooking conditions ranges between 35% for cooked beef and 100% for pork. Range of vitamin D<sub>3</sub> 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 (D <sub>2</sub> )	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 D<sub>3</sub> 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 D<sub>3</sub> 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 D<sub>3</sub>, a further study could assess the effects of storage in various conditions on stability of added vitamin D<sub>3</sub> in vegetable oils. Furthermore, more research using controlled trials is needed to determine effectiveness of fortification of vegetable oils with vitamin D<sub>3</sub> in management of vitamin D<sub>3</sub> deficiency and to estimate sufficient value of vitamin D<sub>3</sub> to obtain meaningful changes in serum vitamin D<sub>3</sub> levels.

## Conclusion

The purpose of the current study was to determine the influence of time and temperature on stability of vitamin D<sub>3</sub> during cooking procedures of fortified vegetable oils. This study has identified that retention rate of added vitamin D<sub>3</sub> 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 D<sub>3</sub>.

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