

The Use of Fractionated Sunflower Lecithins for Encapsulation of Micronutrients

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Abstract

Introduction: The work is dealing with scientific and practical substantiation of the prospects for the use of fractionated sunflower lecithins (FSLs) as an encapsulating agent in relation to hydrophilic and lipophilic physiologically functional micronutrients. **Materials and Methods:** Studies were conducted on the advanced equipment according to the standard techniques. Evaluation of the obtained results was carried out using contemporary methods of calculation of static reliability. **Results:** The article presents the results of comparative studies of the composition, physicochemical indicators and ability to form the FSL-based microemulsions produced by the innovative technology, which consists of processing of cover-free sunflower seeds using a reduced impact technology conditions and ethanol as the extractant. **Discussion:** A certain method is proposed to produce the FSL-based microemulsions with encapsulated hydrophilic and lipophilic micronutrients, consisting of vitamin C and oil solution of β -carotene. It is shown that treatment of the FSL-based microemulsion by an electromagnetic field with magnetic induction of 0.5 T during 5 min provides degrees of encapsulation of vitamin C at a level of 83% and β -carotene at a level of 100%. **Conclusion:** The results obtained can underlay the further research on the creation of a range of encapsulated forms of micronutrient premixes.

Key words: Encapsulation, lecithin, magnetic field, microemulsions, micronutrients, phospholipids

INTRODUCTION

The issues of the nutritional value of foods and their effects on human health in industrially developed countries are on the front burner.^[1-4] Contemporary ideas about diet therapy were supplemented with notions of specialized and functional foodstuffs as well as functional food ingredients.^[5-7]

Most daily diet foodstuffs are characterized by low nutritional density, first of all in terms of the quantitative and qualitative composition of micronutrients.^[8-10] This problem is a consequence of the development of technologies for deep processing of raw materials, their refining, as well as the use of methods and techniques aimed at improving the preservation ability of finished products and reducing the time for cooking. The problem of nutrient deficiency of modern foodstuffs is redoubled by the fact of reducing energy consumption by a human and, accordingly, reducing food intake.^[2,8]

According to leading nutritionists,^[8] even the perfect diet, composed of natural products, cannot meet the physiological needs of the modern human on a number of macro- and micro-nutrients. The proposed solution is to increase the nutritional density of traditional foodstuffs, i.e., to increase the availability of necessary nutrients and especially micronutrients per unit of calorie. As a result of this modification, we get the product, which depending on the quantitative and qualitative composition of nutrients, can be positioned as a specialized or functional product.

Monitoring of enriched foodstuffs, including functional and specialized products, available in the consumer food

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market of the Russian Federation showed that most of them are characterized by the discrepancy of the declared and actual composition of the functional food ingredients. The main reason for this fact is the inactivation of vitamins and other physiologically active substances as a result of mutual antagonism or interactions with other components of foodstuffs.

Effective solution to the problem is the use of encapsulated forms of micronutrients.^[8] The creation of encapsulated forms of micronutrients should not only ensure their preservation in native form before and after enrichment of foodstuffs but also contribute to their effective absorption by the human body.

At present, there are plenty of encapsulation methods available; however, as a rule, they are not universal for hydrophilic and lipophilic micronutrients and they do not provide their stability and adequate bioavailability in the enriched product, as well as display a number of shortcomings related to the type of encapsulated agent and the practical implementation of encapsulation technology.^[9]

Upcoming trend for obtaining complexes of functional food micronutrients in encapsulated form, appropriate to the greatest extent to the assigned task, is their integration into microemulsions formed by lecithins. It is known that fractionated lecithins with a predominance of phosphatidylcholine (PC) in their composition have the best encapsulating ability among the lecithins.^[11-13] It should be noted that fractionated lecithins containing PC in the amount of more than 50% are mainly used as pharmaceutical substances as they are characterized by high cost due to the complex and expensive technology of their separation.^[14,15] Thus, due to economic reasons, lecithins with a content of PC not more than 50% are used in food technology.

Until recently, the Russian consumer market used foreign-made fractionated lecithins, obtained from soya seeds. Although a number of soybean lecithins are positioned by manufacturers as “a non-genetically modified (GM) product” (genetically modified), the authenticity of this assertion can be proved only by the presence of the so-called “hard” certificate of traceability, which is usually missing by the supplier. In this regard, for genetically modified food-free food derivation, the EU prefers to use sunflower lecithins.^[16]

As a result of the conducted applied scientific research on a subject: “Development and transfer of green technologies for deep processing of grain and oilseeds to reduce losses from socially significant diseases” in the framework of the implementation of the measure 1.3 of the federal target program “Research and development in priority growth directions of scientific-technological complex of Russia for 2014-2020,” a manufacturing-oriented comprehensive resource-saving technology for processing of cover-free sunflower seeds was developed to produce physiologically valuable oil, protein products, and fractionated lecithin.^[17] Use

of ethanol as extractant during the extrusion and extraction allows selective retrieving the alcohol-soluble fraction of phospholipid complex, i.e., carrying out the fractionation and producing a fractionated lecithin at the stage of oil extraction and separation of miscella. A comprehensive assessment of quality and safety indicators showed that the produced fractionated sunflower lecithin (FSL) meets the requirements of GOST 32052-2013 “Food Supplements. Lecithins E322. General technical conditions” and can be used as a food ingredient in the manufacture of food products, including specialty and functional products, as well as biologically active supplement. Accordingly, the following objectives were set when conducting the current research: To study the effect of composition characteristics and physicochemical indicators of FSL on characteristics of formed microemulsions; to develop a method for producing microemulsions based on FSL with encapsulated micronutrients.

MATERIALS AND METHODS

The encapsulating agents, lecithins, produced based on microemulsions, as well as microemulsions with encapsulated micronutrients were objects of the current study. The fatty acid composition of the lipid complex was determined according to GOST R 51486 using gas-liquid “Crystal - 5000” chromatograph (ZAO SKB “Chromatek,” Russia), SOLGEL-WAX 30 m × 0.32 mm, and ID SOLGEL-WAX × 0.5 μm column. Group composition of phospholipids was investigated by high-performance liquid chromatography method using “Agilent 1260 Infinity” (Agilent Technology, USA) chromatograph, and “LiChrospher 100” column (250 × 4 mm, diol (5 μm) according to techniques.^[18,19]

Analysis of the physicochemical indicators of lecithins was carried out in accordance with GOST 32052-2013 “Food Supplements. Lecithins E322. General technical conditions.” Peroxide and acid values were determined by titration in accordance with GOST 32052-2013 and techniques.^[20] The phase behavior of FSL was studied by differential scanning calorimetry (DSC) using the NETZCH Maia 200 analyzer.^[21,22] Experimental conditions were as follows: Sample mass ~2.5-4.0 mg, open cell, the inert medium (nitrogen), temperature range 20-500°C, heating rate of the samples - 10°C/min. Determination of the particle size of the emulsion was performed using a laser particle analyzer of “Zetasizer Nano S” series (Malvern, UK) with a measuring range from 0.6 nm to 10 μm. Determination of β-carotene was carried out using “Lovibond” tintometer. The content of water-soluble vitamin C was determined using the system of capillary electrophoresis “CAPEL-105M” (Lumex, Russia).^[23]

All experiments were performed at least in three replicates. Assessment of the obtained results was carried out based on contemporary methods of static reliability calculation using the Statistica 6.0, Microsoft Office Excel 2007, and Mathcad software packages. The confidence level was 0.95.

The studies were carried out on the facilities of the Shared Research Center “Research Center of Food and Chemical Technologies” of Federal State Budgetary Educational Institution of Higher Education “Kuban State Technological University.”

RESULTS AND DISCUSSION

To justify the use of FSL, produced according to innovative technology,^[17] in the capacity of the encapsulating agent, a comparative study was conducted. The comparison objects were “Solec FP-40” soy lecithin, manufactured by the Solae Company (USA), as well as domestic fractionated lecithin “Choline,” produced by fractionation of liquid sunflower lecithin of “NPF Rosma-Plus” (Russia).

The results of the study of physicochemical indicators are presented in Table 1.

It is shown that the FSL sample differs from analogs by a significantly smaller content of substances insoluble in toluene and a lower peroxide value. Tables 2 and 3 present the results of the study of group and fatty acid composition of lecithins.

Table 2 is shown that the group composition of all the studied lecithins is dominated by PC, which is the group of phospholipids, forming mainly stable micelles of spherical configuration when dispersing in water. In terms of the content of PC, FSL exceeds the sample of fractionated soy lecithin Solec FP-40 and is slightly inferior to FSL “Choline.” The ratio of PC/phosphatidylethanolamine (PEA), characterizing the hydrophilic properties of lecithin, and its ability to stabilize a direct (oil in water) emulsions was also higher in the samples of sunflower lecithins as compared to soy lecithins.

As is obvious from the data presented in Table 3, the FSL is characterized by predominance in its composition of oleic acid acyls. First, this determines the greater potential stability of FSL to oxidation, and second, evidences the ability to

Table 1: Physicochemical indicators of lecithins

Indicator	Indicator value		
	FSL	Choline	Solec FP-40
Mass fraction, %	61.8	60.5	62.5
Of substances insoluble in acetone	0.9	0.9	0.9
Moisture and volatile substances insoluble in toluene;	0.01	0.10	0.08
Peroxide value, mmol of active oxygen/kg	0.37	1.10	1.81
Acid value, mg KOH/g	20.7	23.3	19.0

FSL: Fractionated sunflower lecithin

form more solid adsorption layer as a result of more dense packing of phospholipid molecules on the phase interface. At the next stage, we carried out a comparative study of the disperse composition of microemulsions formed by studied lecithins. Microemulsions were produced by dispersing the lecithin sample in demineralized water in a ratio of 1:100 at a temperature of 25°C using the universal apparatus “IKA MagicLab,” equipped with three-stage dispersing element, providing ultrafine emulsification at rotor rotation speed of 230/s. The results are presented in Figure 1.

It is shown that all the studied lecithins in the course of dispersion in water produce microemulsions. Microemulsions formed by FSL differ from emulsions produced by sunflower

Table 2: Group composition of lecithins

The name of the phospholipid groups	Content of phospholipid groups, % of total		
	FSL	Choline	Solec FP-40
PI	8	6	9
PC	60	68	42
PS and LPEA	Lack	Traces	Traces
PEA	25	18	31
FA	1	1	2
DPG	4	6	13
PPA	2	1	3
The ratio of PC/PEA	2.4:1.0	3.8:1.0	1.4:1.0

PI: Phosphatidylinositols, PS: Phosphatidylserine, PC: Phosphatidylcholines, LPEA: Lysophosphatidylethanolamine, PEA: Phosphatidylethanolamine, PhA: Phosphatidic acid, DPG: Diphosphatidylglycerol, PPA: Polypeptide acid, FSL: Fractionated sunflower lecithin

Table 3: Fatty acid composition of lecithins

The name of the fatty acids	Content of the fatty acids, % of total		
	FSL	Choline	Solec FP-40
Myristic C _{14:0}	0.08	Lack	0.05
Palmitic C _{16:0}	7.46	14.82	18.70
Stearic C _{18:0}	4.14	4.16	4.65
Arachidic C _{20:0}	0.29	0.05	0.04
Behenic C _{22:0}	0.73	0.65	0.70
Σ S	12.70	19.71	22.14
Palmitoleic C _{16:1}	0.13	1.10	0.02
Oleic C _{18:1}	32.49	11.91	9.55
Linoleic C _{18:2}	53.38	59.50	56.80
Linolenic C _{18:3}	0.79	2.60	6.50
Eicosenoic C _{20:1}	0.14	0.02	lack
Erucic C _{22:1}	0.14	Lack	lack
Other	0.23	5.16	2.99
Σ US	87.30	80.29	75.86

FSL: Fractionated sunflower lecithin

and soy lecithins in terms of a narrower particles distribution interval and a predominance of smaller particles. This indicates a higher emulsifying ability of FSL and can be explained by the better ratio of PC and PEAs in group composition, as well as a predominance of oleic acid in fatty acid composition.

Thus, based on the results of conducted studies, an FSL produced by the innovative technology was selected as an encapsulating agent.

When conducting research with the aim to develop a production method of FSL-based “enriched” microemulsions, a vitamin C was used as a hydrophilic encapsulated micronutrient, whereas 0.6% oil solution of β -carotene was used as a lipophilic encapsulated micronutrient.

This choice was due to the most frequent use of these micronutrients for the enrichment of various food products, as well as their relatively high variability under the influence of technological factors as well as during storage. It is known that the encapsulation of substances by microemulsions, produced by phospholipids, is most effective when the latter are in the liquid crystalline state. This is due to the fact that phase transition from gel into liquid crystalline phase is accompanied by a lateral expansion of the area occupied by phospholipid molecule and an increase in hydrophobic volume of the phospholipid bilayer. To determine the phase transition temperature of the FSL from gel phase into liquid crystalline phase, a DSC method was employed.

The results are presented in Figure 2.

It is shown that the phase transition of the FSL sample is carried out at a temperature of 37°C. Given this, obtaining microemulsions and subsequent encapsulation must be carried out at a temperature exceeding the phase transition temperature, namely, within a temperature range of 39-40°C. Higher temperatures are impractical to use due to their negative influence on the oxidative stability of lipid systems. It is known that the structure of the double layer or bilayer formed by the phospholipid molecules in aqueous media, as well as the particles size of the dispersed phase of microemulsions significantly depend on the concentration of lecithin in the aqueous phase.

Taking this into consideration, we determined the effective ratio of FSL and water. The results are presented in Figure 3.

From the data presented, it is obvious that with increasing concentration of FSL, both the average particle size and specific content of microemulsions of larger size particles in the dispersion composition significantly increase. The results of the microscopic examination have shown that at the FSL concentration exceeding 10 g/100 g of emulsion, spherical shape of microemulsion particles is deformed while their shell loses its uniformity and elasticity. On the

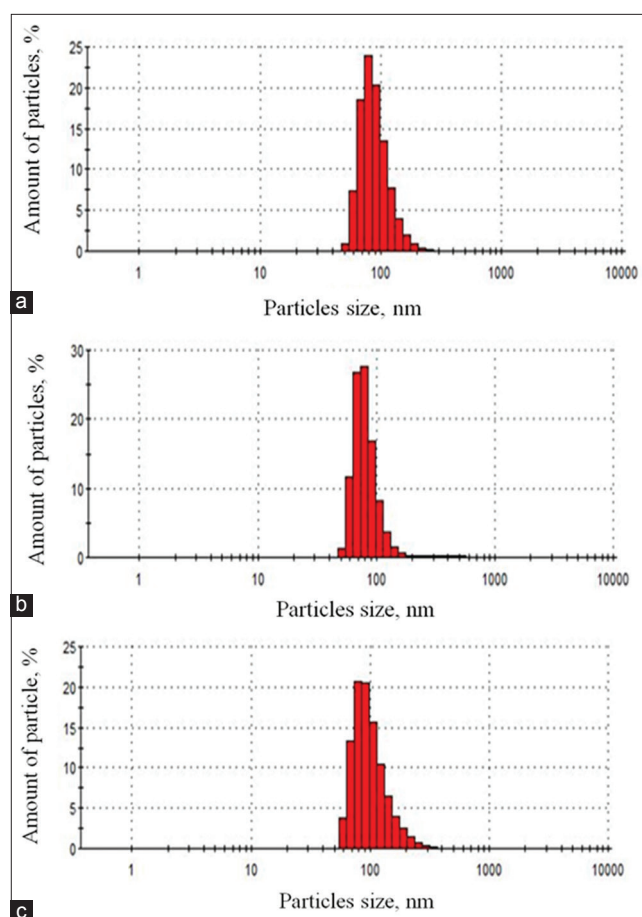


Figure 1: Disperse composition of microemulsions produced by lecithins in water: (a) “Choline;” (b) fractionated sunflower lecithin; (c) “S olec FP-40”

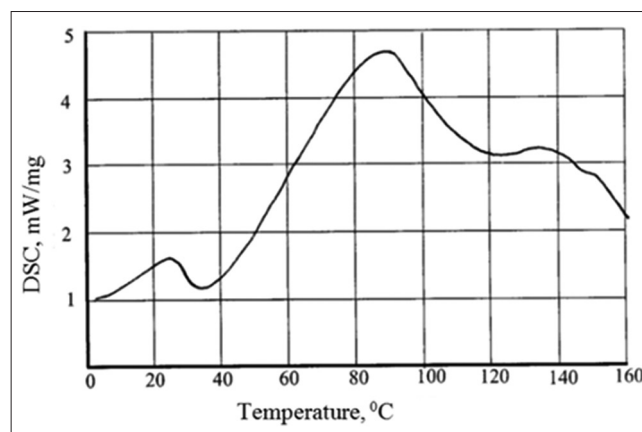


Figure 2: Thermogram of the fractionated sunflower lecithin phase transition

basis of generalization of the data presented in the scientific literature, as well as results of conducted research, we proposed a method of producing microemulsions with encapsulated substances, which is presented in the form of the block diagram in Figure 4. According to the presented diagram, FSL microemulsions were produced in the water by dispensing FSL into the aqueous phase and subsequent

heating of the system to a temperature of 39°C followed by intense homogenization.

While studying the encapsulating capacity and stability of microemulsions produced from the FSL, at the first stage of the experiment, the concentration of vitamin C in the aqueous phase was varied from 0.5% to 4.5%. The amount of oil solution of β -carotene was constant and amounted to 10% of the amount of FSL. During the experiment, we studied the degree of encapsulation of vitamin C and β -carotene. The amount of encapsulated β -carotene was determined using standard methodology as the difference between emulsified and separated oil phase. To determine the degree of encapsulation of vitamin C, we used modified dialysis method.

A membrane module made of polymer fiber with a pore size of 40 nm was used as dialysis chamber. Microemulsion samples with encapsulated vitamin C were placed into the dialysis chamber, which further was installed into a thermostatic

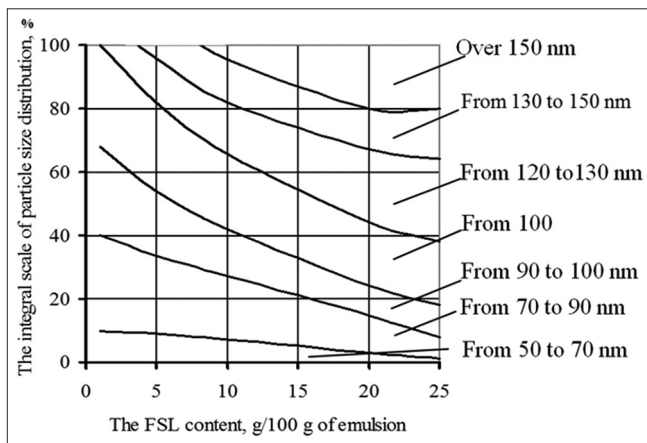


Figure 3: Change in disperse composition of microemulsion depending on the fractionated sunflower lecithin concentration

cell, filled with double distilled water. After exposure of the system at 25°C for 8 h, as shown by preliminary experiments, it was sufficient time to achieve equilibrium concentration of solutions in dialysis chamber and cell; we determined the vitamin C content in the dialysate. The experimental results are presented in Figure 5.

It is shown that with increasing concentration of vitamin C in the aqueous phase over 1.5%; the degree of encapsulation of both vitamin C and an oil solution of β -carotene decreases that is probably associated with the destruction of the emulsion by changing the structure of the adsorption layer, caused by the change of pH. In general, the degree of encapsulation of lipophilic ingredient is significantly superior to the degree of encapsulation of hydrophilic ingredient and reaches a maximum of 100%, whereas the degree of encapsulation of the hydrophilic component is not >67%.

At the second phase of the experiment, we varied the amount of oil solution of β -carotene from 10% to 50% of the amount of FSL at a constant concentration of vitamin C in the aqueous phase, equal to 1.5%. The results are presented in Figure 6.

It is shown that variation in the content of the lipophilic component has no effect on the degree of encapsulation of the hydrophilic component. The degree of encapsulation of lipophilic component decreases with increasing its content in the system over 10% of the FSL content. Study of produced microemulsions stability showed that the emulsions, which are characterized by the highest encapsulation capacity, remain relatively stable within 3 days after their production. At the next stage, we solved the problem of increasing the degree of encapsulation of hydrophilic and lipophilic components.

The approach to the development of a method of improving the encapsulating capability of the FSL was based on the

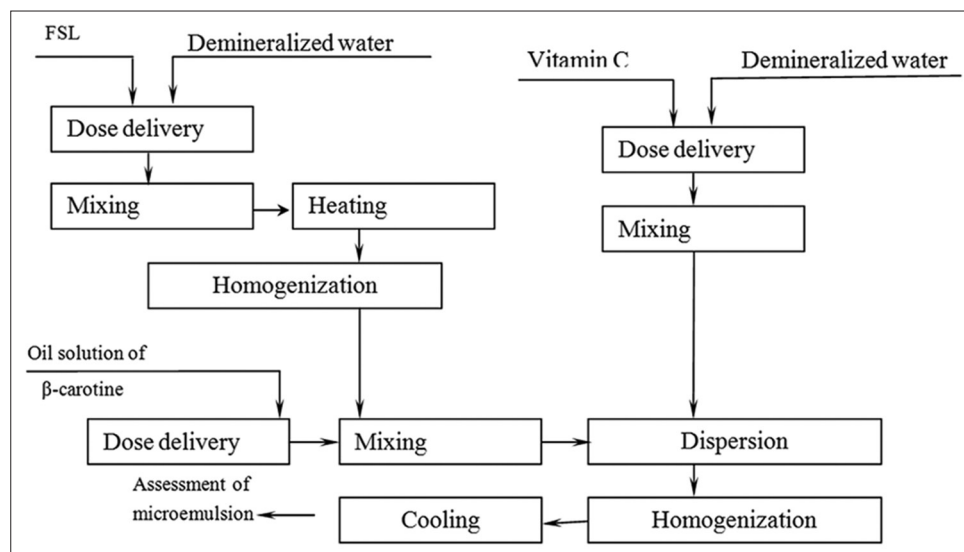


Figure 4: A block diagram to produce microemulsions with encapsulated substances

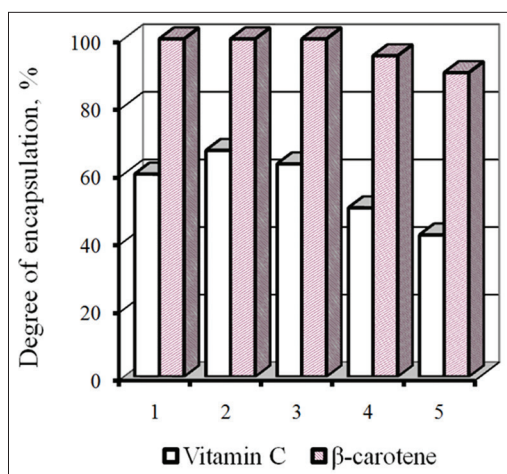


Figure 5: The degree of encapsulation of vitamin C and an oil solution of β-carotene at different concentrations of vitamin C in the aqueous phase: 1-0.5%; 2-1.5%; 3-2.5%; 4-3.5%; 5-4.5%

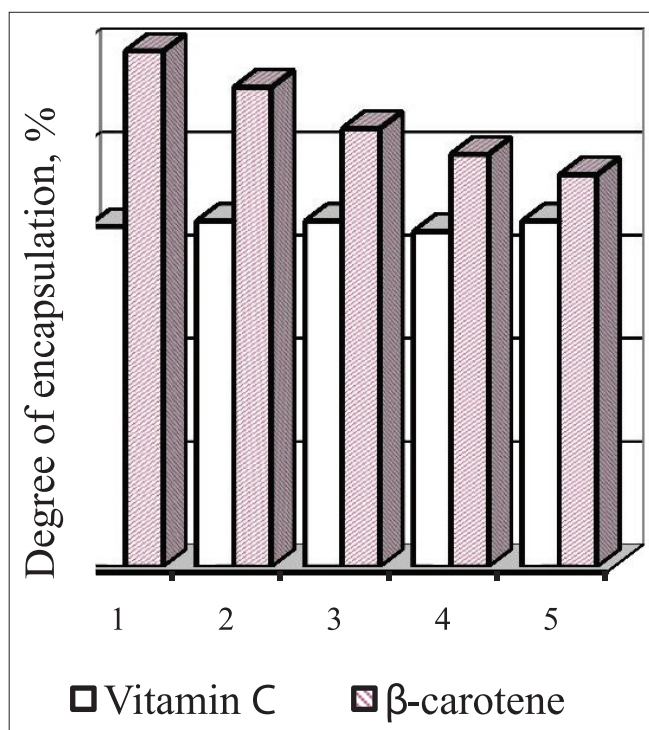


Figure 6: The degree of encapsulation of vitamin C and an oil solution of β-carotene at different contents of oil solution of β-carotene in the fractionated sunflower lecithin oil: 1-10%; 2-20%; 3-30%; 4-40%; 5-50%

idea that the application of alternating electromagnetic field on the “phospholipid-water” system leads to polarization of the molecules of phospholipids and enhances surfactant characteristics. Determination of effective processing modes of the FSL dispersions in the aqueous phase by the electromagnetic field was conducted using AMO-U apparatus by varying the magnetic induction within the range from 0 to 0.8 T through the change of current in the windings of the inductor, as well as by changing the duration of field

application from 1 to 5 min during circulation of the FSL microemulsion through the cell.

The modes were selected on the basis of the experimental results obtained previously by the specialists of the Department of Fats and Cosmetics Technology, Merchandising, Processes and Apparatuses of Kuban State Technological University in relation to systems, similar to that under study.^[24] Constant factors were the amount of lipophilic component, which accounted for 25% of the FSL content, the concentration of vitamin C in the aqueous phase, which was 1.5%, and the processing temperature equal to 40°C, which is close to phase transition temperature and falls within the temperature range that is optimal for electromagnetic processing in the similar apparatus. The degree of encapsulation of vitamin C and β-carotene was used as the response function. The experiment was carried out using mathematical planning methods. Graphical interpretation of the obtained data is shown in Figure 7.

As a result of mathematical processing of experimental results, we have obtained regression equations, adequately describing the process:

$$Z_1 = 64.78 + 1.69x + 34.28y - 0.11x^2 + 0.34xy - 21.78y^2 \quad (1)$$

$$Z_2 = 85.90 + 1.28x + 3.81y - 0.08x^2 + 0.12xy - 9.94y^2 \quad (2)$$

Where Z_1 - is the degree of encapsulation of vitamin C; Z_2 - is the degree of encapsulation of β-carotene; x - is the processing time, and y - is the magnetic induction.

The adequacy of the obtained equations to the real process was checked through a Fisher’s test. The analysis of equation coefficients shows that the effect of electromagnetic treatment intensity and duration on the degree of encapsulation of micronutrients is similar; at that, the cross effect of both factors is insignificant.

As a result of mathematical processing of experimental results and optimization of parameters maximizing the response function, the following technological modes were established that ensured the degree of encapsulation of vitamin C at a level of 83% and the degree of encapsulation of β-carotene at a level of 100%.

The duration of electromagnetic treatment - 5 min.

The intensity of the magnetic treatment determined by a magnetic induction - 0.5 T.

Thus, the conducted studies have shown that FSL produced by the innovative technology from cover-free sunflower kernels, which is an effective encapsulating agent, which can be used as a basis for development of a number of micronutrient premixes in encapsulated form to be used to enrich functional and specialized food

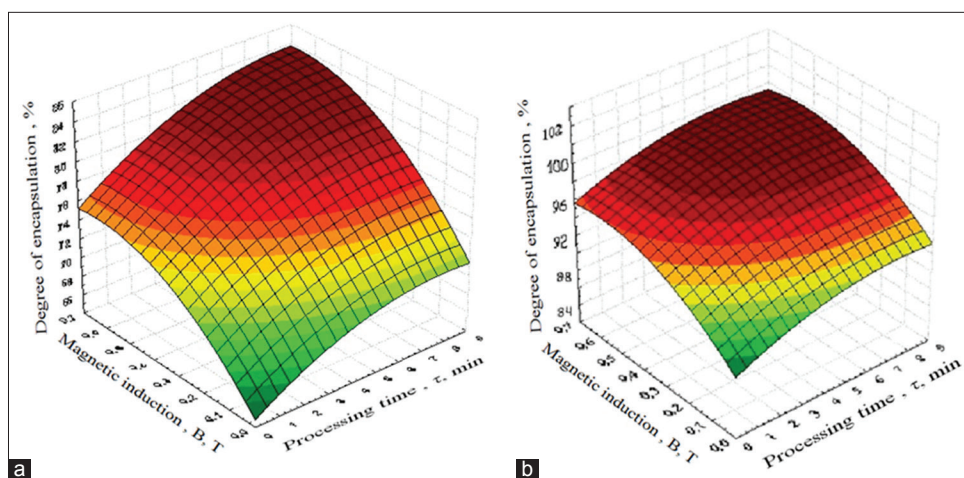


Figure 7: Effect of magnetic treatment on the degree of encapsulation of vitamin C (a) and β -carotene (b)

products, cosmeceuticals, and production of comprehensive biologically active food supplements.

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CONCLUSION

Based on a comparative study of the quality indicators, including composition, physicochemical indicators, and functional properties of fractionated sunflower and soybean lecithins, we justified the use of the FSL as an effective encapsulating agent when producing microemulsions, which are characterized by a high degree of dispersibility and narrow range of particles size distribution.

The FSL--based microemulsions production method is developed, which involves the microemulsions production process and their consistent "enrichment" with lipophilic and hydrophilic components at a temperature, exceeding the phase transition temperature (39-40°C), and the FSL to aqueous phase ratio amounting to 1:10. A method for enhancing encapsulating capacity and the degree of encapsulation of hydrophilic (83%) and lipophilic components (100% if the content in the microemulsion is in an amount of 25% by the FSL weight), which consists of the processing of the FSL-based microemulsion before application of the electromagnetic field with magnetic induction of 0.5 T over 5 min.

The results obtained can serve the basis for further research on the creation of a range of encapsulated forms of micronutrient premixes.

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