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# Effect of cooking time and temperature on vitamin D3 amount in the fortified sunflower oil

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## Abstract

According to WHO report, more than 2 billion people in the world suffer from micronutrient deficiencies caused largely by a dietary deficiency of vitamins and minerals. Fortification of some staple foods is one of the simplest and most practical methods to combat micronutrient deficiencies for both poor and wealthy societies. In this study sunflower oil was fortified with different concentrations (0.1, 0.3, 0.9, 1 and 10 µg/mL) of vitamin D3. Then the reduction of vitamin D3 for concentrations of 0.1 and 0.9 µg/mL at different times (0, 2, 4, and 6h) and cooking temperatures (70, 110, and 150°C) and also at deep-fat frying temperature of 180°C, concentrations of 1 and 10 µg/mL were investigated.

The results showed that the relative resistance of this vitamin observed at 70 °C. Over the specified time, at 110, and 150 °C, the amounts of vitamin D3 were decreased significantly. At 180 °C (deep frying), more than 60% of vitamin D3 was decomposed. Also, after heating, pyro- and iso-pyrocalciferol were detected as vitamin D3 decomposition compounds at deep-fat frying temperature using GC-MS. Based on the obtained results, free- vitamin D3 added to cooking oil can be stable at temperature lower than 100°C but for higher temperature, encapsulation of it is proposed.

**Keywords** Oil; Fortification; Pyrocoleciferol; Isopyrocoleciferol

## Introduction

Micronutrient malnutrition is a major problem in the industrialized nations and also in the developing countries. Although the health of all age groups of people can affect by micronutrient malnutrition, but children and women of reproductive age are at high risk of micronutrient deficiencies [1]. The adverse effects of micronutrient malnutrition on human health include clinically evident and serious detrimental effects on human function. Micronutrient malnutrition is a risk factor for many diseases and can cause to increase the high rates of morbidity and mortality [1]. Vitamins and minerals are important micro-

nutrients and control of these deficiencies is an essential part of the overall effort to fight hunger and malnutrition. Governments should have some food-based strategies such as dietary diversification and food fortification for public health. Food fortification refers to the addition of micronutrients to processed foods for reducing micronutrient malnutrition [1]. Fortification of some staple foods is the one of the simplest and most practical methods to combat micronutrient deficiencies for both poor and wealthy societies [2, 3]. Vitamin D is a micronutrient that is needed for human health. Vitamin D is a group of fat-soluble sterols. There are several forms of vitamin D



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that the two major forms of them are vitamin D3 and vitamin D2 [3]. They differ in their side chain, cholecalciferol in vitamin D3, which is found mainly in foodstuffs of animal origin or can be either synthesized in the human skin upon exposure to the UV light of the sun, or it is obtained from the diet, and ergocalciferol in vitamin D2, which is found in certain fungi such as wild mushrooms. Vitamin D can also be obtained from the diet. Since both forms of vitamin D are metabolized by humans in much the same way, from a nutritional perspective, vitamin D3 and vitamin D2 can be equivalent [3]. Vitamin D3 is metabolized first in the liver to 25-hydroxyvitamin D (25-OH-D3), and then in the kidney to 1, 25-dihydroxyvitamin D (1, 25-(OH) 2-D3), which is the biologically active form of the vitamin [3].

Vitamin D is absorbed by exposure to sunlight that includes ultraviolet B radiation; if ultraviolet B radiation is not available in sufficient amounts, vitamin D needs to be obtained from the diet or dietary supplements [4]. Vitamin D is needed to be increasing the ability to absorb and maintain calcium concentrations within a narrow physiological range that is essential for a large variety of cellular and metabolic processes in the body [5]. Also, vitamin D is one of the most important regulators of calcium and phosphorus homeostasis. It also plays many roles in cell differentiation and in the secretion and metabolism of hormones, including parathyroid hormone and insulin [5]. Some researchers have indicated that vitamin D might help prevent some diseases such as fibromyalgia, rheumatoid arthritis, multiple sclerosis, upper respiratory tract infections, premenstrual syndrome, polycystic ovary disease, psoriasis, muscle weakness, lower back pain, diabetes, high blood pressure, cancer [5], and asthma [6]. Severe vitamin D deficiency produces the bone disease called rickets in infants and children, and osteomalacia in adults, conditions which are characterized by the failure of the organic matrix of bone to calcify. The global prevalence of vitamin D deficiency is uncertain, but it is likely to be fairly common worldwide, and especially among infants and young children, the elderly and those living at high latitudes where daylight hours are limited in the winter months [3].

Vitamin D synthesis in the skin will also be inadequate if the body is consistently covered by clothing, a probable factor in the high prevalence of deficiency among veiled women and their breast-fed infants and children [8].

Vitamin D fortification policy for fluid milks is taken into account at amount of 1 µg/100 g in Finland, United States, and Canada [9]. It is reported that vitamin D fortification of milk reduces the risk of osteoporosis in the elderly, especially in higher latitude regions where levels of incident ultraviolet light are lower during the winter months [9, 10].

Fortification is often more cost-effective than other strategies, especially if the technology already exists and if an appropriate food distribution system is in place [11]. Although it is generally recognized that food fortification can have an enormous positive impact on public health, there are, however, some limitations to this strategy for micronutrient malnutrition control [12]. The nature of the food vehicle, and/or the fortificant, may limit the amount of fortificant that can be successfully added. For example, some iron fortificants change the color and flavour of many foods to which they are added [12]. Vitamin D will not be significantly damaged by most low-heat cooking techniques. Food fortification is a good way to improve vitamin D intake of a population [11].

The aim of this study was to fortification level of vitamin D3 to increases its intake from consuming sunflower oil without compromising safety. For this purpose, fortified sunflower oils with different concentrations of vitamin D3 (0.1 and 0.9 µg/mL) were prepared and exposed to different temperature (70, 110, and 130 °C) for 2, 4, and 6 hours. Also, sunflower oil containing 1 and 10 µg/mL of vitamin D3 were treated at temperature of 180 °C for 15 minutes and decomposed compounds derived from treated fortified sunflower oil at 180 °C were detected using GC-MS analysis.

## 2 Materials and Methods

### 2.1 Materials

Vitamin D3 (powder), vitamin D2 (powder) and BHT (KOH 50%) were purchased from Sigma-Aldrich company. Refined, bleached, deodorized vegetable oil samples of sunflower were provided by Kouros Food Industry (Safadasht, Iran). Ethanol and methanol and other chemicals were from Merck (Germany). All materials and reagents were of analytical grade, and double distilled water was used for preparation of solutions. TLC silica gel 60G F254 25 plates 20 × 20 cm were obtained from Merck (Berlin, Germany).

### 2.2. Preparation of stock standard solutions, internal standard solutions and working standard solutions of vitamin D3

For preparing the stock solution of vitamin D3 or vitamin D2 (1 mg/mL), 100 mg of vitamin D3 or vitamin D2 was dissolved in 100 mL ethanol in a volumetric flask separately and kept in a dark glass bottle. A standard solution containing 10 µg/mL of methanolic solution of vitamin D3 or vitamin D2 was prepared from each stock solution by dilution. For preparing the internal standard solution of vitamin D2 and vitamin D3, 10 mL of a standard solution was dissolved in ethanol in a volumetric flask (100 mL). These solutions should be prepared freshly on the

day of use. Working standard solutions of vitamin D3 were prepared by transferring internal standard solutions into a series of volumetric flasks and dilution them in the ethanol to obtain 0.1, 0.3, 0.6, 0.9, 1, 5 and 10 µg/mL of vitamin D3. Then, these solutions of vitamin D3 were injected to HPLC for obtaining calibration curve.

In addition, other calibration curve was prepared at concentrations of 0.1, 0.3 and 0.9 µg/mL of vitamin D3 in sunflower oil (as a real matrix) which recoveries, and LOD and LOQ were estimated using this calibration curve.

## 2.3. Methods

### 2.3.1. Fortification of sunflower oil

For fortification of sunflower oil, concentrations of 0.1, 0.3, 0.9, 1, and 10 µg/mL of vitamin D3 were used. The of recovery percentage of vitamin D3 in the sunflower oil containing 0.1, 0.3 and 0.9 µg/mL of vitamin D3 were determined. The effects of time and temperature (cooking temperatures) on vitamin D3 content of fortified sunflower oil (0.1 and 0.9 µg/mL) were studied. Also, the effect of the deep-frying temperature on vitamin D3 content of sunflower oil samples in concentrations of 1 and 10 µg/mL of vitamin D3 was investigated. Each fortified sample was well mixed and shaken at 400 rpm for 10 min at room temperature. The sunflower oil without vitamin D3 was used as the blank sample.

### 2.3.2. Effect of different cooking temperatures on degradation of vitamin D3

The reduction rate of added vitamin D3 (in concentrations of 0.1 and 0.9 µg/mL) in fortified sunflower oils was determined during the different cooking temperatures at 70°C, 110°C and 150 °C. The selected temperatures were set using a heat sensor and a thermometer for further control of temperature. The temperature in the middle of the samples were measured by a digital thermometer (Tešto 735-2, Tešto AG, Lenzkirch, Germany). After 0, 2, 4 and 6 hours, the sampling was done for assessment of vitamin D3 reduction. Vitamin D3 extraction was performed as the described steps in the 2.3.4. section. The extracted vitamin D3 was injected to the HPLC.

### 2.3.3. Effect of deep-frying temperature on reduction of vitamin D3 in fortified sunflower oils

Effect of deep-frying temperature (180 °C) on vitamin D3 reduction in the fortified sunflower oils containing 1 and 10 µg/mL of vitamin D3 was investigated. For this purpose, some chipped potato was added to hot fortified sunflower oil containing 1 or 10 µg/mL of vitamin D3. After frying potatoes, the amount of remained vitamin D3 in the cooled fortified sunflower oil and cooled fried

chipped were determined. For extraction of vitamin D3 absorbed by fried chipped potatoes, 100 mL hexane was added to the fried potatoes, shaken for 24 hours at 4°C in the dark place and then, passed through the folded filter paper. The filtrate was evaporated by a rotary evaporator under vacuum condition. The vitamin D3 in the cooled remaining oil was determined as described in the 2.3.4. Each treatment was performed in triplicate.

### 2.3.4. Extraction and determination of vitamin D3 in oil samples

Vitamin D3 was determined in the samples by high performance liquid chromatographic (HPLC) [13]. All internal standard solutions and working standard solutions were prepared based on the descriptions in DIN EN 12821 - 2009-08 [13]. Extraction and determination of vitamin D3 in the fortified sunflower oil were performed according to Rashidi et al., [14]. Briefly, 24 g of sunflower oils containing vitamin D3 were weighed. Then 0.5 g of sodium L-ascorbate, sodium sulfide (0.04 g), 1mL of vitamin D2 (1 µg/mL solution) as an internal standard, and 90 mL of ethanol were added to each weighted fortified sunflower oil and mixed completely. Thirty mL of potassium hydroxide solution (50 % (w/w)) was added and the mixture solution saponified for 45 minutes at 100 °C. Then, the liquid- liquid extraction was used for extraction of remained vitamin D3 using a decanter and diethyl ether (for each extraction, 100 mL) and also, water added for washing of saponified fraction completely until it became neutral. The extracted diethyl ether was evaporated in a rotary at 25 °C. Then, 2 mL of hexane was added to remained extract samples and spotted on the TLC plate (20× 20 cm) by a syringe (TLC was treated in a developing tank containing hexane/methanol/acetone (10:2:88) and BHT (KOH 50%) before using TLC for separation of vitamin D3). The area of vitamin D3 was recognized by the UV lamp. This area was scratched off and eluted by the mixture solution of hexane and diethyl ether (60:40) five times using a glass chromatographic column. After washing the column, the filtrate solution was evaporated under vacuum at 25 °C. Then, 2 mL of hexane was added to the residual material and 20 µL of it was injected to HPLC-UV. Vitamin D3 content was determined by reversed phase HPLC equipped with an UV detector. A Yung Lin 9120 HPLC system (Korea) equipped with a C18 column (250 mm × 4.6 mm, 5 µm) was applied. The mobile phase was methanol (99.999%) with a flow rate of 1 mL/min at ambient temperature. The working standard solutions of vitamin D3 were injected too. Vitamin D3 was detected at 265 nm. A four-point calibration curve was obtained for vitamins and checked for linearity and used for quantification of vitamin D in the

samples. LOD and LOQ were determined by using standard deviation. Also, the recoveries of vitamin D3 were determined by spiked vitamin D3 in the fortified samples. LOD and LOQ were calculated according to the standard deviation ( $\sigma$ ) of lowest concentration and slope (S) of the calibration curve (Eqs. 1 and 2).

Extraction recovery (ER %) was estimated by Eq. (3), where Cr experimentally determined concentration in fortified samples (Cr=0 in blank samples), and Cf is the final concentration after extraction.

$$\text{LOD} = 3 \times \sigma S \quad (1)$$

$$\text{LOQ} = 10 \times \sigma S \quad (2)$$

$$\text{ER}\% = \frac{C_f - C_r}{C_f} \times 100 \quad (3)$$

Vitamin D3 and D2 concentrations (in micrograms per gram) were calculated by comparing peak heights or peak areas of vitamins in the test samples with controlled standards. Also, the average recoveries and relative standard deviation of the analytical methods applied for vitamin D3 in oil samples were investigated.

### 2.3.5. Detection of vitamin D3 degradation compounds using GC-MS

Extraction of vitamin D3 from the oil sample after deep

frying which contained 10  $\mu\text{g}/\text{mL}$  of vitamin D3, and the extraction of sunflower oil without vitamin D3 as a blank sample were investigated by GC-MS. A GC system: 7890A from Agilent technologies, mass selective detector: 5975C VL MSD with triple-axis detector, electron impact (EI) with quadrupole analyzer and Rtx 5 MS column (30 m  $\times$  0.250 mm  $\times$  25  $\mu\text{m}$ ) at 250  $^\circ\text{C}$  for injection port and 230  $^\circ\text{C}$  for ion source and He as a carrier gas was used [15].

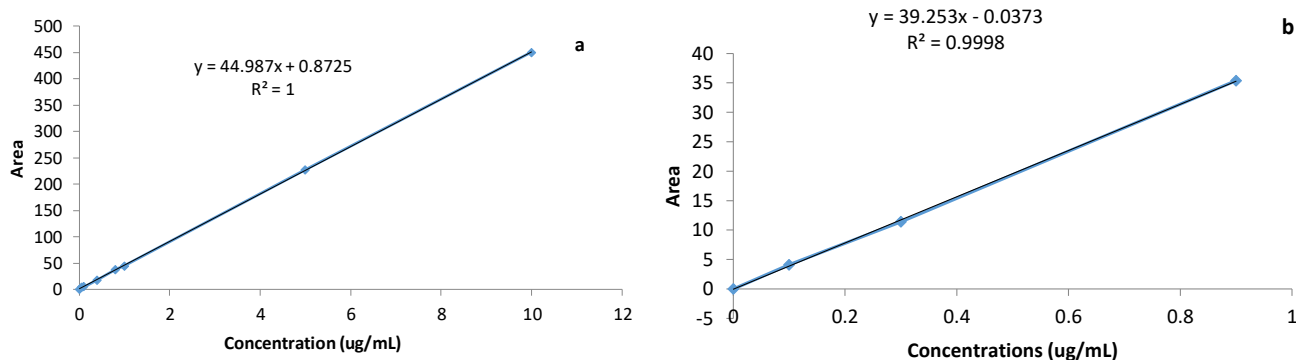
### 2.3.6. Statistical analysis

The one-way analysis of variance (one-way ANOVA) was applied for evaluation of the experimental data and statistical analyses, for a confidence interval of 95%. All tests were performed in triplicates.

## 3. Results and discussion

### 3.1 Determination of recovery of vitamin D3 in control samples

Fig. 1 shows the six-point calibration curve of vitamin D3 in ethanol in concentrations of 0.1, 0.3, 0.9, 1, 5, and 10  $\mu\text{g}/\text{mL}$  (a), and fortified sunflower oil with vitamin D3 in concentrations of 0.1, 0.3, and 0.9  $\mu\text{g}/\text{mL}$  (b).



**Figure 2.** Calibration curves of vitamin D3 in the ethanol (a) and in the fortified sunflower oil (b)

**Table 1.** The extraction recovery percentages of vitamin D3

The concentration of added vitamin D3 into sunflower oil ( $\mu\text{g}/\text{mL}$ )	The concentration of determined vitamin D3 by HPLC ( $\mu\text{g}/\text{mL}$ )	Extraction Recovery (%)
0.1	0.071 $\pm$ 0.01	71.00 $\pm$ 0.05
0.3	0.227 $\pm$ 0.03	78.12 $\pm$ 0.03
0.9	0.778 $\pm$ 0.09	85.02 $\pm$ 0.01

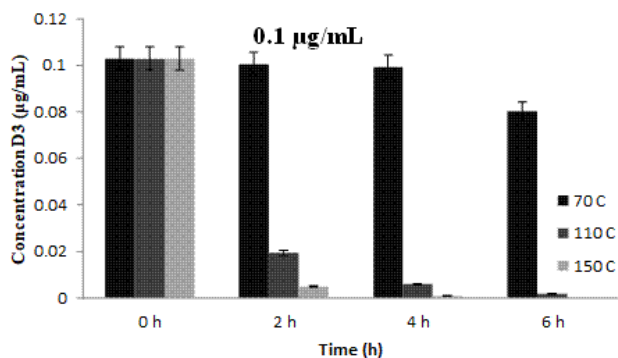
The extraction recovery percentages of Vitamin D3 in the samples fortified with 0.1, 0.3, and 0.9  $\mu\text{g}/\text{mL}$  of vitamin D3 were obtained from 71 to 85% (Table 1). In addition, LOD and LOQ were obtained 0.03 and 0.108  $\mu\text{g}/\text{mL}$ , respectively.

### 3.2 Effect of heating time and temperature on vitamin D3 reduction

The concentration of vitamin D3 in the fortified sunflowers was determined after heating treatment which sampling was performed in the defined times (0, 2, 4, 6 hours) and temperatures (70, 110 and 150  $^\circ\text{C}$ ) (Fig. 3). Results showed that in the fortified sample with 0.1  $\mu\text{g}/\text{mL}$  of vitamin D3 at different temperatures, more than 80% of vitamin D3 lost after 2 hours at 110  $^\circ\text{C}$  and 150  $^\circ\text{C}$  while at 70 $^\circ\text{C}$ , vitamin D3 has not been reduced significantly. The reduction of vitamin D3 at 70  $^\circ\text{C}$  was reached lower than 0.10  $\mu\text{g}/\text{mL}$  after 2 h, 0.099  $\mu\text{g}/\text{mL}$  after 4 h, and 0.08  $\mu\text{g}/\text{mL}$  after 6 h, which it showed that after 6h, only 20% of vitamin D3 was reduced. At 110  $^\circ\text{C}$ , the amounts of

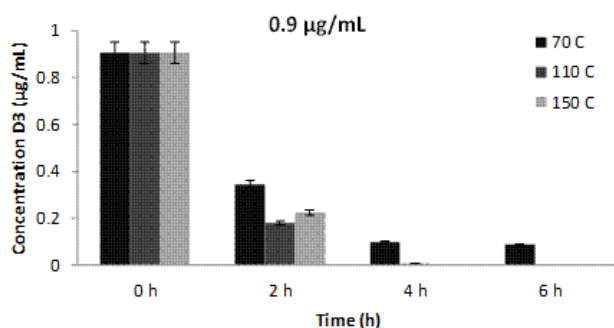


the remained vitamin D3 were obtained 0.019, 0.006, and 0.002  $\mu\text{g/mL}$  after passing time of 2h, 4h, and 6h, which respectively indicated 81%, 94%, and 98% of vitamin D3 reduction. At 150  $^{\circ}\text{C}$ , the amounts of the remained vitamin D3 were 0.005, 0.0, and 0.0  $\mu\text{g/mL}$ , after 2h, 4h, and 6h, respectively, which showed vitamin D3 was disappeared in the media.



**Figure 2.** Effects of time and temperature on the reduction of vitamin D3 in the fortified samples of sunflower at 0.1  $\mu\text{g/mL}$

Fig. 2 shows decreasing of vitamin D3 in the sunflower fortified with 0.9  $\mu\text{g/mL}$  at 70  $^{\circ}\text{C}$ , 110 $^{\circ}\text{C}$ , and 150 $^{\circ}\text{C}$ . Results showed that the amounts of vitamin D3 at 70  $^{\circ}\text{C}$  were decreased to 0.344  $\mu\text{g/mL}$ , 0.099  $\mu\text{g/mL}$ , and 0.087  $\mu\text{g/mL}$  after 2, 4, and 6 hours, respectively. At 110 $^{\circ}\text{C}$ , the concentration of vitamin D3 from 0.9  $\mu\text{g/mL}$  decreased to 0.18  $\mu\text{g/mL}$ , 0.008  $\mu\text{g/mL}$ , and 0.00  $\mu\text{g/mL}$ , while at 150  $^{\circ}\text{C}$ , the amount of vitamin D3 from 0.9  $\mu\text{g/mL}$  was decreased to 0.224  $\mu\text{g/mL}$ , 0.00  $\mu\text{g/mL}$ , and 0.00  $\mu\text{g/mL}$ , after 2h, 4h, and 6h, respectively, which showed that after 2 hours all of vitamin D3 was disappeared. All results showed that more than 80 % of vitamin D3 was lost at the temperature higher than 100  $^{\circ}\text{C}$ .



**Figure 3.** Effect of time and temperature on reduction of vitamin D3 in fortified samples of sunflower at 0.9  $\mu\text{g/mL}$

### 3.3 Effect of deep frying on reduction/destruction of vitamin D3 in sunflower oil samples

The reduction of fortified sunflower oil samples at con-

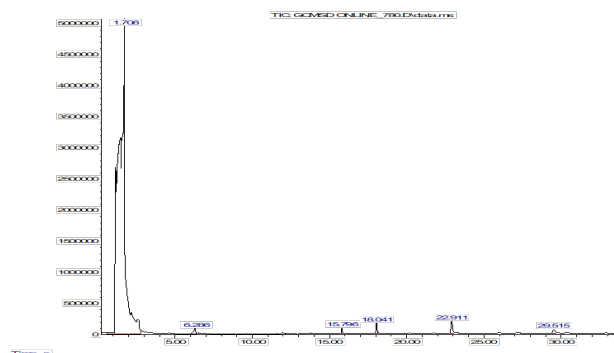
centrations of 1 and 10  $\mu\text{g/mL}$  of vitamin D3 after frying potatoes at 180  $^{\circ}\text{C}$  was investigated (Table 2). The results showed that the amount of absorbed vitamin D3 in the fried potatoes surface was more than remained vitamin D3 in the oil after frying of potatoes. After frying, about 37.7% of vitamin D3 was absorbed by the fried potatoes when the amount of added vitamin D3 into sunflower oil was 10  $\mu\text{g/mL}$  but for sunflower oil containing 1  $\mu\text{g/mL}$  of vitamin D3, 39.6% of vitamin D3 absorbed by fried potatoes. The results showed that the amount of remained vitamin D3 in the sunflower oil after frying was very low and more than 80% of vitamin D3 was lost after 15 minutes deep frying of potatoes.

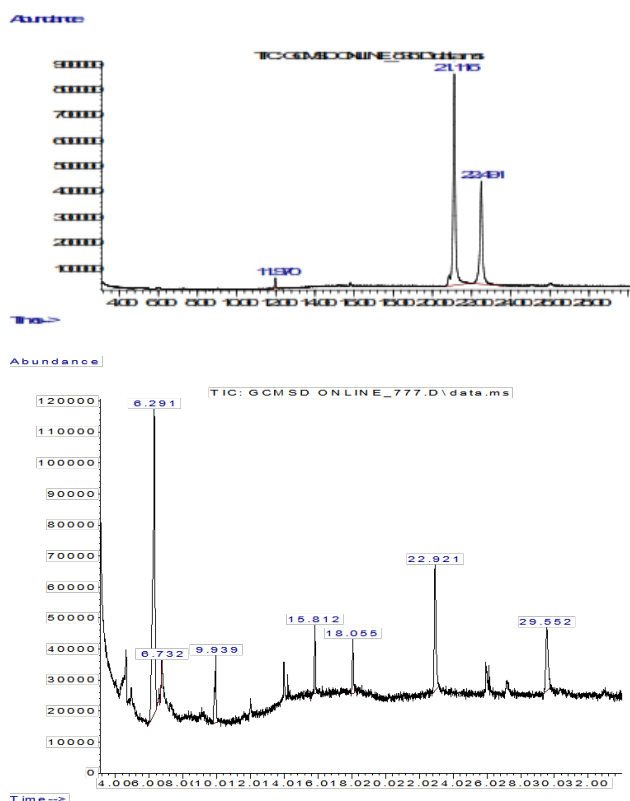
**Table 3.** The recovery percentages of vitamin D3 after deep-frying

Samples	Vitamin D <sub>3</sub> concentration ( $\mu\text{g/mL}$ )
Control sample containing 1 $\mu\text{g/mL}$ of vitamin D <sub>3</sub>	0.91 $\pm$ 0.08
Sunflower oil containing 1 $\mu\text{g/mL}$ of standard vitamin D <sub>3</sub> after frying of potatoes at 180 $^{\circ}\text{C}$	0.125 $\pm$ 0.09
Vitamin D <sub>3</sub> absorbed on the surface of fried potatoes (at concentration of 1 $\mu\text{g/mL}$ ) at 180 $^{\circ}\text{C}$	0.36 $\pm$ 0.04
Control sample containing 10 $\mu\text{g/mL}$ of vitamin D <sub>3</sub>	8.01 $\pm$ 0.55
Sunflower oil containing 10 $\mu\text{g/mL}$ of standard vitamin D <sub>3</sub> after frying of potatoes at 180 $^{\circ}\text{C}$	1.50 $\pm$ 0.10
Vitamin D <sub>3</sub> absorbed on the surface of fried potatoes (at concentration of 10 $\mu\text{g/mL}$ ) at 180 $^{\circ}\text{C}$	3.02 $\pm$ 0.15

### 3.4 Detection of compounds' consequent of decomposing of vitamin D3 in sunflower oil samples

By using GC-MS, Fig. 4a shows the chromatograph of sunflower oil without vitamin D3 which a small peak at retention time of 22,911 was attributed to vitamin E. Fig 4b shows the chromatograph of GC-MS related to the sunflower oil fortified by vitamin D3 (10  $\mu\text{g/mL}$ ). The retention time of vitamin D3 was detected at 21.115 min. The retention time of cholesterol was detected at 22.491. Fig. 4C is shown many peaks which confirmed degradation of vitamin D3. Two peaks including pyro" and "iso-pyro" cholecalciferol were observed at retention time of 15.81 and 18.05, respectively.





**Figure 4.** GC-MS chromatographs of (a) sunflower oil without vitamin D3 (b) fortified sunflower oil by vitamin D3, (c) fortified sunflower oil (10 $\mu$ g/mL) after deep-frying (at 180  $^{\circ}$ C)

#### 4. Discussion

In industrialized countries, food fortification is a good way to successful control of deficiencies of vitamins and minerals [16]. Vitamin D3 is a micronutrient that is needed for human health due to its role in regulation of calcium and phosphorus homeostasis and also in cell differentiation and in the secretion and metabolism of hormones, including parathyroid hormone and insulin.

Vitamin D3 can be either synthesized in the human skin upon exposure to the UV light of the sun, or it is obtained from the diet. Vitamin D deficiency causes the bone disease in infants and children, and osteomalacia in adults [17].

Most of the vitamin D is produced in the skin. But Vitamin D3 synthesis in the skin is inadequate especially if the body is consistently covered by clothing. In the elderly person, the ability of the skin to synthesize vitamin D decreases and dietary requirements for vitamin D3 are increased. But vitamin D from natural food usually supplies only a small fraction of the daily requirements for it. So, in industrialized countries most dietary vitamin D comes from fortified food [1]. The Codex general principles for

the addition of essential nutrients to foods (CAC/GL 07-1987, amended 1989, 1991) established a uniform set of principles for the rational addition of essential nutrients to foods. According to this guideline, the essential nutrients [18]:

- should be present at a level that will not result in an excessive or an insignificant intake of the added nutrient considering the amounts from other sources in the diet;
- should not result in an adverse effect on the metabolism of any other nutrient;
- should be sufficiently stable in the food during packaging, storage, distribution and use;
- should be biologically available from the food;
- should not impart undesirable characteristics to the food, or unduly shorten its shelf-life.

So, it is important to take into account how much of the supplied vitamin D will be absorbed. Vegetable oils can be considered to be potential candidates for fortification with vitamin D especially vitamin D3. Exposure to high temperatures can cause adverse effects in vitamin D3 [19] content of food products and determination of the stability of added vitamin D3 in vegetable oils during cooking procedures is essential. In this study, we compared the effect of three different temperatures (70, 110 and 150  $^{\circ}$ C) after 2, 4 and 6 hours of cooking fortified sunflower oil at concentrations 0.1 and 0.9  $\mu$ g/mL of vitamin D3. Heating at 70  $^{\circ}$ C for 2 hours didn't show any destructive effect on vitamin D3. Results of our study were consistent with other research that showed the heat treatment above 100  $^{\circ}$ C, caused some decomposition in vitamin D3 content of cooked foodstuffs [20]. Also, it is recommended to investigate the effect of storage time on stability of fortified vegetable oils with vitamin D3 and vitamin D concentrations in other fortified foods and dietary supplements for determination vitamin D intake. In a study, different parameters including temperature, iodine, acidic conditions, and oxidation on the isomerization of vitamin D3 were studied by HPLC-DAD and UHPLC-MS/MS which results showed vitamin D3 reversibly and thermally changed to pre-vitamin D3 type isomers [20]. The effect of processing and storage on the fortified wheat flour and oil by vitamin D and B12 were also, investigated, and results showed that retention of vitamin D3 was 10% during frying which was agreed with our results [21]. Effects of time and temperature on stability of fortified vegetable oil with vitamin D3 during cooking temperature (at 105  $^{\circ}$ C for 0, 60, 120, and 180 minutes) were investigated by Saghafi et al., (2018) [22]. Results showed that retention rate of added vitamin D3 in sunflower, corn and canola oils during cooking temperature modified 68.6% to 87.4% [21] which were different compared with our study. It may be that the procedure of vitamin D3 extraction and analysis were different. Also, Zareie, Abbasi and Faghieh (2019)

studied thermal stability and reduction rate of vitamin D3 in canola oil during heating process (100, 150, and 180°C) for 30 minutes using the HPLC. They obtained the thermal kinetic degradation of vitamin D3 in the fortified canola oil samples. They reported that vitamin D3 had no significant reduction at 100°C but the fortified samples treated at 150 and 180°C depending on the initial amount of vitamin D3 indicated the maintenance of 67.5% to 72.97% and 33% to 40.35% of vitamin D3, respectively [23].

Vitamin D2, D3 and their 25-hydroxy metabolites were determined in veterinary feeds (incubated at 70°C using Gas Chromatography Tandem Mass Spectrometry (GC/MS/MS) which results showed that dog foods had 44% toxic amounts of vitamin D3. In addition, the small levels of pyro-, isopyro-, and iso-vitamin D and isotachysterol forms were detected from each vitamin D-related compound upon extraction and GC/MS/MS analysis [24].

### Conclusion

This study has shown that the stability of added vitamin D3 in sunflower oil depends on the range of natural vitamin D3 retention in cooking of foodstuffs and temperature. Regarding to our results, vitamin D3 was stable at temperature lower than 70 °C and more than 80% of it decomposed after heating at temperature higher than 100 °C for two hours. In frying temperature (180 °C), more than 60% of vitamin D3 was destroyed during 15 min, but in short time frying vitamin D3 absorbed to food material such as potatoes. Vitamin D3 is unstable, and it can be used in cooking process below temperature of 100°C. Also, it is recommended to determine the amount of claimed vitamin D3 added to the fortified foods and dietary supplements for determination vitamin D intake. In addition, the allowance limit for adding this vitamin for a defined food should be specified in the national standards.

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### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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