International Journal of Engineering Trends and Technology
ISSN: 2231 – 5381 /doi:10.14445/22315381/IJETT-V68I10P201
Volume 68 Issue 10, 1-9, October 2020

Production of Water-Dispersible Forms of Phytosterols

Ivan Alekseevich Bugaets[1], Elena Aleksandrovna Butina[1], Evgeny Olegovich Gerasimenko[1], Sergey Aleksandrovich Sonin[1], Albina Anatolievna Kopteva[2]

[1]*Kuban State Technological University, Krasnodar, Russia*

[2]*K.G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russia*

Abstract *— The research is devoted to the development of a method for producing phytosterols in a water-dispersible form by encapsulating a finely dispersed suspension of phytosterols with a nonionic surfactant. As a surfactant, it is proposed to use powdered sunflower lecithin. The results of studies of producing emulsions and a powdered encapsulated form of phytosterols are presented.*

Keywords — *phytosterols, lecithin, emulsions, encapsulation.*

I. INTRODUCTION

Phytosterols are accompanying substances of triacylglycerols of vegetable oils and belong to the group of unsaponifiable lipids [1].

The chemical structure of phytosterols, similar to cholesterol, vitamin D, bile acids and their salts, as well as some hormones and cardiac glycosides belong to the group of steroids [2].

The main physiologically functional properties of phytosterols are due to their structural similarity with endogenous cholesterol and consist in the ability to reduce the intestinal absorption of the latter. Phytosterols replace cholesterol by integrating into fatty micelles through which fats are absorbed in the intestine [3], [4]. This leads to a decrease in cholesterol absorption and promotes its excretion from the body. The absorption of phytosterols in the intestine is limited and usually does not exceed 5-10% [5].

There are numerous data in the scientific literature that the regular inclusion of phytosterols in the diet can reduce the level of atherogenic cholesterol in the composition of low-density lipoproteins [4], [6]–[9].

According to [10] the inclusion of phytosterols in the diet in an amount of 2-3 g per day provides a reduction in atherogenic cholesterol by 10 %. At the same time, according to (3), a decrease in total content of cholesterol by 1% will reduce the risk of coronary vascular disease by 3 %.

The U.S. National Cholesterol Education Program recommends daily intake of foods enriched with phytosterols, phytostanols, and their derivatives at the rate of 2 grams per day in order to reduce plasma cholesterol levels [11]. In EU countries the recommended level of phytosterols consumption and their esters in foods is from 0.8 to 2.4 g per day [12], [13]. In Russia the adequate level of phytosterols consumption is set at 0.2 g per day [14].

Other important physiologically functional properties of phytosterols are anti-inflammatory, antioxidant and oncoprotective activity and their advantage over substances with similar properties is the absence of pronounced negative side effects on the body [4], [15].

Phytosterols are not synthesized in the human body. The main source of phytosterols are vegetable oils, in which, the content of phytosterols is from 0.3 to 0.8 % depending on the type of oil seeds and oil processing technology [4].

Given that the intake of phytosterols with traditional foods is quite small, they are used as a physiologically functional food ingredient to enrich foods, to create the products of specialized and personalized nutrition, dietary supplements and pharmaceuticals. An important advantage of phytosterols as an enriching microingredient is their stability in the composition of products with different pH values [5], [16].

The main uses of phytosterols in food technologies include the production of functional and specialized food products, such as yogurts, milk, margarine, spreads, flour, sausage, bakery and pasta products [5], [16]–[18].

In pure form, phytosterols are insoluble in water and practically insoluble in oils and fats. Phytosterols dissolve quite well in chloroform and in low molecular weight alcohols such as ethanol and methanol [19]. Given this to ensure the effective use of phytosterols in food technologies, including in the production of functional and specialized foods, phytosterols have to be transformed into a bioavailable form.

Ways are known to improve the solubility of phytosterols in water and their bioavailability through chemical modification, for example by creating a complex compound with hydroxypropyl-β-cyclodextrin [20], or by forming a chemical compound with polyethyleneglycol [21].

To increase the bioavailability of phytosterols, it has been proposed that they be used in the form of nano-dispersed powders, which are prepared by dissolving them in an organic solvent, mixing them with water and then removing the organic solvent under vacuum [22] or using a method based on supercritical carbon dioxide drying [23].

Known forms of phytosterols technological transformation is their esterification to obtain fatty acid esters, as well as the production of emulsion substances, including liposomes, in particular phytosterols or their esters with fatty acids [24]–[27].

The creation of liposomal or emulsion forms of phytosterols and other biologically active substances with the use of lecithin as an encapsulating agent is the most promising method, as it was evidenced by a numerous scientific studies [25]–[29].

To date, soy lecithins have been used to create a lecithin-based emulsions. However, given that most soy lecithins are produced from genetically modified raw materials, as well as taking into account the existing restrictions in some countries on the use of such raw materials in the production of healthy foods or organic products, recently an interest in the replacement of soy lecithins with sunflower lecithins has increased [30].

Thus, it is actual to develop a method for producing water-dispersible forms of phytosterols by encapsulating them with emulsions formed by sunflower lecithins.

II. MATERIALS AND METHODS

The objects of the study were samples of powdered sunflower lecithin produced by LLC "JUVIX-PHARM" (10, Lenin st., Afipsky village, Seversky district, Krasnodar region, Russia, 353235).

A mixture of phytosterols produced at LLC "Khimtekhlab" (12, Nauki ave., building «A», St. Petersburg, Russia, 195257) was used as a sample of phytosterols.

Powdered sunflower lecithin had the following characteristics: acetone insoluble substances - 97.5%; acid value 22.3 mg KOH/g; peroxide value 0.3 mEq/kg; mass fraction of moisture - 0.45%.

The mixture of phytosterols was a fine crystalline powder of white color, the total content of phytosterols was 95.2%, including stigmasterol 15.1 %; brassicasterol 13.1%; campesterol 25.7%; beta-sitosterol 41.3%.

Distilled and deionized water was used to prepare solutions and emulsions.

The content and composition of phytosterols were determined by using the gas chromatography-mass spectrometer Crystal 5000 GC-MS (Chromatek, Russia) and the NIST library. Cholesterol was used as an external standard for the quantitative determination of phytosterols. Cholesterol was produced at Sigma-Aldrich (St. Louis, USA); the content of the main substance (purity) - more than 99.0 %, appearance - white crystalline powder).

IR spectral analysis was performed by using the Cary 630 IR Fourier spectrometer (Agilent Technologies, USA).

Preparation of emulsions was carried out as follows.

The calculated amount of powdered sunflower lecithin was weighed into beaker with a capacity of 250 cm^3, then distilled water was added in a ratio of 1:10 and homogenized using Ultra-Turrax T25 homogenizer (IKA, Germany) at a 40±2 ^{0}C and the dispersant shaft rotation speed of 7200 min^{-1} during 1 min. The subsequent exposure of the system was carried out by stirring by RW 20 digital (IKA, Germany) with propeller stirrer at a speed of 240 min^{-1} during 30 minutes.

An Ultrasonic Cleaner (UD50 SH-2LD, China) was used to increase the dispersion degree of phytosterols suspension.

To determine a sedimentation stability of dispersions and emulsions, the MPW – 260 RH centrifuge (Poland, AWTech) was used.

The dispersion efficiency was monitored by using a Micromed 3 optical microscope with a digital camera DCM 510 (5 M pixels) at 450x magnification. The fractional compositions of produced emulsions were determined by using the Leica Application Suite software. The particle diameter of each fraction was calculated as an average of at least three measurements.

All experiments were carried out in no less than three repetitions. The obtained results were evaluated using modern methods of static reliability calculation - Statistica, Microsoft Office Excel and Mathcad programs. A confidence level was 0.95.

The research was carried out on the equipment of the CCU (Centre of collective usage) "Research Center for Food and Chemical Technologies", "Kuban State Technological University".

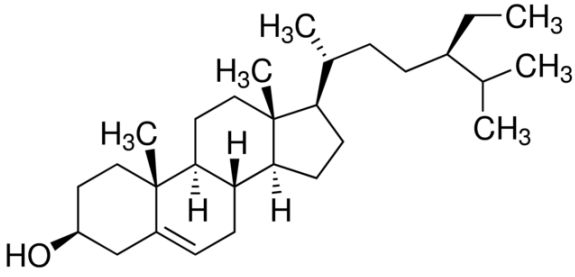
III. RESULTS AND DISCUSSION

It is known that phytosterols in their pure form are insoluble in water, slightly soluble in vegetable oils and nonpolar solvents. This determines the complexity of their microencapsulation, since phytosterols cannot be attributed to either hydrophilic or lipophilic substances.

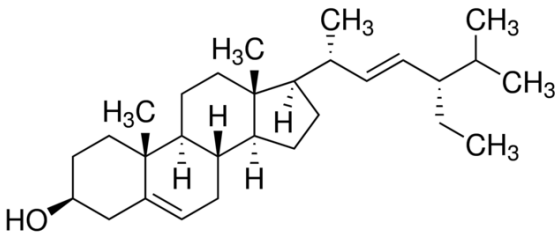
An analysis of the scientific literature and patent information has shown that the implementation of phytosterols microencapsulation by using surfactants supposes at the first stage a preparation of a finely dispersed suspension in an aqueous phase [25], [31]–[33].

The patent [33] describes a method for producing a stable homogeneous suspension of microcrystalline phytosterol and a sweetener in an aqueous solution by double homogenization. The degree of dispersion of the resulting system is from 0.1 to 100 micrometers.

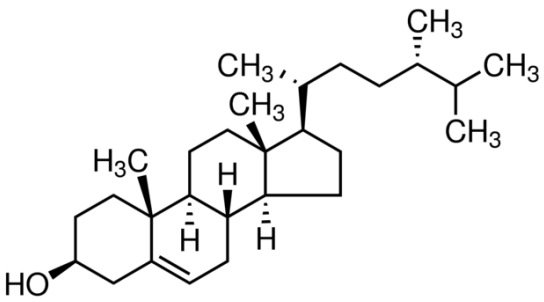
The structural formulas of phytosterol molecules are shown on the Figure 1 [34].



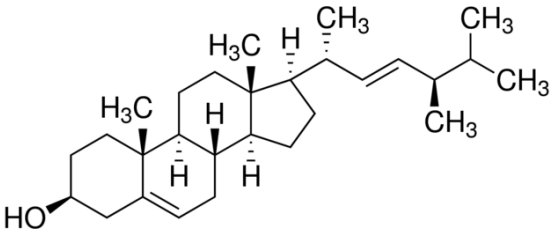
β-Sitosterol



Stigmasterol



Campesterol

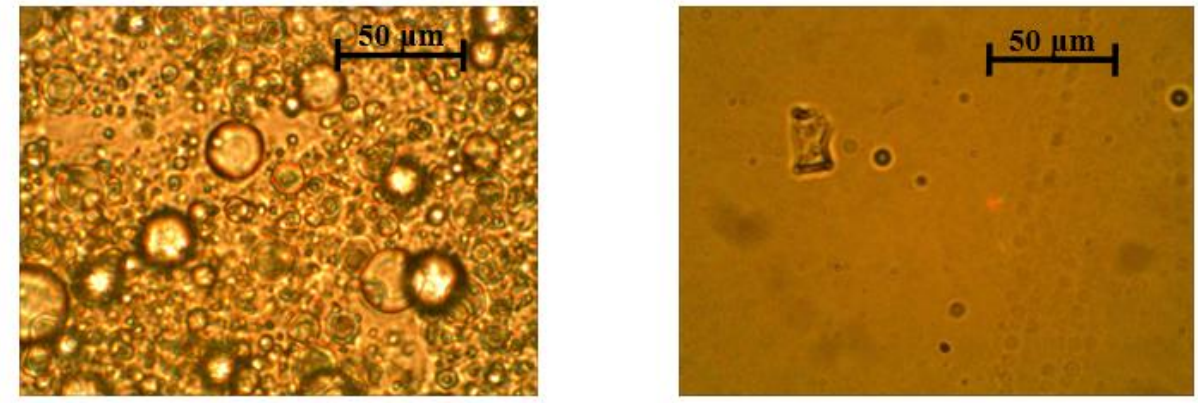


Brassicasterol

Fig. 1. Structural formulas of phytosterols

Taking this into account, the dispersion of the initial mixture of phytosterols in water with a concentration of 10% was prepared at the first stage of research and then it was injected into the emulsions formed by lecithins in an amount providing a lecithin : phytosterols ratio of 100:10.

For example, the results of emulsion microscopy produced by the described above method are shown on the Figure 2.



(a) (b)

Fig. 2. Emulsion formed by powdered sunflower lecithin with the injection of phytosterols:

(a) - before dilution; (b) - after dilution of 1:250

The analysis of microphotographs presented in Figure 2 shows that the emulsion contain polydispersed particles of phytosterols, some of which were immobilized by phospholipid vesicles, and the other part remained in free form in a dispersed medium.

Analysis of the sedimentation stability of emulsions by centrifugation of the resulting system at 14000 min^{-1} at a temperature of (23±2ºC) during 5 minutes showed that 40% of the injected phytosterols amount are precipitated.

Taking this into account, a method was developed for preparing phytosterols for injection into a emulsion formed by lecithins, the purpose of which was to obtain a fine suspension of phytosterols in the aqueous phase.

A phytosterol suspension was prepared in two stages. At the first stage a coarse suspension was prepared by distributing a portion of powdered phytosterols in water at a temperature of 40 °C, at the second stage the system was homogenized using a T 25 digital ULTRA - TURRAX dispersant at 7200 min^{-1}. In the resulting system the dispersity degree was determined. The data are presented on the Figure 3.

As it can be seen from, the resulting suspension is characterized by the presence of polydisperse particles, the size of which varies widely: from 1.0 to 40 micrometers, which determines their low sedimentation stability.

A polar solvent – ethanol mixed with water – was used as a technique to reduce the dispersity degree with described above conditions of homogenization. Based on the preliminary experiments, it was found that the use of 45% ethanol solution in water allows to increase the dispersity degree of the resulting suspension: the particle size does not exceed 10 µm, at the same time the prevailing fractions are the particles of 3-4 µm (Figure 4).

To further increase of the dispersity degree, the resulting suspension of phytosterols was treated with ultrasound. The treatment was carried out, varying in duration from 15 seconds to 3 minutes. It is established that the greatest effect is achieved with a processing time of 50 seconds, after which the dispersity degree practically does not change. The characteristics of the resulting dispersion are shown on the Figure 5.

It was shown that ultrasonic treatment can significantly increase the dispersity degree of the suspension: more than 80% are particles, the size of which is smaller than 0.35 μm.

At the next stage, there were produced emulsions formed by lecithins with encapsulated phytosterols.

A fine water-ethanol suspension of phytosterols after described above ultrasonic treatment with a concentration of 20%, in an amount of 60% by weight of lecithin, was injected into the emulsion formed by lecithin. Then the system was homogenized using a T 25 digital ULTRA - TURRAX dispersant at 12000 min^{-1} during 1 min. Next the system was exposed with stirring at a speed of 240 min^{-1} for 30 minutes by RW 20 digital (IKA, Germany) with propeller stirrer.

The resulting system was centrifuged at 14000 min^{-1} at ambient temperature of (23±2 °C) for 10

minutes and the supernatant was drained. The amount of sediment did not exceed 1 %.

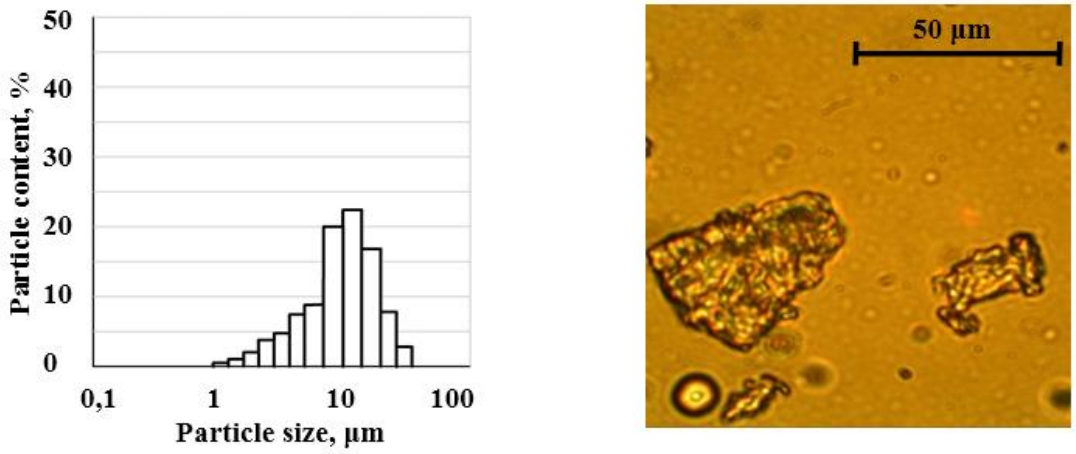


Fig. 3. Phytosterols suspension in water

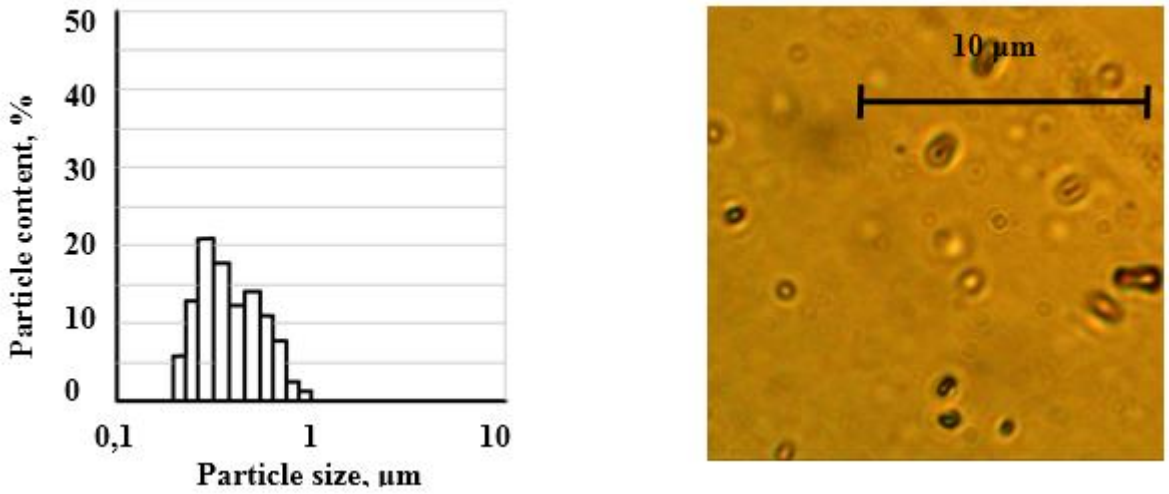


Fig. 4. Phytosterols suspension in water-ethanol solution (45%)

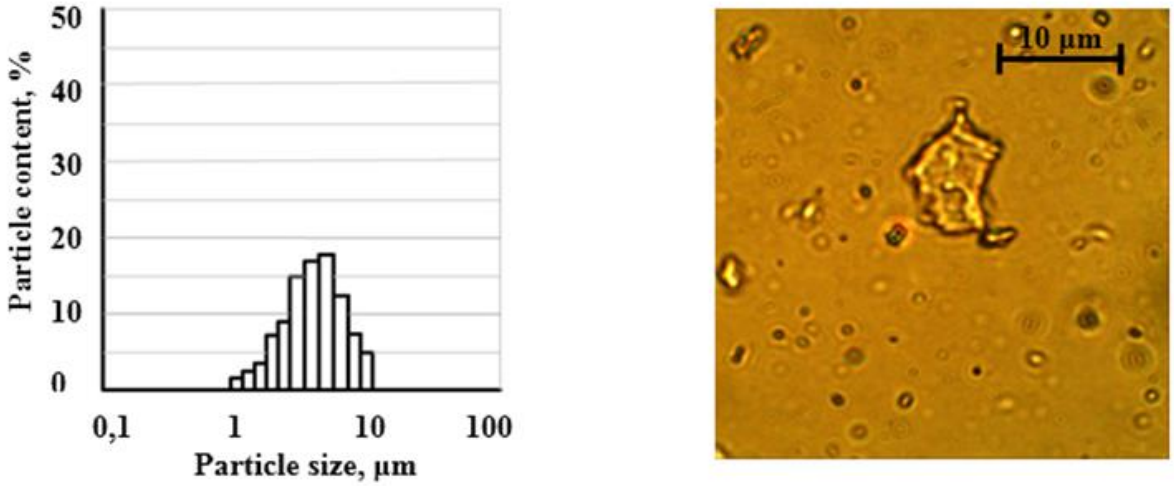
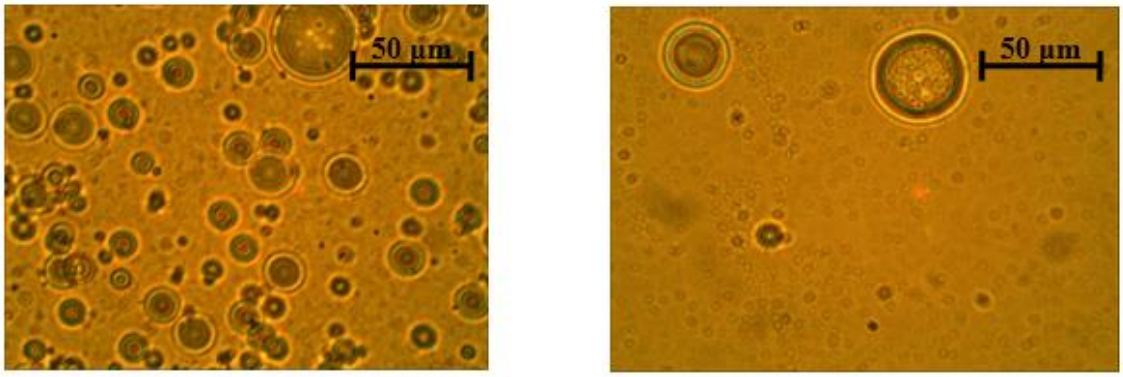


Fig. 5. Phytosterols suspension in water-ethanol solution (45%) after ultrasonic treatment

The supernatant was microscopically exanimated (Figure 6).



(a) (b)

Fig. 6. Emulsion formed by powdered sunflower lecithin with the injunction of a prepared phytosterols suspension: (a) - before dilution; (b) - after dilution of 1:100

As it can be seen from the presented data all suspended phytosterol particles were immobilized by phospholipid vesicles.

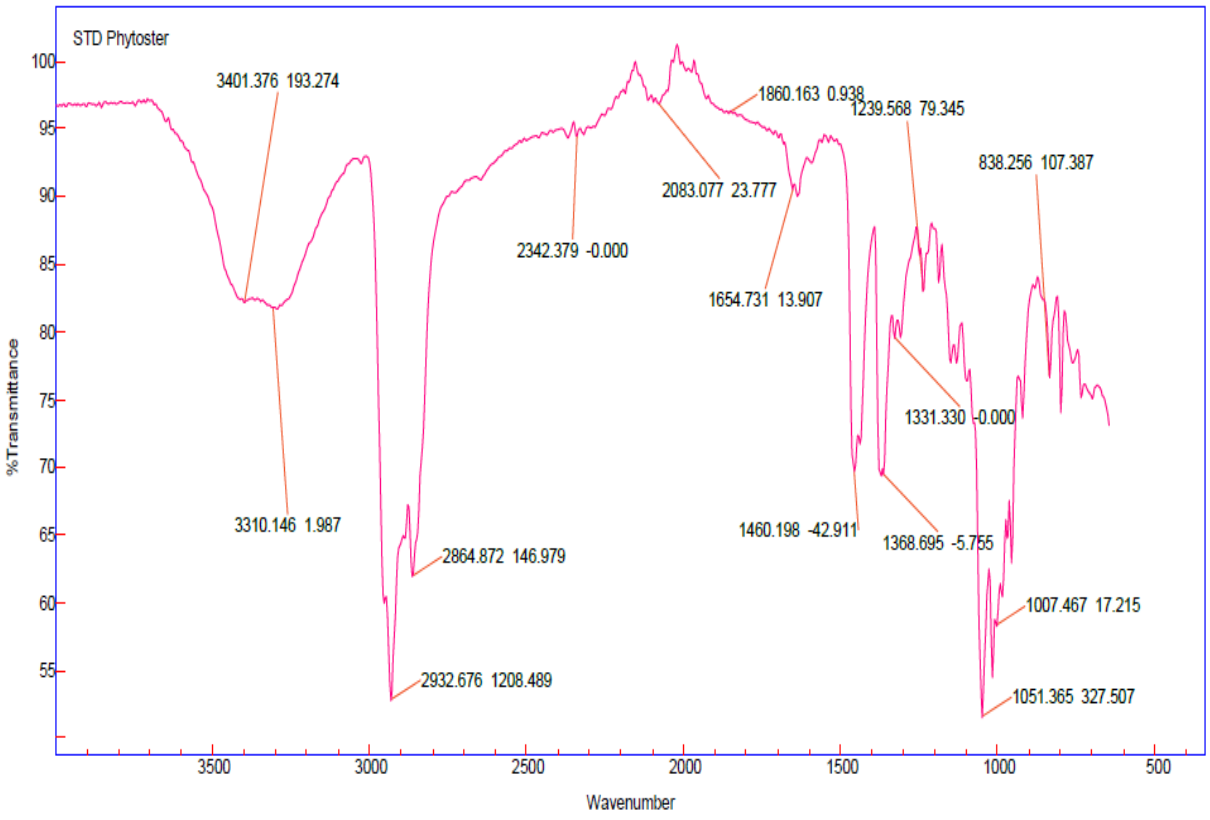
The supernatant was dried to a constant weight at a temperature of 50°C in a vacuum oven, after which the resulting powder was analyzed.

It was found that the content of phytosterols in the supernatant is an average of 10.56 % in terms of absolutely dry matter, which corresponds to 88% of the initial amount of injected phytosterols.

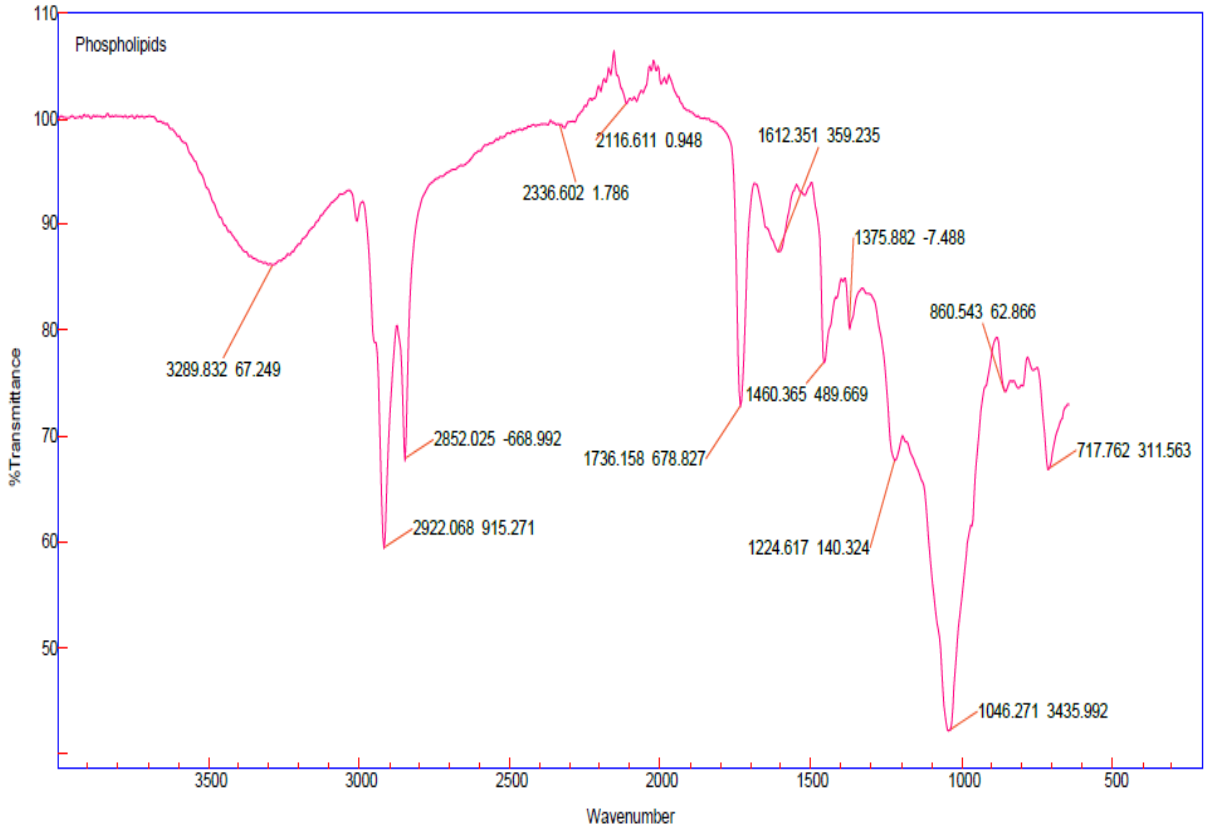
The IR spectra of the initial mixture of phytosterols (a), powdered lecithin (b) and the substance produced after removing moisture from the supernatant (c) are shown on the Figure 7

A comparative analysis of the presented spectra shows that the IR spectrum (in the wavelength range from 500 to 1500 nm) of the powder surface after drying of the supernatant is similar to the IR spectrum of sunflower lecithin powder, which indicates the encapsulation of phytosterol suspension particles by phospholipid vesicles.

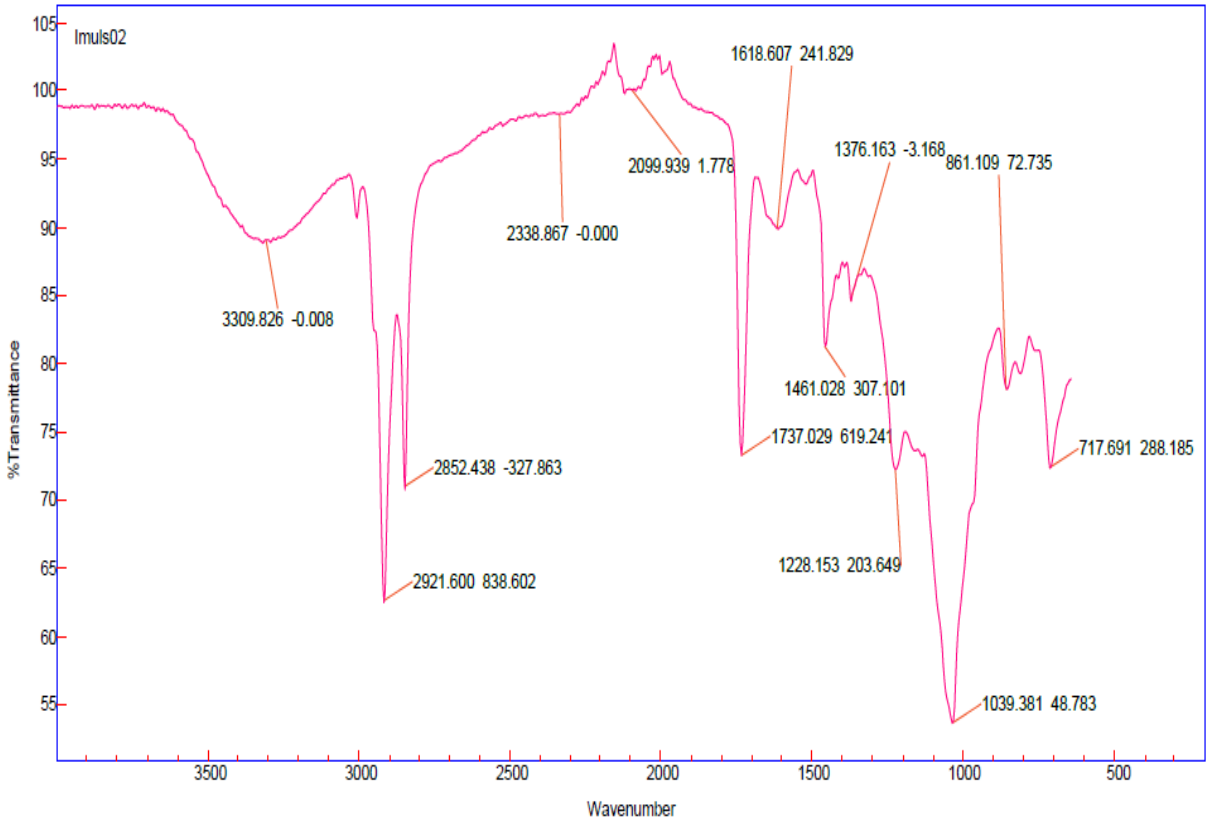
The next step was the restoration of emulsions when they were dispersed in water.


STD Phytoster
3401.376 193.274
1860.163 0.938
1239.568 79.345
838.256 107.387
2083.077 23.777
2342.379 -0.000
1654.731 13.907
1331.330 -0.000
3310.146 1.987
1460.198 -42.911
1368.695 -5.755
2864.872 146.979
1007.467 17.215
2932.676 1208.489
1051.365 327.507
%Transmittance
Wavenumber

(a)


Phospholipids
2116.611 0.948
1612.351 359.235
2336.602 1.786
1375.882 -7.488
860.543 62.866
3289.832 67.249
1460.365 489.669
2852.025 -668.992
1736.158 678.827
717.762 311.563
2922.068 915.271
1224.617 140.324
1046.271 3435.992
%Transmittance
Wavenumber

(b)



(c)

Fig. 7. IR spectra of phytosterols mixture (a), powdered sunflower lecithin (b), powder after drying of the supernatant (c)

Microphotographs of emulsions produced after recovery are presented on the Figure 8.

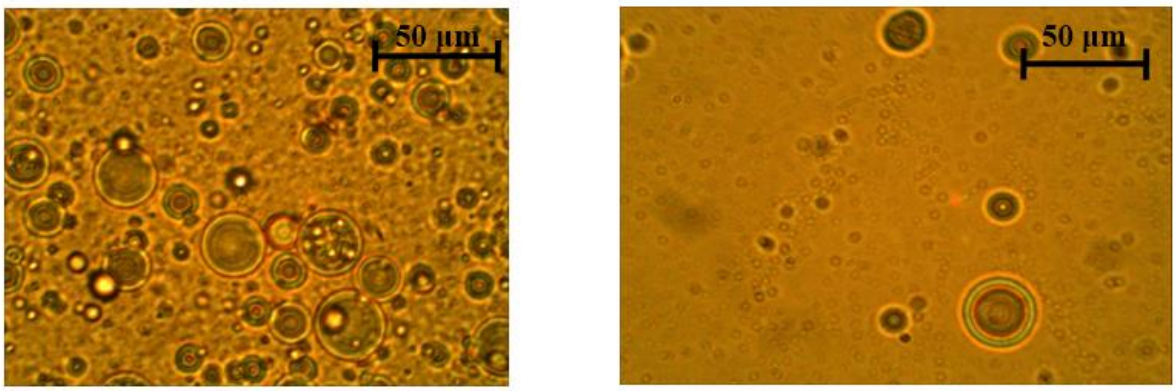


Fig. 8. Recovered emulsion formed by powdered sunflower lecithin with encapsulated phytosterols: (a) - before dilution; (b) - after dilution of 1:100

It was found that the dispersion of encapsulated phytosterols in water at a ratio from 1:10 to 1:50 at a temperature of 23±2ºC is a complete recovery of emulsions remaining stable for at least 24 hours.

The proposed method can be used for production water-dispersible forms of phytosterols intended for use as a physiologically functional ingredient in food technologies, as well as in the production of complex biologically active food additives.

IV. CONCLUSION

Phytosterols are a perspective physiologically functional ingredient.

The widespread use of phytosterols in food technologies implies the need for their technological transformation into a water- or fat-soluble form.

The combination of hydromechanical and ultrasonic effects during the dispersion of phytosterols in water-ethanol solution allows to get a fine dispersed suspension (more than 80% of particles are less than 0.35 μm).

The injection of a fine dispersed suspension of phytosterols into a emulsion formed by powdered sunflower lecithin provides a stable system, subsequent dehydration of which allows to produce a powdered product, which is encapsulated phytosterols with a mass fraction of the last 10%.

When the encapsulated phytosterols are dispersed in water at a ratio from 1:10 to 1:50 at temperature of 23±2°C, the emulsions are completely restored remaining stable for at least 24 hours.

ACKNOWLEDGMENT

The work was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation, project FZEZ-2020-0004.

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