

Functional approach to D-fortification of sunflower oil as a factor of nutritional compensation of vitamin deficiency and immunoprophylaxis

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Abstract.In modern conditions of the spread of new strains of microorganisms, along with sanitary and hygienic issues of prevention, measures aimed at immunoprotection play an important role. Insufficient micronutrient supply of vitamin D contributes to a high susceptibility of a person to an infectious agent. Insufficient supply of vitamin D contributes to a high human susceptibility to an infectious agent. Adequate compensation for chronic deficiency of fat-soluble vitamin D in food belongs to a promising field of nutritionology. In the structure of oilseed production of Russian farmers, about 60% is occupied by sunflower oil, which practically lacks vitamin D. The development of fortified sunflower oil that compensates for this need solves a two-pronged task: as a preventive measure and as a biologically valuable food product. According to the research results, it has been established that the fortification of unrefined sunflower oil with the addition of 0.04... 0.20 ml of vitamin D3 oil solution with an activity of 50,000 M.E. provides coverage of 10-50% of the daily requirement for vitamin D3. In the samples of unpacked unrefined sunflower oil, the indicators of oxidative spoilage during storage up to 60 days corresponded to the normalized requirements for premium vegetable oil with lower indicators with an increase in the amount of vitamin D3 oil solution added.

1Introduction

Accumulating evidence from scientific research shows the role of vitamin D in various physiological functions of the human body, when its insufficient intake can provoke complete or partial suppression of functions leading to osteoporosis, rickets, calcium-phosphorus imbalance, cardiovascular and neurological diseases, endocrine disorders, as well as the severity of the coronavirus infection COVID-19 [1-5]. A number of authors emphasize [6] that vitamin D deficiency can negatively affect the course of the disease.

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During infectious diseases and in the early stages of COVID-19, vitamin D intake can be considered as a new immune protective strategy. Currently, it is known that about 1 billion inhabitants of the globe (about 12% of the population) suffer from vitamin D deficiency or deficiency [7, 8]. In Russia, 80% of the population is deficient in vitamin D, against which there may be a decrease in the human body's resistance to bacterial and viral diseases [9, 10]. Vitamin D with the regulatory function of immunity [11], along with folic acid and omega-3 fatty acids, improves the reproductive functions of women [12] and promotes bone mineralization in the postoperative period [13]. It is used in the diet to prevent the development of Alzheimer's disease [14] and endothelial dysfunction of the lower urinary tract [15]. At the same time, up to 91.4% of vitamin D comes from food and 8.6% comes from dietary supplements.

The first mention of food fortification dates back to 4000 BC. In the United States, vitamin D was added to milk to control its deficiency in the diet of people [16]. The consumption of animal products (eggs, dairy products, fish,) and plant foods (mushrooms, cabbage, zucchini, lettuce, spinach, etc.) rich in vitamin D does not fully satisfy the physiological need, which is 10 mcg per day for children and adults, for people over 60 years of age - 15 mg/day, according to MP 2.3.1.2432-08 "Norms of physiological energy and nutrient requirements for various population groups of Russian Federation".

The recommended daily dose of vitamin D in other countries is almost the same. The International Osteoporosis Foundation (IOF) developed dietary recommendations for vitamin D intake for the elderly in 2010 [17]. The American Society of Endocrinologists (2011) recommends a daily vitamin D intake of 400 IU for infants under 1 year old, 600 IU for children over 1 year old and adults under 70 years old, 800 IU for adults over 70 years old. According to the clinical recommendations of the American Society of Endocrinologists (2011) and the Global Consensus Recommendation on the Prevention and Treatment of Foodborne Rickets (2016), it is recommended to take 600 IU/ day to ensure the formation of normal human bone tissue [7, 18]. The maximum permissible intake of vitamin D for the population of different age groups was determined by the European Food Safety Authority (EFSA) in 2012 [19]. In Europe, according to the practical recommendations on vitamin D intake and treatment of its deficiency in Central Europe (2013), the daily norms for infants are similar - 400-600 IU/day, for children from 2 to 18 years old, the daily norm is from 600 to 1000 IU/ day, depending on age. depending on body weight, for adults it is 800-1000 IU/day [20].

In Russia, especially in regions with insufficient solar activity, there is a deficiency of vitamin D, the lack of which can lead to various diseases. The use of vitamin complexes for medical reasons makes up for the lack of vitamin D [21]. Adequate compensation for chronic vitamin D deficiency refers to immunoprophylaxis measures in Russian Federation [22].

Vegetable oils extracted from seeds of different oilseeds differ in the content of fat-soluble vitamins A and E, but do not contain vitamin D. In the medium term, the role of nutritionology in choosing a rational nutrition strategy is increasing, which requires a meaningful approach to the development of functional food products. A promising direction is the enrichment of vegetable oils with fat-soluble vitamin D, consisting of up to 98% of triglycerides of fatty acids. The development of an enriched vegetable oil that compensates for this need for vitamin D achieves a dual goal: for prevention and at the same time, the oil is a biologically valuable food product. The enrichment of vegetable oils with fat-soluble vitamins helps to make up for the deficiency of vitamins in the human diet.

According to [23], it is possible to provide from 3.9% to 21% of the estimated daily requirement as a result of fortification of widely consumed food products, such as vegetable oil, at the level of 10 IU g/100 g. Russia occupies a stable position in the world as the largest producer of sunflower oil. Unrefined sunflower oil, valuable as a food product due

to the content of phospholipids and free fatty acids, is used for direct consumption, food production and industrial processing. When storing vegetable oil, the key conservation factors are the air temperature, the darkened storage areas and the lack of oxygen [24, 25]. Unrefined sunflower oil has a short shelf life of up to 4 months, and unpackaged oil has a shelf life of up to 1.5 months. It is necessary to continue modeling quantitative consumption indicators and conduct a study on the safety of valuable fat-soluble vitamin during oil storage. The aim of the study is to develop a new functional food product – unpackaged unrefined sunflower oil enriched with vitamin D3, with an extended shelf life and functional value. Mixing oils or adding vegetable components to ready-made edible oil is most often used in the fat and oil industry to increase nutritional value and extend shelf life. The issues of product enrichment are of a point-by-point experimental nature. The scientific novelty of the work is the proposed technology for the production of unrefined sunflower oil enriched with vitamin D3 for industrial production and expansion of the range of functional food products. The proposed fortified oil can be used to make up for vitamin D3 deficiency. Such oil, being a product of mass consumption, increases the body's resistance to bacterial and viral diseases.

2 Materials and Methods

The object of the study was unrefined sunflower oil of the highest grade of industrial production, obtained by pressing at a fat processing plant. The samples were divided into 6 groups: No. 1 – control group (samples of unrefined sunflower oil); № 2, 3, 4, 5 and 6 are experimental groups. The experimental groups consisted of samples of unrefined sunflower oil, to which an oil solution of vitamin D3 with an activity of 50,000 IU/ml was added at the rate of 0.04 ml; 0.08 ml; 0.12 ml; 0.16 ml and 0.20 ml per 1 liter. The calculation scheme of oil enrichment is based on the forecast calculation of coverage of the daily requirement for vitamin D3 from 10 to 50 % when using 20 g of unrefined sunflower oil.

The quality of the unrefined sunflower oil samples was assessed by organoleptic and physico-chemical parameters. The following research methods were used: acid number – by titration of free fatty acids; peroxide content – by titrimetric method; fatty acid composition – by gas chromatography; fat-soluble vitamins content - by high-performance liquid chromatography in the ultraviolet (UV) spectral region with a given wavelength. The peaks of the solutions of the vitamin samples of the control and tested samples on the chromatogram were identified by the coincidence of the retention time with the retention time of the peaks of vitamins on the chromatograms of standard solutions under the same chromatographic conditions; the content of vitamins A, E and D – by high-performance liquid chromatography (HPLC). The liquid chromatography method is also the most effective according to researchers [26]. The measurements were carried out using a four-channel modular system Agilent 1260 Infinity II (Agilent Technologies, CIIIA). Elution was performed under isocratic conditions for 11 minutes. The determination of each fat-soluble vitamin was carried out separately: vitamin A was determined at a wavelength of 325 nm, vitamins D and K1 – at 270 nm, vitamin E - at 285 nm. It has been experimentally established that it is possible to detect and achieve selective separation of vitamins at a detection wavelength of 270 nm with the formation of specific spectra and retention time of 2.51 ± 0.03 min for vitamin A, 8.52 ± 0.02 min for vitamin D, 9.63 ± 0.04 min for vitamin K, 10.34 ± 0.05 min for vitamin E. The studies were conducted in a laboratory with artificial lighting at an air temperature of $(17 \pm 1)^{\circ}\text{C}$. During the experiment, darkened laboratory glassware was used. The obtained results were processed using standard MS software packages and the method of variation statistics. Quantitative indicators of vitamin content were determined using software Agilent OpenLab CDS. The studies were carried out in five-fold repetition

3 ResultsandDiscussion

According to organoleptic parameters, the control and experimental groups of oils met the standard requirements: the oil is transparent, with slight turbidity, the smell and taste of the oil are corresponding to sunflower unrefined oil, without foreign smell and taste. The indicators of oxidative spoilage were evaluated during the storage period after 45 days; 52 days and 60 days according to the requirements of MUC 4.2.1847-04 "Control methods. Biological microbiological factors. Sanitary and epidemiological assessment of the validity of shelf life and storage conditions of food products. Methodological guidelines". The acid and peroxide numbers in all the studied samples on the 60th day of storage were within the normal range, with a higher indicator in the samples of the 1st control group: 1.69 ± 0.04 mg KOH/g and 8.32 ± 0.12 mmol ac. oxygen/ kg, respectively, as shown in the graphs (Fig. 1 and 2).

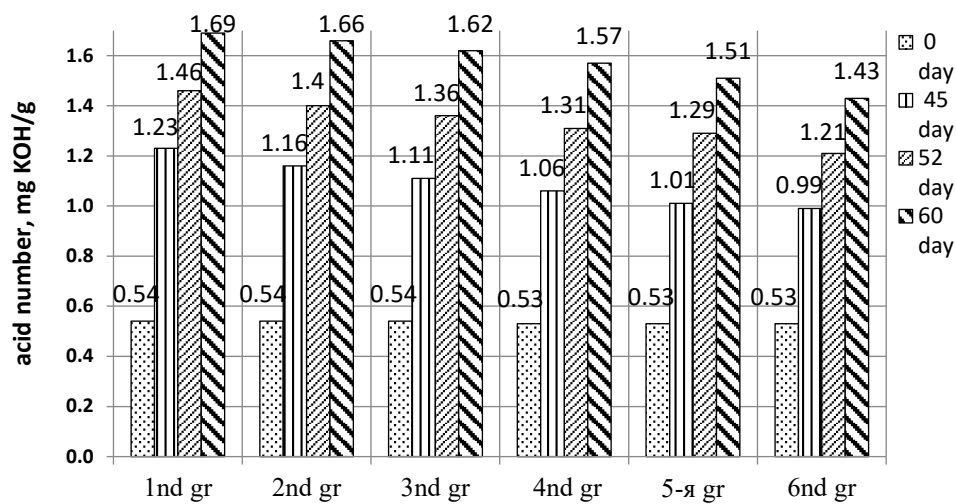


Fig. 1. Dynamics of changes in the acid number in oil samples, mg KOH/g.

During storage up to 60 days. in the control samples, the acid number increased by 3.13 times and in the experimental samples by 2.70-3.07 times. As a result of the addition of an oil solution of vitamin D3 to the test samples, a slowdown in the activity of hydrolytic enzymes and the formation of free fatty acids is observed: the acid number in group 2 was 1.66 ± 0.05 mg KOH/g; in group 3 – 1.62 ± 0.06 mg KOH/g; in group 4 – 1.57 ± 0.04 mg KOH/g; in group 5 - 1.51 ± 0.04 mg KOH/g and in group 6 – 1.43 ± 0.02 mg KOH/g with high confidence 0.997-0.999 ($p \leq 0.05$) (Fig.2).

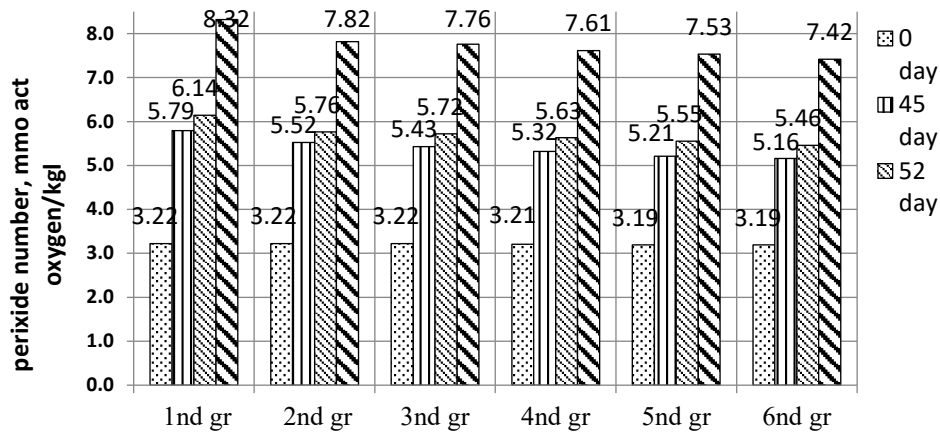


Fig. 2. Dynamics of changes in the peroxide number in samples of unpacked unrefined sunflower oil, mmol act. oxygen/ kg.

The peroxide number during storage is up to 60 days. in the control samples, it increased by 2.58 times and in the experimental samples – by 2.33-2.43 times. As a result of the addition of an oil solution of vitamin D3 to oil prototypes, a slowdown in oxidative processes and the accumulation of peroxide compounds and the formation of free fatty acids in vegetable oil prototypes is observed: the peroxide number in the 2nd group was 7.82 ± 0.12 mmol ac. oxygen/ kg; in the 3rd group - 7.76 ± 0.16 mmol ac. oxygen/ kg; in the 4th group – 7.61 ± 0.08 mmol ac. oxygen/ kg; in the 5th group – 7.53 ± 0.11 mmol act. oxygen / kg and in the 6th group - 7.42 ± 0.12 mmol act. oxygen / kg with a high confidence of 0.997-0.999 ($p \leq 0.05$).

In the experimental groups, with an increase in the amount of vitamin D3 oil solution added, a decrease in the peroxide number was recorded. At the same time, during 45 days of storage, the peroxide content increased by 6.5% from 5.52 ± 0.08 mmol ac. oxygen/kg in group No. 2 to 5.16 ± 0.07 mmol ac. oxygen/kg in group No. 6. Over 52 days of storage, the peroxide content increased by 5.2% from 5.76 ± 0.10 mmol ac. oxygen/kg in group No. 2 to 5.46 ± 0.10 mmol act. oxygen/kg in group No. 6. Over 60 days of storage, the peroxide content increased by 5.1% from 7.82 ± 0.12 mmol act. oxygen/kg in group No. 2 to 7.43 ± 0.12 mmol act. oxygen/kg in group No. 6 with a high correlation stability of 0.951-0.993 ($p \leq 0.05$).According to the fatty acid composition, unrefined sunflower oil belonging to the oleic group was characterized by an increased content of omega-6 fatty acids (linoleic acid), necessary for the formation of the human immune system (Table 1).

Table 1. Structure of fatty acid composition of unrefined sunflower oil, % (n=5, $p \leq 0.05$)

Name of the fatty acid	Content	Name of the fatty acid	Content
Myristic C _{14:0}	0.15±0.01	Oleic C _{18:1}	33.34±0.09
Palmitic C _{16:0}	6.66±0.03	Gondoic C _{20:1}	0.08±0.01
Stearic C _{18:0}	2.92±0.03	Erucic C _{22:1}	0.10±0.01
ArachinicC _{20:0}	0.36±0.02	Monounsaturated fatty acids (MUFA), total	33.70±0.08
BegenicC _{22:0}	0.83±0.01	Linoleic C _{18:2}	54.62±0.11
Lignoceric C _{24:0}	0.56±0.02	Alpha-linolenic C _{18:3}	0.20±0.02
Saturated fatty acids (SFA), total	11.48±0.02	Polyunsaturated fatty acids (PUFA), total	54.82±0.08
Palmitoleic C _{16:1}	0.18±0.01		

The separation of fat-soluble vitamins in the isocratic mode of operation of the chromatograph was established (Fig. 3). The obtained chromatogram peaks were compared with peaks of standard solutions of vitamins of certain concentrations in the automatic mode. For this, the program of processing of chromatographic data according to a complete set of the chromatograph was used.

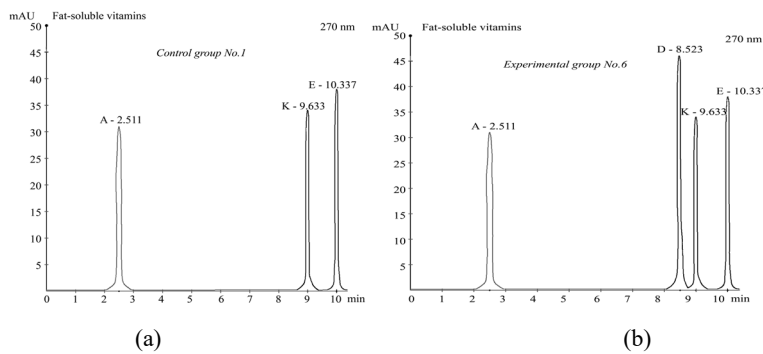


Fig.3. Chromatogram of sunflower oil samples from control group No. 1 (a) and experimental group No. 6 (b).

The control and experimental samples of vegetable oil did not show any significant differences in the content of vitamins A, E, and K1 (Table 2).The addition of vitamin D to the experimental samples of vegetable oil did not affect the change in the content of vitamins A, E and K compared to the control samples. The measurement error in the control and experimental samples of different groups was within the confidence limits of errors.

Table 2. Vitamin content in the control and experimental groups of unrefined sunflower oil (n=5, p < 0.05)

Groups	Vitamin A, mcg per 100 g	Vitamin E, mg per 100 g	Vitamin K ₁ , mcg per 100 g	Vitamin D ₃ , mcg per 100 g
Control No.1	0.02±0.01	42.30±0.56	5.60±0.11	-
No.2	0.02±0.01	42.30±0.49	5.60±0.09	5.01±0.01
No.3	0.02±0.01	42.30±0.52	5.60±0.10	9.98±0.02
No.4	0.02±0.01	42.30±0.53	5.60±0.09	15.03±0.04
No.5	0.02±0.01	42.30±0.51	5.60±0.09	19.89±0.02
No.6	0.02±0.01	42.30±0.55	5.60±0.09	24.96±0.07

The results obtained showed, that vitamin D₃ was not detected in the samples of the control group No.1. The samples of the experimental group No.2 contained 5.00-5.02 µg of vitamin D₃ per 100 g of oil, which provided a 10% daily requirement for this vitamin. The samples of the experimental group No.3 contained 9.96-10.01 µg of vitamin D₃ per 100 g, which provided 20% of the daily requirement. The samples of the experimental group No.4 contained 14.99-15.07 µg of vitamin D₃ per 100 g, which provided 30% of the daily requirement. The samples of the experimental group No.5 contained 19.87-19.91 µg of vitamin D₃ per 100 g, which provided 40% of the daily requirement. The samples of the experimental group No.6 contained 24.89-25.03 µg of vitamin D₃ per 100 g, which provided 50% of the daily requirement. The obtained samples of unrefined sunflower oil enriched with vitamin D₃ met the requirements of TR TS 024/2011.

At the same time, after 60 days of storage of unpackaged unrefined sunflower oil, all samples showed a decrease in the content of vitamin D₃: by 6.79% to 4.67 ± 0.1 µg per 100

g in the group No.2; by 3.41% to 9.64 ± 0.02 µg per 100 g in the group No.3; by 3.13% to 14.56 ± 0.04 µg per 100 g in the group No.4; by 2.21% to 19.45 ± 0.02 µg per 100 g in the group No.5, and by 2.16% to 24.42 ± 0.06 µg per 100 g in the group No.6 with high reliability of 0.95-0.99 ($p \leq 0.05$) (Fig. 4). This could be explained by the natural storage conditions (under the influence of natural light and air oxygen). The obtained data are comparable with the results of [21].

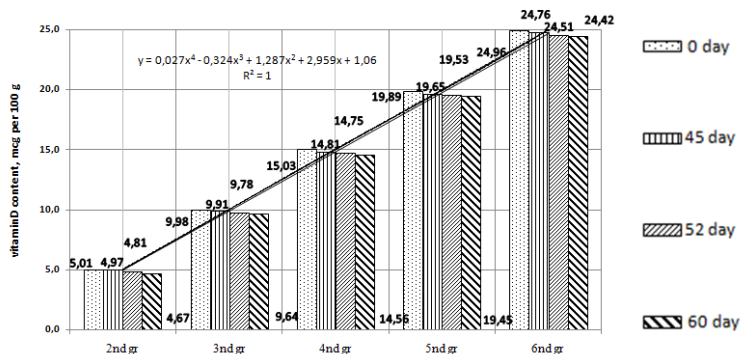


Fig. 4. Vitamin D3 content in unrefined sunflower oil samples during storage ($n=5$, $p \leq 0.05$)

The polynomial model is presented in the form of the following equation:

$$Y = 0.027 \cdot X^4 - 0.324 \cdot X^3 + 1.287 \cdot X^2 + 2.959 \cdot X + 1.06 \quad (1)$$

Several authors [27, 28], noting the importance of following a healthy diet, which has a multicomponent protective effect on human health, determine that a healthy diet alone cannot provide protection against the penetration of the virus into the body. Instead, they argue that a balanced and nutritious diet creates the conditions for the formation of a timely and adequate immune response.

Thus, a valuable fortified product was developed by adding vitamin D3 oil extract (which is used for the prevention and treatment of viral diseases, including COVID-19) to unrefined sunflower oil. A slowdown in oxidative processes in the oil was observed, which contributed to an increase in shelf life and a decrease in vitamin D3 losses in oil samples with an increase in the amount of oil extract added.

4 Conclusion

According to research results, it was found that the fortification of unrefined sunflower oil rich in omega-6 fatty acids by adding 0.04-0.20 ml of a vegetable solution of vitamins with an activity of 50,000 mg provides coverage of 10-50% of the daily need for this vitamin when consuming 20 g of vegetable oil per day (as a dressing for vegetable salads and when preparing second lunch dishes). In the samples of unpacked unrefined sunflower oil, the indicators of oxidative spoilage during storage up to 60 days met the requirements of the current GOST 1129-2013 standard for premium grade oil with lower indicators with an increase in the amount of added vitamin D3 oil solution. A slight decrease in vitamin D3 content during storage in the range of 2.16-6.79% is determined by storage in darkened conditions at a temperature of 17 °C. The results obtained are of practical importance for the production of mass-consumption oil with high biological value and the use of such vitamin D-enriched oil as a prevention of the spread of infections

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