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Research Article

Influence of Storage Conditions on the Stability of Vitamin D_3 and Kinetic Study of the Vitamin Degradation in Fortified Canola Oil during the Storage

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Nowadays, fortified vegetable oils with vitamin D_3 are widely available in different countries. In this study, the influence of storage conditions including light, air, storage temperature, and time on vitamin D_3 retention in fortified canola oil was evaluated. Moreover, a kinetic study on vitamin D_3 degradation in the oil was done. To this aim, fortified canola oil was prepared at two initial concentrations of 6.87 $mg{\cdot}kg^{-1}$ and 13.8 $mg{\cdot}kg^{-1}$ and then filled in transparent and dark-brown polyethylene terephthalate bottles at two filling levels of 50% and 100%. Samples were kept in two temperatures of 4°C and room temperature (27°C). The retention of vitamin D_3 in different samples showed that the vitamin content was affected by the packaging type, storage temperature, and initial concentration. Vitamin D_3 in the samples with a lower concentration of the vitamin which was stored in the refrigerator showed the highest retention (91%) after 70 days of storage, and the samples with higher initial concentration packed in transparent containers which were stored at room temperature (RT) showed the greatest loss (55.6%). Results of the kinetic study also showed that vitamin D_3 was affected by storage condition. The half-life of the vitamin D_3 differed from 96 to 577 days depending on the storage condition.

1. Introduction

Vitamin D_3 (cholecalciferol) is an essential fat-soluble vitamin that plays a crucial role in human health as a hormone precursor. This vitamin is involved in many processes, including bone health metabolism, stimulating intestinal calcium and phosphorus absorption, immune system function, and protection against certain cancers [1–6]. Nowadays, production of some fortified foods with vitamin D such as margarine, dairy products, orange juice, and breakfast cereals has become customary in the world. However, research is going on to find out more suitable carrier for vitamin D [7]. Because of the presence of double bonds in the structure of vitamin D_3, this vitamin is known to be sensitive to isomerization under various conditions such as pH, temperature, iodine, oxidation, and light exposure [8]. By now, some researchers studied the stability of vitamin D_3 during storage in different fortified foods such as dairy products, bread, and vegetable oil [7, 9–11]. Results of these studies revealed that the retention of vitamin D_3 may be affected by some environmental factors such as light, oxygen or oxidation status, and temperature [7, 10, 12]. Tabibian et al. who assessed the stability of vitamin D in fortified flatbread during process and storage showed that the vitamin decreased in fortified flatbread after one day. They suggested that storage temperature has an effect on vitamin stability during the storage [11]. Another study found that the added vitamin D_3 in both yoghurt and doogh samples packed in the opaque bottles was more stable than in the similar products packed in the translucent bottles [7].

Kazmi et al. [4] indicated that vitamin D_3 was stable during the process of ice cream production as they found that atmospheric oxygen did not have an important effect on vitamin D_3. Some authors studied the stability of fat-soluble vitamins in fortified vegetable oils; Hemery et al. [9] investigated the stability of vitamin D_3 in fortified soybean oil under the storage conditions of the factory and retailers. They found that the initial quality of the oil and light exposure during the storage affected the vitamin content of fortified soybean oil. Canola oil is a widely consumed edible oil in Iran. Additionally, the production of fortified vegetable oils with vitamin D_3 is considered as a practical solution to mitigate vitamin D_3 deficiency. Since in a fortification process assessment of the stability of the micronutrient is a crucial matter, the aims of the present study were (i) investigation of the stability of vitamin D_3 in fortified Canola oil stored under different storage conditions, (ii) evaluation of the effects of different environmental factors including light and oxygen exposure, the oxidative status of the oil, and storage temperature on retention of vitamin D_3, and (iii) providing a kinetic study on vitamin D_3 degradation in fortified canola oil during the storage to the prediction of the vitamin behavior in the fortified oil.

2. Materials and Methods

2.1. Materials and Reagents. All HPLC grade solvents and analytical reagent were obtained from Merck (Darmstadt, Germany). Vitamin D_3 (Cholecalciferol, 47763) was purchased from Sigma-Aldrich Chemical Co. (USA). Vitamin D_3 solved in soybean oil at a concentration of 1 MIU·g^{-1} was provided by Iranian pharmaceutical company and used as a premix. Canola oil was obtained from a local market.

2.2. Methods

2.2.1. Fortification of Canola Oil with Vitamin D_3. For fortification of the oil, the premix of vitamin D_3 was added to it and the concentration of vitamin was adjusted at two levels of 6.87 mg·kg^{-1} and 13.8 mg·kg^{-1}. These two groups were named as low concentration (LC) and high concentration (HC), respectively. Since the purpose of this study was to provide a simulated fortified oil in which vitamin loss could be clearly monitored during the storage, the oil was fortified with higher concentrations of the vitamin compared with the real fortification. The two concentrations were also used to figure out if there is a relation between the vitamin concentration and its destruction rate. The characteristics of the oils are shown in Table 1.

2.2.2. Storage Conditions. Fortified oils (LC and HC) were stored at six different conditions. To determine the effect of light, the fortified oils were packaged in 20 ml transparent and dark-brown bottles; transparent bottles were exposed to the artificial light, and in order to eliminate the light effect, dark-brown bottles were kept in a dark place until analysis. To evaluate the effect of the air content of the bottles on the oxidative status of the oil and the vitamin degradation during the storage, samples were filled at two levels of 50% (with 50% air between oil and the cap) and 100% volume of bottles. Then, all of the samples were stored at two temperatures of 4°C and 27°C. The study models are summarized in Table 2. Vitamin D_3 content, peroxide value, and p-anisidine value of the prepared samples were analyzed every 7–14 days for ten weeks (70 days).

2.2.3. Quantification of Vitamin D_3 by High-Performance Liquid Chromatography. Retention of vitamin D_3 was determined by reverse-phase HPLC (Waters-1525, USA). One milliliter of the fortified oil was mixed with acetone in an amber glass tube 10 ml and then homogenized. The mixture was filtered through a Q-MAX-Model RR-syringe filter 13 mm, 0.45 μm PTFE-philic (Germany) and injected to the L1-ProntoSIL 120-3-C 18 H 250 × 4.6 mm column. According to the method of Kumar and Tripathi combination of acetonitrile, methanol (95 : 5, v/v) at a flow rate of 1.2 ml·min^{-1} was used as a mobile phase [13]. Vitamin D_3 was detected by a UV detector (Waters-2487, USA) at 265 nm. The standard stock solution of vitamin D_3 was prepared by dissolving 10 mg of vitamin D_3 standard in 100 ml of ethanol. The calibration curve was obtained by diluting the stock solution at a range of 0.3125 to 40 μg·ml^{-1}. For the determination of the accuracy of the method, the recovery of the added vitamin D_3 was calculated. It was determined by analyzing one milliliter of fortified oil samples spiked with 5, 10, 15 μg of vitamin D_3 standard. The results showed that the accuracy was 80.4 ± 5.84% ($n = 3$), 82.83 ± 4.11% ($n = 3$), and 85.97 ± 3.11% ($n = 3$), respectively.

The retention percentage of vitamin D_3 is calculated as follows:

$$\%\text{retention} = 100 \times \frac{[\text{vitamin}]t}{[\text{vitamin}]\text{ref}}, \quad (1)$$

where [vitamin]t is the vitamin content after storage time t and [vitamin]ref is the initial content of the vitamin.

2.2.4. DPPH Measurement. Antioxidant activity of the oil was determined by the DPPH method according to the procedure described by Ramadan and Moersel [14]. First, 10 mg of the oil sample was mixed with 100 μl ethyl acetate and 390 μl of DPPH solution (10^{-4} M in ethyl acetate). The mixture was shaken and placed in a dark place for 30 min. The difference between the absorbance of the sample solution and pure ethyl acetate was measured at 515 nm using a double-beam UV/Vis spectrophotometer (Halo DB-20R) Dynamica. The percentage of inhibition was calculated from the following equation:

Table 1: Initial characteristics of the oil samples.

Sample	Vitamin D_3 content ($\mu g\ ml^{-1}$)	DPPH assay (% inhibitory)	Peroxide value (mEq $O_2 \cdot kg^{-1}$)	Anisidine value	Acid value (g KOH kg^{-1})
LC	6.87 ± 0.16	8.85 ± 0.27	0.96 ± 0.04	3.53 ± 0.39	1.31 ± 0.09
HC	13.84 ± 0.25	8.93 ± 0.25	0.97 ± 0.05	3.17 ± 0.26	1.36 ± 0.09
P value	0.019*	0.435	0.589	0.486	0.543

Means ± SD ($r = 2$); LC: oil samples fortified with low concentration; HC: oil samples fortified with high concentration. *Statistically significant ($P < 0.05$).

Table 2: Storage conditions for fortified oil samples.

Model*	Storage condition
A	Samples in 20 ml transparent bottles exposed to usual artificial light for 9 hours a day with filling level of 50%, stored at the room temperature ($27 \pm 3^{\circ}C$)
B	Samples in 20 ml transparent bottles exposed to usual artificial light for 9 hours a day with filling level of 100%, stored at the room temperature ($27 \pm 3^{\circ}C$)
C	Samples in 20 ml dark-brown bottles with filling level of 50%, stored at the room temperature ($27 \pm 3^{\circ}C$)
D	Samples in 20 ml dark-brown bottles with filling level of 100%, stored at the room temperature ($27 \pm 3^{\circ}C$)
E	Samples in 20 ml transparent bottles with filling level of 50%, stored at the refrigerator ($4 \pm 1^{\circ}C$)
F	Samples in 20 ml transparent bottles with filling level of 100%, stored at the refrigerator ($4 \pm 1^{\circ}C$)

*All samples were prepared for both the initial concentrations of LC and HC.

$$\%\text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100. \quad (2)$$

(1) Determination of Peroxide (PV), p-Anisidine (p-AV), and Acid Value. Antioxidant activity, PV, p-AV, and acid value AV of all samples were determined. The PV was determined using iodometric titration, and p-AV was performed according to the AOCS cd 8-53 Official Method [15]. The acid value was determined according to the AOAC.

2.3. Statistical Analysis. Means, standard deviation (SD), and linear regression analysis were calculated using Microsoft Excel 2016. Independent t-test was carried to determine if there were differences between initial characteristics of samples HC and LC. The difference among means at P value <0.05 was considered statistically significant.

3. Results and Discussion

3.1. Vitamin D_3 Retention. As Figure 1 shows vitamin D_3 content decreased in all models during the storage. The degradation rate of vitamin D_3 depending on the storage conditions varied (<0.05). Generally, the retention percentage of the vitamin in samples LC was more than samples HC. The highest retentions of vitamin D_3 depending on the initial concentrations of the vitamin were about 88–91%, which belonged to the samples stored in the refrigerator (models E and F), without the intervention of two influential factors of light and temperature. On the other hand, the least retention belonged to the samples stored at model A that after 70 days were 65% and 56% for samples LC and HC, respectively. The results showed that the storage condition deeply affects the vitamin stability in the oil.

3.2. Oxidative Status of the Oil and Its Effect on Vitamin D_3 Degradation. To investigate the oxidative changes of the oil samples, oxidation indexes including PV and p-AV were determined during the storage (Figure 2).

As the results showed, oxidative changes in the samples during 70 days of storage were limited to the generation of primary oxidation products (PV), while p-AV were not changed significantly. The p-AVs in all models were still lower than the allowable maximum (less than 10) [16]. However, PV in all models depending on the severity of the lipid oxidation increased. The highest PV was observed in model A. The correlations between oxidative products and vitamin D_3 retention in samples stored in different models are shown in Table 3. No significant difference was observed between PV and p-AV of the same models of samples LC and HC ($P > 0.05$).

Oxidation seemed to be an effective factor in vitamin D_3 destruction [17]. The effect of oxidation on vitamin D_3 has been shown by some researchers previously [9, 10]. In the present study, we assessed the effect of air exposure on oxidation deterioration of the oil and vitamin D_3 degradation during the storage. To this aim, a comparison was made between the oxidative status and vitamin D_3 degradation of the models which were filled without any headspace (models B, D, and F) and the models containing air as much as 50% of bottle volume (A, C, and E). The other storage conditions were quite similar. According to the results of models A and B that were exposed to the light, after 70 days of storage, no significant differences were observed between vitamin D_3 content in models C and D or models E and F. It may reveal the importance of the light effect on vitamin D_3 oxidative degradation. The results showed an inverse relationship between the generation of oxidative

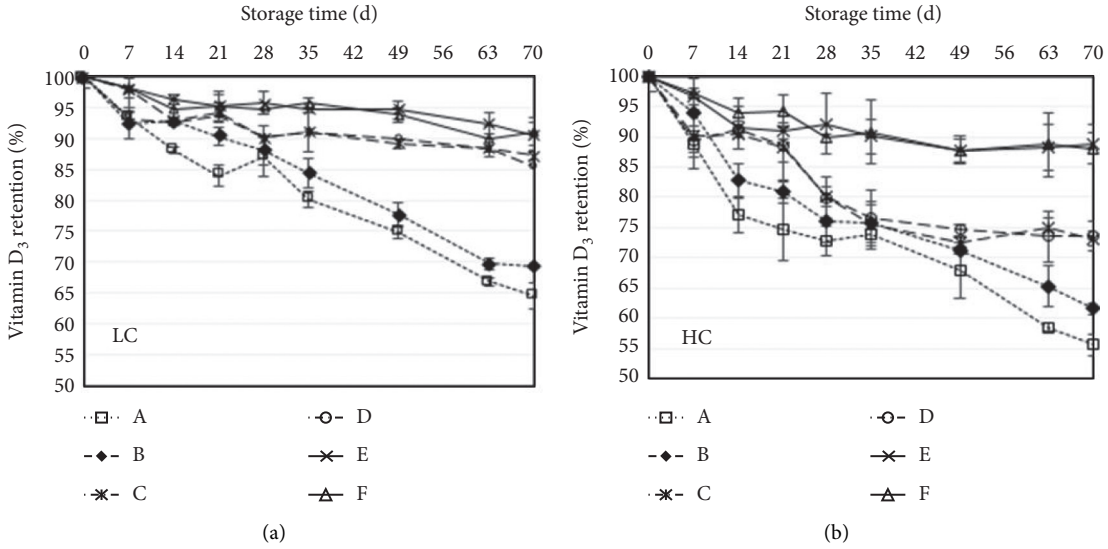

Figure 1: Evolution of vitamin D_3 retention in the fortified oils during storage. A: samples stored in transparent bottles at room temperature with filling level of 50%; B: samples stored in transparent bottles at room temperature with filling level of 100%; C: samples stored in dark-brown bottles at room temperature with filling level of 50%; D: samples stored in dark-brown bottles at room temperature with filling level of 100%; E: samples stored in dark-brown bottles at 4°C with filling level of 50%; F, samples stored in dark-brown bottles at 4°C with filling level of 100%.

products and vitamin D_3 retention during the storage, especially in models exposed to light (Table 3). Our results suggested that the differences between retention of vitamin D_3 in different models of this study might be attributed to the differences in the oxidative status of these models. As the models with the least oxidation showed more retention of the vitamin during the storage. These results are close to the results reported by Andarwulan et al. who evaluated the effect of PV on vitamin loss in fortified oil with retinyl palmitate [18]. Given the effect of oxidation status of the oil on the vitamin stability, it is highly probable that antioxidant compounds enhance the quality of oil and so prevent vitamin from oxidation as the beneficial effect of antioxidants on oxidative stability of vitamin has been demonstrated by some previous studies [9, 19]. One study that evaluated the effect of different oxidation status of the initial oils on the stability of vitamin D_3 showed that the antioxidant compounds could reduce peroxide and free fatty acid productions and also vitamin D_3 degradation in the oil during the storage [9]. They also reported the correlations of −0.74 to −0.88 between the content of vitamin D_3 and PV during the storage of fortified soybean oils with different initial PV. Another study also found good correlations between thiobarbituric acid reactive substances (TBARS) concentration and vitamin D_3 degradation in milk powder (0.92 to 0.99 depending on the storage condition) [10]. These observations show that the vitamin D_3 loss in fortified oil probably depends on the progress of lipid oxidation during storage and consumer handling. There are very few studies on the effect of oxidation on vitamin D_3 content in fortified food products. Kazmi et al. [4] investigated the stability of vitamin D_3 in some dairy products. They observed no reduction in vitamin D_3 content of ice cream during the aeration step (ice cream making process) even though more than 50% of the volume of ice cream consists of air. Therefore, it can be concluded that in the absence of the other effective factors like the light, vitamin D_3 seems to be rather stable when being exposed to the air at RT or lower temperatures [20, 21].

3.3. Effect of Storage Temperature. According to the results of our previous study, the increment of temperature during cooking causes a decrease in vitamin D_3 content of fortified oil, and the reduction rate of the vitamin profoundly depends on the temperature and the oxidative status of the oil [22]. Studies that evaluated the influence of the temperature on vitamin D_3 in fortified foods during cooking also reported similar results [11, 23, 24]. No published scientific data was not found on the effect of storage temperature on vitamin D_3 stability in fortified food products. One study which assessed the effect of the temperature on the stability of 25(OH)-vitamin D_3 in human blood or serum samples found that the vitamin was more stable when samples were stored at 6°C in comparison to samples which were kept at 25°C [25]. Another study found that vitamin D_3 in the dietary supplement was more stable when the samples were stored at a lower temperature [26]. The higher stability of vitamin D_3 in models E and F might be attributed to the lack of light, and lower temperature during the storage.

3.4. Effect of Light Exposure. Results of our study demonstrated that the highest degradation of vitamin D_3 occurred in the models which were exposed to light (samples packaged in transparent bottles) (Figure 1). Light degrades vitamin D_3 through isomerization [27] and also can exacerbate the effects of oxidation by an increment in the production of reactive oxygen species or even generation of singlet oxygen in the presence of sensitizers like chlorophyll or riboflavin [28, 29].

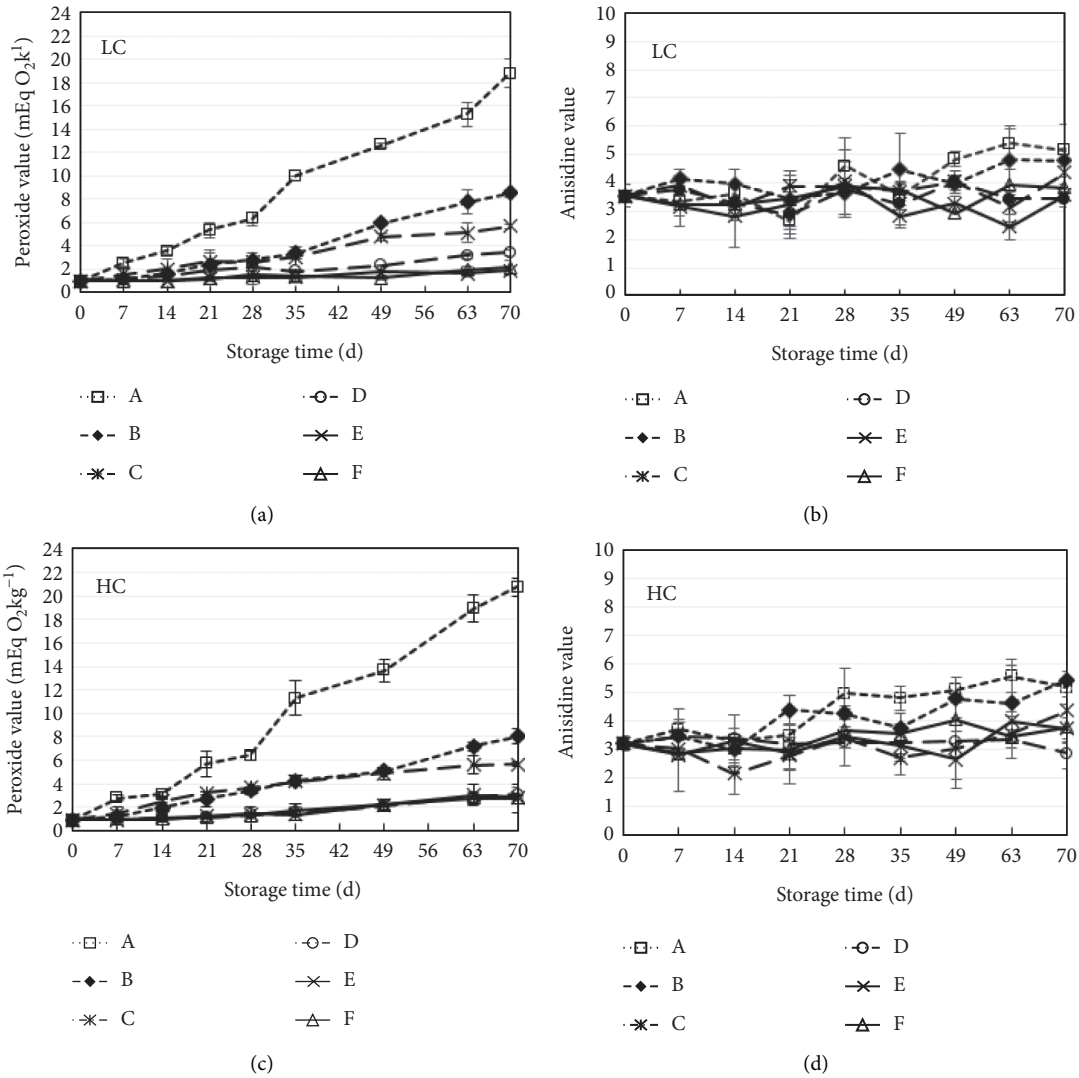

Figure 2: Evolution of peroxide and p-anisidine values of fortified oils during storage. A: samples stored in transparent bottles at room temperature with filling level of 50%; B: samples stored in transparent bottles at room temperature with filling level of 100%; C: samples stored in dark-brown bottles at room temperature with filling level of 50%; D: samples stored in dark-brown bottles at room temperature with filling level of 100%; E: samples stored in dark-brown bottles at 4°C with filling level of 50%; F: samples stored in dark-brown bottles at 4°C with filling level of 100%.

Some previous authors also have shown the adverse effect of the light exposure on vitamin D_3 stability; our results were close to the results reported by [9]. They fortified soybean oil with vitamin D_3 and stored them at dark, semidark, and natural light. Their results showed more loss of vitamin D_3 content in samples exposed to the light in comparison with the sample stored at the other condition. Jafari et al. showed that the stability of vitamin D_3 in doogh or yoghurt packaged in transparent bottles (71–74%) was less than those packed in opaque bottles (92–94%) [7]. Another study also observed a reduction of 65% for vitamin D_3 content in milk samples packed in clear pet bottles, which was more than the reduction of the vitamin in the pigmented bottles [30]. Therefore, although light exposure as an effective factor reduces vitamin D_3 in samples stored under light, in the products containing high-fat content such as vegetable oils, generation of more oxidative products during peroxidation of the oil might be contributed to more degradation of vitamin D_3 contents. However, obviously, the results related to the effect of light might depend on the type of light and the exposure time.

3.5. Degradation Behavior of Vitamin D_3 and Kinetic Study. The aim of a kinetic study during the storage is quantifying a change as a function of the time. In the present study, the kinetic parameters including rate constant, activation energy, half-life, and quotient indicator were evaluated. The kinetic order is determined due to the goodness of fit of the observations to a preselected kinetic order model. Zero-order and first-order models were used to evaluate the degradation rate of vitamin D_3. These kinetic types were obtained by the following equations, respectively:

Table 3: Correlation coefficients (P value) between vitamin D_3 retention and oxidation products in different storage conditions.

		Correlation (P value)	
		Peroxide value	p-Anisidine
LC	A	−0.959 (<0.001)	−0.730 (0.001)
	B	−0.965 (<0.001)	−0.749 (0.003)
	C	−0.930 (<0.001)	−0.379 (<0.001)
	D	−0.709 (<0.001)	−0.427 (0.008)
	E	−0.783 (0.004)	−0.043 (0.009)
	F	−0.664 (0.011)	−0.423 (0.01)
HC	A	−0.892 (<0.001)	−0.620 (<0.001)
	B	−0.894 (<0.001)	−0.397 (0.001)
	C	−0.858 (<0.001)	−0.233 (0.001)
	D	−0.821 (<0.001)	−0.117 (0.086)
	E	−0.519 (0.001)	0.129 (0.037)
	F	−0.593 (0.003)	0.078 (0.386)

$$\text{zero} - \text{order} : C = C_0 - k_0 t, \tag{3}$$

$$\text{first} - \text{order} : \ln = \ln C_0 - k_1 t, \tag{4}$$

where C is the vitamin D_3 content studied at time t, C_0 is the vitamin D_3 content at time zero, k_0 is the zero-order kinetic constant, and k_1 is the first-order kinetic constant.

To estimate the degradation rate constant of vitamin D_3 in samples during the storage, the slope of the linearized plot of $\ln(C/C_0)$ versus time was used. Vitamin D_3 reduction was described properly by both zero and first kinetic orders. However, because of the better correlation coefficients, the first-order model was preferred.

Temperature sensitivity of the rate constant was determined using the Arrhenius equation:

$$\ln\left(\frac{k}{k_{\text{ref}}}\right) = -\frac{E_a}{R\left(T - T_{\text{ref}}\right)}, \tag{5}$$

where k is the rate constant (min^{-1}), k_{ref} is the frequency factor (min^{-1}), E_a is the activation energy ($\text{kJ}\cdot\text{mol}^{-1}$), T is the absolute temperature (K), T_{ref} is the reference temperature (K), and R is the universal gas constant ($8.314\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$).

Another way to describe the dependency of the rate of quality changes on temperature is calculating the quotient indicator (Q_{10}) through the following equation:

$$Q_{10} = \left(\frac{k_1}{k_0}\right)^{\left(10/\left(T_1 - T_0\right)\right)}, \tag{6}$$

where k_1 is the rate constant at the temperature of T_1 and k_0 is the rate constant at the temperature of T_0.

One way to determine the reduction rate of the vitamin is to specify the amount of time that is required to reduce the vitamin content to half of its original value which for the first order is calculated using the following equation:

$$T_{1/2} = -\ln\left(\frac{1}{2}\right) \cdot K^{-1}, \tag{7}$$

where $T_{1/2}$ is the half-life of vitamin D_3 and k is the rate constant.

The first-order plots of vitamin D_3 degradation for all samples are shown in Figure 3, and the results related to the kinetic study are summarized in Table 4.

The differences between the rate constant of different models clearly represented the influence of storage conditions on vitamin D_3 stability. The results summarized in Table 4 showed that depending on the initial concentration, vitamin D_3 in the samples stored in the refrigerator with a half-life of about 407–577 days underwent the least decrease. According to the rate constants (Table 4), the reduction rate of vitamin D_3 in models A and B was higher than the other models in both LC and HC, while the vitamin content of oils in models E and F declined very slowly. Since the distinguishing factor in models A and B was light exposure, it can be concluded that light played the most important role in vitamin decomposition. As it has been explained previously (Section 3.4.), in the fortified oil samples, light reduces the vitamin through isomerization and causes more reduction by increasing the oxidation products which in turn react with the vitamin and reduce that. The effect of light on the oil quality and the influence of the oil quality on vitamin content have been shown in some studies; Manzocco et al. evaluated the effect of light on the oil quality and reported a higher rate constant of oxidation for the oil samples exposed to a higher light intensity rather than those protected from light [31]. Malau et al. also found a higher reaction rate constant for vitamin A degradation in the fortified palm oil that had a higher level of oxidation products (PV) [32]. As Table 4 shows, the reaction rate constants were close for the samples which were protected from light and their difference was only the air content of the bottles (models C and D and also models E and F). These results indicated that the air content of the bottles does not have a significant effect on vitamin D_3 content. One study also assessed the effects of the air on vitamin D_3 retention during the storage of fortified coconut powder. They found out that there was no significant difference between the degradation rate of the vitamin in the coconut powder packaged in the atmospheric air (0.0004, 0.0004, and 0.0006 (day^{-1})) and the samples packaged with N_2 (0.0003, 0.0004, and 0.0005 (day^{-1})) in three different storage temperatures, respectively [33]. The values of activation energies (E_a) (Table 4) clearly showed that with the increment of storage temperature, the reaction rate constant also increased. The result reported by Lucas Aguirre et al. was close to our observations as they obtained activation energy (E_a) of $8.505\ \text{kJ}\cdot\text{mol}^{-1}$ for the first-order reaction for the vitamin D_3 decomposition that represented the temperature-dependent nature of vitamin D_3 degradation during the storage [33]. The higher values of the activation energy (E_a), quotient indicator (Q_{10}), and rate constant and the lower half time in samples HC revealed that the degradation rate of the vitamin in samples HC was more and faster than samples LC. Therefore, according to these results, although the final vitamin content in samples HC in all models was still more than samples LC, vitamin D_3 content in samples HC was more affected by the time in all storage models. Given the fact that increment of the concentration of a particular reactant in a chemical reaction increases the rate of the reaction [34], in samples HC, the higher concentration of vitamin D_3 probably led to more proximity with oxidation products and as a result led to more

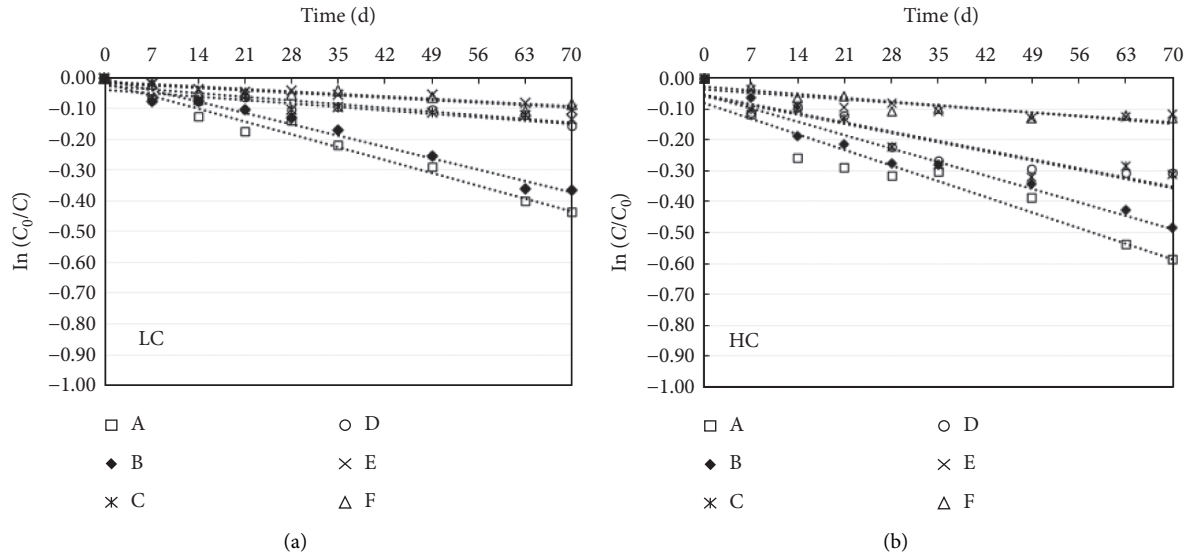

Figure 3: First-order plots of vitamin D_3 degradation during storage. A: samples stored in transparent bottles at room temperature with filling level of 50%; B: samples stored in transparent bottles at room temperature with filling level of 100%; C: samples stored in dark-brown bottles at room temperature with filling level of 50%; D: samples stored in dark-brown bottles at room temperature with filling level of 100%; E: samples stored in dark-brown bottles at 4°C with filling level of 50%; F: samples stored in dark-brown bottles at 4°C with filling level of 100%.

Table 4: Effect of storage conditions on the activation energy (E_a), reaction rate constant (k), Q_{10}, and half-life of vitamin D_3 in LC and HC.

Oil	Model	Temperature (°C)	$K_1 \times 10^3$ (day^{-1})	R^2	$T_{1/2}$ (days)	*Q_{10}	*E_a (kJ·K^{-1}·mol^{-1})
LC	A	27 ± 3	5.9	0.97	117.48		
	B	27 ± 3	5.2	0.97	133.29		
	C	27 ± 3	1.7	0.84	407.73		8.22
	D	27 ± 3	1.6	0.8	433.21		7.97
	E	4 ± 1	1.2	0.9	577.62	1.16	
	F	4 ± 1	1.2	0.81	577.62	1.13	
HC	A	27 ± 3	7.2	0.92	96.27		
	B	27 ± 3	6.3	0.95	110.02		
	C	27 ± 3	4.3	0.82	161.19		25.71
	D	27 ± 3	4.2	0.85	165.0		28.53
	E	4 ± 1	1.7	0.82	407.73	1.49	
	F	4 ± 1	1.5	0.71	462.09	1.55	

*Since we had no models in the refrigerator (4°C) which were exposed to light, Q_{10} and E_a just were calculated for models E and F that their parallel in RT was models C and D, respectively.

degradation of vitamin D_3. Our previous study also was a good agreement with these results because it also showed that during cooking, the rate constant and activation energies for samples with higher vitamin concentration were more than samples with lower vitamin content [22]. There are not many published papers on the degradation kinetics of vitamin D_3 to make a comparison. One study, however, was found in this regard that reported a different result about the effect of the vitamin concentration on vitamin stability during storage [35]. This contradiction is probably due to the different experimental conditions as they evaluated the vitamin stability at two concentrations in the aquatic medium. In such media, oxidation products resulting from oil oxidation, which played an important role in the vitamin degradation in our study, are not a matter. Hence, they attributed their results to the greater impact of particular destabilizing factors like pH, storage temperature, and so on in the samples with the lower vitamin concentrations. Nonetheless, it seems that more studies are needed to evaluate the effect of vitamin D concentration on the vitamin degradation rate in various food products during storage.

4. Conclusion

This study showed that vitamin D_3 retention in fortified oil profoundly depends on storage conditions. Among all samples, the most vitamin stability belonged to the samples stored in the refrigerator 4°C without light exposure, while the least stability was observed in the samples with the air in the bottle, exposed to light, and stored at RT. In all environmental variables, light exposure was found to be the most effective factor in vitamin degradation. It may be due to its

adverse effect on the chemical structure of vitamin D_3 and exacerbation of oxidative degradation of the vitamin. This paper concluded that canola oil can be fortified with vitamin D_3; however, in order to increase the vitamin retention, this study suggests that the fortified oil should be packed in the light protective bottles and be stored at lower temperatures to reduce the oxidation potential of the oil. The kinetic study showed that the degradation rate of the vitamin in samples with a higher initial concentration (HC) of the vitamin was higher, but, totally, the final vitamin content of these samples was higher than the samples with lower initial vitamin content. The kinetic study also demonstrated that depending on the initial concentration and the examined storage conditions of the present study, the half-life of vitamin D_3 in fortified canola oil could range from 96 to 577 days.

Data Availability

All data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors have declared no conflicts of interest for this article.

Authors' Contributions

A. Abbasi designed the study and interpreted the results. M. Zareie collected test data and drafted the manuscript. S. Faghih had contribution in the design of the study.

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