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THERMAL STABILITY AND DIGESTIBILITY OF A BIOPOLYMER SYSTEM FOR THE DELIVERY OF MINOR NUTRIENTS IN ENRICHED MEAT PRODUCTS

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Keywords: *supramolecular complex, enriched meat products, eicosapentaenoic acid, docosahexaenoic acid, vitamin D3, in vitro digestion, oxidation*

Abstract
The study examined thermal stability and digestibility of a biopolymer delivery system for the liposomal form of minor nutrients (omega-3 polyunsaturated fatty acids, vitamin D3, essential oil of clove buds) in enriched meat products. A fraction of encapsulated liposomes in the biopolymer delivery system, i. e. in the supramolecular complex with sodium caseinate (SC), was more than 74%. The difference between the number of bound liposomes before and after freeze-drying is statistically insignificant. The study of the fatty acid composition in samples of enriched meat product containing a supramolecular complex (EPSC) and enriched meat product containing components of the supramolecular complex (EPC) showed that the total omega-3 fatty acids content in EPC was 0.079 ± 0.002 g/100 g, while in EPSC it was 0.207 ± 0.002 g/100 g. The data obtained made it possible to state that EPSC sample was a source of omega-3 fatty acids. Product fortification with the supramolecular complex made it possible to meet the daily requirement of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by 70%, and vitamin D3 by 470%. A study of the in vitro digestion of EPC and EPSC enriched meat products, it was revealed that in both samples, release of fat in the gastric phase was almost identical, in contrast to the intestinal phase, where the released fat in EPSC was found to be 2 times higher than in EPC. This indicates that the use of physiologically functional ingredients in encapsulated form to fortify meat products is more effective and does not violate the general principles of lipid digestion. At the same time, the mass fraction of released fatty acids in the intestinal phase was higher by 74.4% and 48.5%, respectively, when using physiologically functional ingredients in the form of a supramolecular complex in comparison with a product containing these ingredients in their native form. Use of high temperature treatment did not affect the bioavailability of EPA and DHA, as well as the organoleptic parameters or oxidative stability of EPSC.

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Introduction

Currently, due to the lack of various bioactive substances (BAS) in the human diet, product fortification with physiological and functional ingredients is becoming increasingly popular.

Long-chain omega-3 polyunsaturated fatty acids (PUFAs), which are mainly concentrated in fish oil, represent one of the most physiologically important nutrients. The deficiency of these nutrients among children and adults in the Russian Federation is quite high with a prevalence of 80% [1]. Therefore, it is of the utmost importance to develop the production of fortified, functional and specialised food products with the aim of increasing daily consumption of PUFAs [2].

The biochemical functions of omega-3 PUFAs are associated with the transmembrane transmission of synaptic signals, the synthesis of prostaglandins, as well as vasodilatory, antithrombotic and antiatherogenic effects. These functions underlie the successful functioning of the cardiovascular, central nervous, visual systems and

the regulation of lipid metabolism [3]. However, the use of polyunsaturated fatty acids in food production is complicated by their low solubility in aqueous media (due to their hydrophobic nature) and high susceptibility to oxidation and degradation (due to the presence of unsaturated carbon-carbon bonds) [4]. The incorporation of PUFAs into meat products is of paramount importance, given that the lipid profile of meat is characterized by a high content of saturated fatty acids and a low content of PUFAs [5]. At the same time, the negative effect on organoleptic parameters is a limitation for application of PUFAs in quantities to achieve their claimed physiological effect [6,7]. This is associated with increased oxidative processes during heat treatment and storage. Unlike PUFAs [8], vitamin D3 does not affect the taste of the product, but has low solubility in aqueous media, including body fluids of the gastrointestinal tract, due to its hydrophobic nature [9]. One way to overcome these problems is to use the encapsulated forms of these bioactive substances in food products [10]. This approach can help to slow down the oxidation of PUFAs, increase the bioavailability of vitamin D3, neutralize undesirable odors and extend the shelf life of food products, thereby improving their quality and safety [11].

Phospholipid liposomes are a form of unique membrane-forming constructs for encapsulating hydrophobic bioactive substances (BAS), which are considered as a promising form for BAS delivery to cells and tissues [12]. This is due to the similarity of their bilayers to the cell membrane, as well as their ability to form mixed micelles with bile salts in the small intestine [13,14]. In addition, it is important that after entering the body, liposomes are metabolized and do not accumulate in the body. However, along with these properties, phospholipid liposomes have a number of disadvantages, i. e. instability of liposome structure and the associated uncontrolled release of loaded bioactive substances, as well as the tendency of liposomes to autoxidize with atmospheric oxygen due to the presence of PUFAs in them, especially at high temperatures in food production and during storage. It is now well known that additional encapsulation of phospholipid liposomes with loaded bioactive substances using food biopolymers may help solving these problems in practice [15].

A number of works are devoted to the use of encapsulated bioactive substances in the composition of meat products, in which the following food biopolymers were used as an encapsulating agent: sodium alginate, chitosan, maltodextrin, carrageenan, inulin, sodium caseinate, and whey protein concentrate [16]. Mohamed K. Morsy studied the stability of functional beef hamburgers enriched with microencapsulated fish oil [17]. Jimenez-Martin studied the effects of microencapsulated omega-3 PUFAs on the oxidative stability and sensory properties of frozen chicken nuggets [18]. Solomando investigated the effect of fish oil microcapsules in meat systems on the bioavailability of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) using *in vitro* models [19]. All these studies focused on the effect of microencapsulation on the bioavailability of the introduced components, as well as on the physicochemical and sensory properties of short-term heat-treated meat products. In this regard, it is of interest to study the effect of prolonged high-temperature exposure, on the preservation and bioavailability of the liposomal form of minor nutrients, in the form of a biopolymeric delivery system in the production of enriched meat products.

The purpose of this study was to study thermal stability and digestibility of a biopolymer system for the delivery of omega-3 PUFAs and vitamin D3 in liposomal form as a new functional ingredient for meat product fortification.

Objects and methods

The objects of the research were as follows:

— the *biopolymer supramolecular complex* consisting of phosphatidylcholine (PC) liposomes, with an amount of 0.156% w/v, 99.5% purity (Lipoid GmbH, Germany) coated with food-grade sodium caseinate (SC), with an amount of 2.44% w/v (88% protein, 3% carbohydrates, 1.5% fat, Targis Moloko LLC, Russia);

The following biologically active substances were encapsulated into bilayers of phosphatidylcholine (PC) liposomes:

- fish oil (FO), in the amount of 0.146% w/v ("Omega-deti Omega-3 Concentrate", Ruskaps, Russia), containing 20% DHA, 27.6% EPA according to gas-liquid chromatography data [20]);
- vitamin D3, in the amount of 5×10^{-6}% w/v ("Vitamin D3, drops" food supplement, Mirolla LLC, Russia), D3 content 250 μg/ml;
- essential oil of clove buds (EOC), in an amount of 0.006% w/v (IP Repicheva T. D., Russia).

Supramolecular complex (SC-PC-FO-EOC-D3) was prepared in an aqueous medium and then freeze-dried using AK 4–50 freeze-dryer (Proflab, Russia).

The supramolecular complex was formed by electrostatic and hydrophobic interactions, as well as by the formation of hydrogen bonds between the functional groups of sodium caseinate (SC) and PC-FO-EOC-D3 liposomes.

Previously obtained data indicated that the degree of encapsulation for fish oil and the essential oil of clove buds in a liposome bilayer reached 100 ± 1%, while for vitamin D3 it was 82 ± 2% [21].

The use of sodium caseinate for liposome encapsulation is due to its amphiphilic nature, which promotes the formation of various non-covalent bonds with phosphatidylcholine liposomes [22].

— *samples of heat-treated meat products*:

1) enriched meat product containing components of the supramolecular complex (EPC): sodium caseinate (SC) 2.75 g, phosphatidylcholine (PC) 0.156 g, fish oil (FO) 0.146 g, essential oil of clove buds (EOC) 0.006 g, vitamin D3 0.017 g;

2) enriched meat product containing a supramolecular complex (EPSC).

The technology of meat products was as follows: after grinding in a grinder, raw meat (54 g/100 g) was blanched for 5 minutes at 80 °C and homogenized to obtain a homogeneous finely ground paste, to which the recipe components were added, i. e. water (40 g/100 g), butter (3 g/100 g), nutmeg (0.05 g/100 g), supramolecular complex (3 g/100 g) or individual components included in the complex: sodium caseinate (SC) 2.75 g, phosphatidylcholine (PC) 0.156 g, fish oil (FO) 0.146 g, essential oil of clove buds (EOC) 0.006 g, vitamin D3 0.017 g. After mixing, the paste was packed in glass jars and heat treated at a temperature of 120 °C for 30 minutes.

The amount of functional ingredients added, i. e. EPA+DHA and vitamin D3, is determined by meeting at least 15% of physiological needs for adults established in Methodological recommendations of the MR 2.3.1.0253–21[1] and the TR CU 022/2011[2].

Determination of fat mass fraction in enriched meat products was carried out using the Soxhlet method in accordance with the GOST 23042–2015[3].

To assess the degree of lipid peroxidation, the peroxide value (PV) was determined by a method based on the reaction of the primary products of fat oxidation with potassium iodide, followed by titration and quantitative determination of the released iodine according to GOST 34118–2017[4], and the thiobarbituric acid value was determined by a method based on the reaction of thiobarbituric acid with malonic dialdehyde and the subsequent measurement of the absorbance of the product of this reaction on a spectrophotometer according to the GOST R 55810–2013[5].

The composition of fatty acids was determined by gas chromatography according to the GOST R 31663–2013[6] on an Agilent 7890A automatic gas chromatograph (Agilent Tech., USA) with a flame ionization detector. To determine fatty acids, a Supelco SP 2560 100 m × 0.25 mm × 0.2 mkm chromatographic column (Supelco, USA) was used;

Determination of vitamin D3 mass fraction was carried out according to the GOST 32307–2013[7];

An organoleptic analysis was carried out according to the GOST 9959–2015[8].

Determination of supramolecular complex particle size using laser light scattering in a dynamic mode

The effective hydrodynamic radius for sodium caseinate, PC-FO-EOC-D3 liposomes and SC-PC-FO-EOC-D3 supramolecular complex was determined in aqueous buffer solutions by dynamic laser light scattering using an LS-01 apparatus (Scientific Instruments, Russia), having a He-Ne laser with a vertically polarized beam (λ = 633 nm). All measurements were carried out in a thermostatic cell at 25 °C. The method is based on the analysis of autocorrelation function G(t) of fluctuations in the intensity (number of photons) of scattered light, which correspond to fluctuations in the local concentration of matter particles caused by their Brownian motion.

$$G(t)=\Sigma_j A_j A_{(j+n)} \quad (1),$$

where $t = n\tau$ is the delay time equal to the time interval of $\tau = 10^{-7}$ seconds, during which the intensity of light scattering is measured, multiplied by n which is the correlator channel number; A_j is the number of photons on the j-channel of the autocorrelator (high-speed computer). The light scattering intensity itself has a very strong fluctuation, but the product of the light scattering intensities $A_j A_{j+n}$ from two intervals separated by a fixed time period (the delay time t) is well averaged using an autocorrelator if the measurement procedure is repeated 10^5 to 10^6 times. Thus, j_0 corresponds to the start of measurements.

The time-correlation function may be associated with the time-correlation function of the electric field $g(t)$ of the scattered wave of light (determined by fluctuations in the number of photons in time) according to Seigert relation, chosen to interpret the empirical autocorrelation function in most cases:

$$G(t) = A + [Bg(t)]^2 \quad (2),$$

where

$$g(t) = exp(-D_{trans} q^2 t) \quad (3),$$

or

$$ln\, ln\, g(t) = -D_{trans} q^2 t \quad (4),$$

where

D_{trans} is the translational diffusion coefficient, m²/s;

t is time, s;

$q = \frac{4\pi}{\lambda} sin\frac{\theta}{2}$ is wave scattering vector, m⁻¹.

The effective hydrodynamic radius R_h was calculated from the time-correlation function of the light scattering intensity,

[1] Methodological recommendations MR 2.3.1.0253–21 "Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation". Moscow: Garant, 2021. Retrieved from https://www.garant.ru/products/ipo/prime/doc/402716140. Accessed April 15, 2024. (In Russian)

[2] TR CU 022/2011 Technical Regulations of the Customs Union "Food products regarding their labeling". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/902320347. Accessed April 15, 2024. (In Russian)

[3] GOST 23042-2015 "Meat and meat products. Methods of fat determination" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133107. Accessed April 15, 2024. (In Russian)

[4] GOST 34118-2017 "Meat and meat products. Method for determination of peroxide value". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146654 Accessed April 15, 2024. (In Russian)

[5] GOST R 55810-2013 "Meat and meat products. Method for determination of tiobarbituric acid reactive assay" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107008. Accessed April 15, 2024. (In Russian)

[6] GOST R 31663-2013 "Vegetable oils and animal fats. Determination of methyl esters of fatty acids by gaz chromatography method". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200104486. Accessed April 15, 2024. (In Russian)

[7] GOST 32307-2013 "Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107182. Accessed April 15, 2024. (In Russian)

[8] GOST 9959-2015 "Meat and meat products. General conditions of organoleptical assessment" Moscow: Standartinform, 2016. Retrieved from https://docs.cntd.ru/document/1200133106. Accessed April 15, 2024. (In Russian)

which was measured at the scattering angle θ = 90°. Then, from it, the dependence of the natural logarithm of the time-correlation function of the electric field $g(t)$ on the delay time (t) was calculated using DYNALS Release 1.5 software (all rights belong to A. Golding and N. Sidorenko). Then, from the tangent of the angle of initial section, translational diffusion coefficient D_{trans} was found. Next, the value of R_h was calculated from D_{trans} using Stokes-Einstein equation:

$$D_{trans} = \frac{kT}{6\pi\eta R_h} \quad (5),$$

where

k is Boltzmann's constant, $1{,}38 \cdot 10^{-23}$ J/K;

T is absolute temperature, K;

η is the dynamic viscosity of the liquid (in a dilute solution, usually it is solvent), Pa · s;

R_h is hydrodynamic radius, m.

The calculation results were presented as the size distribution of light-scattering particles in the form of a histogram, the ordinate axis of which was the light scattering intensity values, and the abscissa axis was the size (hydrodynamic radius) of the particles in microns.

Determination of the degree of liposome encapsulation with sodium caseinate

PC-FO-EOC-D3 liposomes not bound in the supramolecular complex with sodium caseinate before and after freeze-drying, as well as their control variant (without protein), were extracted from their aqueous solutions with diethyl ether (Kuzbassorgkhim LLC, analytical grade, Russia). 3 ml of ether was added to 1 ml of sample solution, stirred, kept for 30 minutes at a room temperature and then left for 24 hours at a temperature of 5–7 °C. The upper organic phase was separated and the absorbance value of extracted lipids was measured using SF 2000 spectrophotometer (Spectrum, Russia) at λ = 210 to 215 nm. Diethyl ether was used as a reference solution (the method was developed by the Laboratory of Functional Properties of Biopolymers at Emanuel Institute of Biochemical Physics of Russian Academy of Sciences) [20].

The efficiency of encapsulation of PC-FO-EOC-D3 liposomes with sodium caseinate in their supramolecular complex was calculated using the equation:

$$E = \frac{A_{control} - A_{SC}}{A_{control}} \times 100 \quad (6),$$

where

E is the efficiency of liposome encapsulation with sodium caseinate (SC), %;

$A_{control}$ is absorbance value measured for the diethyl ether extract of PC-FO-EOC-D3 liposomes from the control buffer solution, taken as 100%;

A_{SC} is absorbance value measured for the diethyl ether extract of non-SC-encapsulated liposomes in SC-PC-FO-EOC-D3 supramolecular complex solution.

In total, 3 independent experiments were carried out, the experimental error in which did not exceed 5%.

Simulation of experimental in vitro digestion

The release of fat and omega-3 fatty acids, EPA and DHA, from SC-PC-FO-EOC-D3 supramolecular complex and enriched meat products was assessed under conditions simulating *in vitro* digestion.

To simulate digestion process of the sample, the Infogest 2.0 digestion model was used in accordance with the method [23]. First, to simulate chewing, the sample was crushed in a porcelain mortar. Then 5 g of the test sample was mixed with 3.5 ml of simulated oral fluid (pH 7) consisting of 0.5 ml of α-amylase 1500 U/ml (Sigma, China), 25 μl of 0.3 M calcium chloride (PanReac, Spain), 10.6 ml of phosphate-buffered saline (PanReac, Spain) and 0.09 ml of 6 M hydrochloric acid (Component-Reaktiv, Russia). The mixture was thoroughly mixed at 250 rpm at 37 ± 1 °C for 2 minutes using ImmunoChem-2200 thermal shaker (Helena Biosciences Europe, USA). After that, a sample was taken for further research.

Simulation of gastric digestion was done by mixing 10 ml of liquid oral contents with 7.5 ml of simulated gastric juice (pH 3) consisting of 1.6 ml of porcine pepsin 25,000 U/ml (PanReac, Spain), 1 ml of lipase 1200 U/ml (ABBOTT LABORATORIES, Russia), 5 μl of 0.3 M calcium chloride (PanReac, Spain), 0.8 ml of 1 M hydrochloric acid (Component-Reaktiv, Russia) and 10.0 ml of phosphate-buffered saline (PanReac, Spain). The mixture was thoroughly mixed at 250 rpm at 37 ± 1 °C for 120 minutes using ImmunoChem-2200 thermal shaker (Helena Biosciences Europe, USA). At the end of the incubation time, samples were taken for further studies.

Next, intestinal digestion was simulated, for which 20 ml of gastric chyme was mixed with 10 ml of simulated intestinal fluid (pH 7), which contained 5.0 ml of porcine pancreatin 800 U/mg (PanReac, Spain), 1.5 ml of preserved bovine bile (Samson-med, Russia), 40 μl of 0.3 M calcium chloride (PanReac, Spain), 10.6 ml of phosphate-buffered saline (PanReac, Spain). The mixture was thoroughly mixed at 250 rpm at 37 ± 1 °C for 120 minutes using ImmunoChem-2200 thermal shaker (Helena Biosciences Europe, USA). Then samples were also taken for further research.

In parallel with the simulation of experimental digestion of the studied samples, a similar experiment was carried out, where instead of 0.5 g of the sample, distilled water was added to take into account the determined indicators of the introduced reagents (background).

After simulation, the resulting substance was centrifuged on LISTON C2201 (LISTON, Russia) at 3500 rpm for 15 minutes to sediment undigested particles. The supernatant was collected and frozen at minus 40 °C to inactivate the enzymes for at least 12 hours.

The fatty acid composition of the obtained samples was determined. Results were resented as the percentage of released fat, EPA and DHA relative to the initial content (before digestion) at the end of each digestion stage.

Statistical analysis

Statistical analysis was performed using Mann-Whitney U-test ($p<0.05$) and STATISTICA 10.0 software.

Results and discussion

The results of measuring the particle size of SC-PC-FO-EOC-D3 supramolecular complex and its components separately, i. e. sodium caseinate (SC) and PC-FO-EOC-D3 liposomes, measured by the dynamic laser light scattering showed that the average value of the hydrodynamic radius R_h for liposomes was 60 nm, for sodium caseinate it was 180 nm, and for supramolecular complex it was 210 nm (Figure 1).

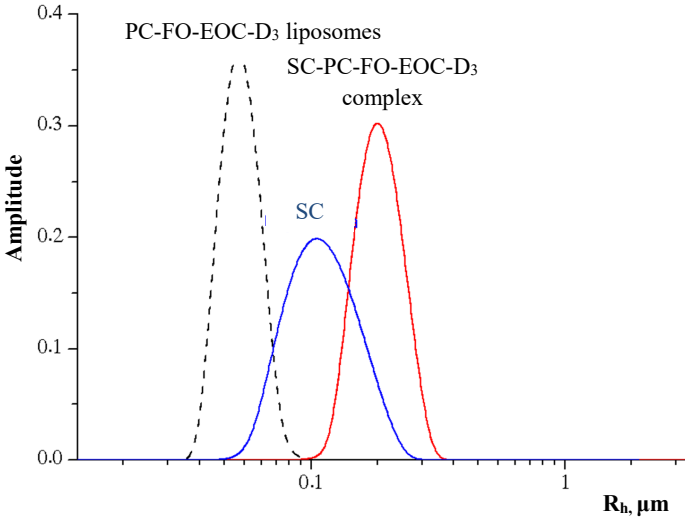

Figure 1. Size distribution for the studied samples: native sodium caseinate (blue), PC-FO-EOC-D3 liposomes (black dotted line) and their supramolecular complex, SC-PC-FO-EOC-D3 (red) in an aqueous medium (pH 7.0, 25 °C)

The shift of the complex peak from the peaks of the protein solution and liposomes to the region of a larger hydrodynamic radius indicates the successful formation of a submicron-sized complex in an aqueous medium.

The results of evaluating the effectiveness of liposome encapsulation with sodium caseinate in their supramolecular complex before and after freeze drying are presented in Table 1.

Table 1. The degree of liposome encapsulation with sodium caseinate in their supramolecular complex before and after freeze drying

Parameters	Free liposomes, %	Bound liposomes, %
The degree of liposome encapsulation in supramolecular complex before freeze drying	23.9 ± 0.3^b	76.1 ± 0.7^a
The degree of liposome encapsulation in supramolecular complex after freeze drying	25.3 ± 0.1^a	74.7 ± 0.9^a

Note: Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values determined for the two samples (comparing the values in the same column).

The degree of liposome encapsulation in supramolecular complex was more than 74%, while the difference between the number of bound liposomes before and after freeze drying was statistically insignificant.

The results of total fat and omega-3 PUFA (EPA and DHA) content in SC-PC-FO-EOC-D3 supramolecular complex and in thermally processed meat products, i. e. enriched meat product containing components of the supramolecular complex (EPC) and enriched meat product containing a supramolecular complex (EPSC), are presented in Table 2.

The results (Table 2) indicate that samples of enriched meat products differ slightly in fat content. When omega-3 fatty acids, EPA and DHA, were added in equal amounts to the formulations of EPC and EPSC samples, the content of ∑EPA+DHA in finished products was 21.6% and 70% of the adequate daily intake for adults and children over two years of age, respectively, which is 250 mg DHA+EPA per day[9]. Probably, the identified difference is associated with the oxidation and further degradation of fatty acids introduced into EPC sample in a free (non-encapsulated) form. The data obtained indicate the advantage of using SC-PC-FO-EOC-D3 supramolecular complex for fortification of meat products subjected to heat treatment and are consistent with the work [24].

A study of the fatty acid composition of the samples showed that the sum of all omega-3 fatty acids in EPC was 0.079 ± 0.002 g/100 g, and in EPSC it was 0.207 ± 0.002 g/100 g. The data obtained allow to state that EPSC sample as a source of omega-3 fatty acids, because in accordance with the technical regulations TR CU 022/2011 "Food products regarding their labeling", a product in which the amount of omega-3 fatty acids is at least 0.2 g per 100 g may be labeled as a "source" of omega-3 fatty acids.

The vitamin D3 content in EPC and EPSC samples was 231% and 470% of the recommended daily intake, respectively[10]. Despite the fact that the research results showed an excessively high content of vitamin D3 in both samples, its amount does not exceed the maximum permissible daily intake [25]. The results are consistent with those obtained

[9] Methodological recommendations MR 2.3.1.0253–21 "Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation". Moscow: Garant, 2021. Retrieved from https://www.garant.ru/products/ipo/prime/doc/402716140. Accessed April 15, 2024. (In Russian)

[10] TR CU 022/2011 Technical Regulations of the Customs Union "Food products regarding their labeling". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/902320347. Accessed April 15, 2024. (In Russian)

Table 2. Total fat and omega-3 fatty acids (EPA and DHA) content in the studied samples

Parameters	SC-PC-FO-EOC-D_3	EPC	EPSC
Fat, g/100 g of sample	10.76 ± 0.79	4.2 ± 0.4^a	4.6 ± 0.8^a
EPA, g/100 g of sample	1.136 ± 0.01	0.029 ± 0.002^b	0.101 ± 0.003^a
DHA, g/100 g of sample	2.025 ± 0.01	0.025 ± 0.001^b	0.074 ± 0.002^a
∑EPA+ DHA, g/100 g of sample	3.161 ± 0.01	0.054 ± 0.001^b	0.175 ± 0.002^a
Vitamin D_3, μg/100 g of sample	210.49 ± 31.57	11.6 ± 2.9^b	23.5 ± 5.9^a

Note: Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values determined for the two samples (comparing the values in the same row).

by Rabelo et al. [26], who believe that encapsulating vitamin D3 in colloidal nanocarriers may maintain its effectiveness, biological activity and bioavailability, thereby increasing its physiological benefits.

The effectiveness and bioavailability of supramolecular complex and enriched meat products in an in vitro model simulating the processes in the gastric and intestinal phases of digestion was determined by studying the content of released fat and omega-3 fatty acids, EPA and DHA, directly from SC-PC-FO-EOC-D3 supramolecular complex (Figure 2), and from EPC and EPSC samples (Figure 3).

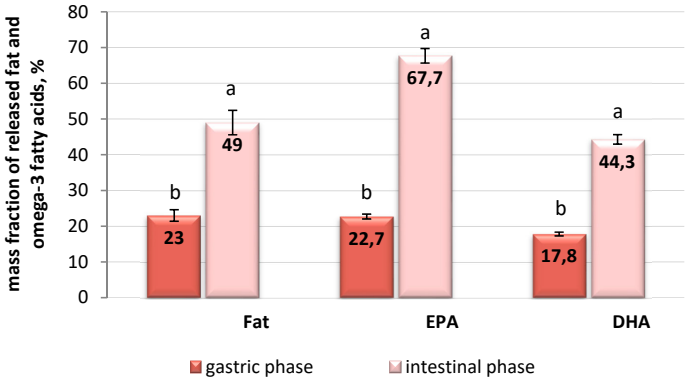

Figure 2. Mass fraction of released fat and omega-3 fatty acids during in vitro digestion of SC-PC-FO-EOC-D3 supramolecular complex, %

Note: Data scatter bars show the standard deviation from the average value of the measured parameter. Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values measured in the gastric and intestinal phases.

The results showed that most of the fat released from SC-PC-FO-EOC-D3 supramolecular complex during the intestinal phase (49%), while during the gastric phase, it was 23% (Figure 2).

Since lipolytic enzymes (pancreatic lipase, phospholipase and sterol esterase) are secreted at the intestinal level producing most of the hydrolyzed lipid compounds, fat found in the gastric phase is due to partial hydrolysis and dissolution of the superficial fat of the capsule, as a result of the gastric lipase action, which probably also indicates the effectiveness of liposome encapsulation in a supramolecular complex with sodium caseinate.

These results are consistent with the studies by Solomando et al. [27], who showed that during digestion, depending on the nature of the lipid compound, about 10% to 30% of fats are hydrolyzed in the gastric phase and 50% to 90% of fats are hydrolyzed in the intestinal phase.

The results of bioavailability studies of EPA and DHA fatty acids in SC-PC-FO-EOC-D3 supramolecular complex showed an increase in their content by 2.9 and 2.4 times, respectively, in the intestinal phase relative to the data obtained in the gastric phase.

When studying in vitro digestion of EPC and EPSC meat products, it was revealed that the release of fat for both samples in the gastric phase was almost the same, in contrast to the intestinal phase, where in EPSC, released fat was found to be 2 times higher than that of EPC (Figure 3).

This indicates that the use of physiologically functional ingredients in encapsulated form for meat product fortification is more effective and does not violate the general principles of lipid digestion.

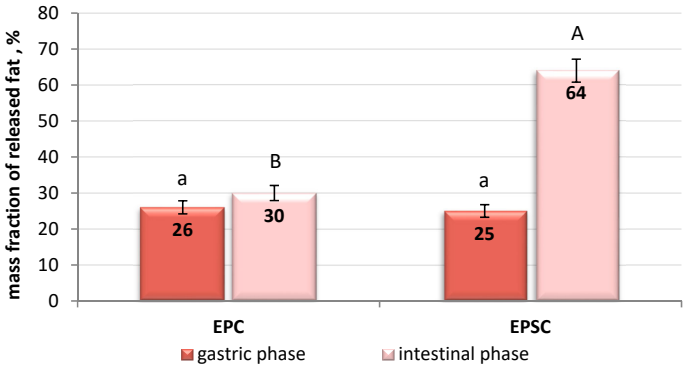

Figure 3. Mass fraction of released fat during in vitro digestion of enriched foods, %.

Note: Data scatter bars show the standard deviation from the average value of the measured parameter. Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different letters indicate statistically significant differences in parameter values, enriched meat product containing components of the supramolecular complex, EPC, and enriched meat product containing a supramolecular complex, EPSC, namely lowercase letters in the model gastric environment and capital letters in the model intestinal environment.

Regarding the release of EPA and DHA from enriched meat products when simulating in vitro digestion, the mass fraction of released EPA and DHA in the intestinal phase was 3.9 and 1.9 times higher, respectively, when using physiologically functional ingredients in the form of SC-PC- FO-EOC-D3 supramolecular complex compared to a product containing these ingredients in their native form (Figure 4).

The relatively low bioavailability of EPA and DHA from non-encapsulated fish oil is due to the higher release of these fatty acids in the gastric phase by gastric lipase.

Lipid oxidation is a common type of spoilage in foods containing fats and oils, resulting in undesirable changes in taste, discoloration, and reduced nutritional value. Wang et al. [28] and Solomando et al. [29] believe that the use of encapsulation not only protects the polyunsaturated fatty acids (EPA and DHA) in fish oil from oxidation, but also increases their solubility and hides any undesirable odors.

The results of using encapsulation in enriched meat products are presented in Table 3.

Table 3. Parameters of fat oxidation in enriched meat products

Parameters	EPC	EPSC
Peroxide value, meq/kg	1.6 ± 0.2^{a}	1.1 ± 0.1^{b}
Thiobarbituric acid value, mg of MA/kg	< 0.039	< 0.039

Note: **Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values determined for the two samples (comparing the values in the same row).**

Data analysis showed that the use of encapsulation led to a decrease in peroxide value by 1.5 times in EPSC relative to EPC. No changes in thiobarbituric acid value were detected. The results correlate with the data on the sensory

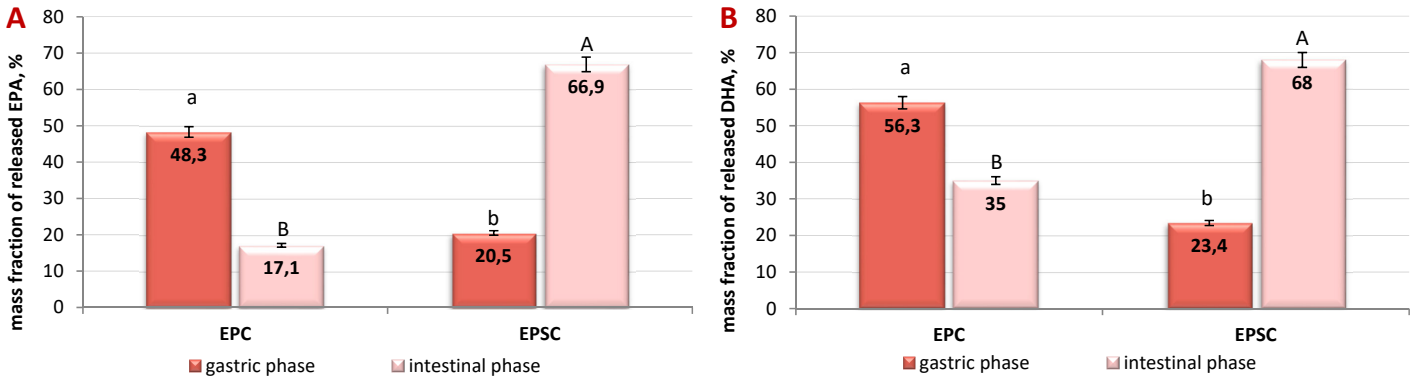

Figure 4. Mass fraction of released omega-3 fatty acids (A — EPA; B — DHA) during in vitro digestion of the studied samples, %.

Note: Data scatter bars show the standard deviation from the average value of the measured parameter. Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different letters indicate statistically significant differences in parameter values, enriched meat product containing components of the supramolecular complex, EPC, and enriched meat product containing a supramolecular complex, EPSC, namely lowercase letters in the model gastric environment and capital letters in the model intestinal environment.

evaluation of the samples. It is also important to note that additional inhibition of PUFA autoxidation in the composition of the fats was due to adding one of the most effective plant antioxidants to the liposomes, i. e. essential oil of clove buds [20].

Sensory evaluation of the enriched meat products showed that EPSC had an intense meaty taste and no taste or smell of fish oil, while EPC had a weak taste and smell of fish oil, which intensified when heated. It was also noted that the presence of SC-PC-FO-EOC-D3 supramolecular complex in the composition of the products had a stabilizing effect on the texture of the product.

A number of studies have shown that, in general, the addition of encapsulated fish oil has a little effect on the sensory characteristics of meat products.

Solomando et al. [29] noted that the addition of fish oil microcapsules to boiled and dry-cured sausages had a slight effect on taste and color parameters.

In studies by Aquilani et al. [4] and Solomando et al. [5], a slight reduction in odor and taste intensity was observed in hamburgers fortified with microencapsulated fish oil. But during storage, these products showed no changes in sensory characteristics and no increase in rancidity, in contrast to the control product without fortification [5].

In addition, Jiménez-Martín et al. [18] and Solomando et al. [29] noted that the use of fish oil microcapsules resulted in increased salt taste in fortified chicken nuggets and dry-cured sausages, which may be related to the hypothesis that increasing the oil phase improves mixing with saliva, thereby accelerating the transport of salt to taste buds [5]. According to Solomando et al. [29], this hypothesis may be used to develop salt-reduced meat products fortified with fish oil microcapsules without reducing taste perception.

Conclusion

EPSC fortification with physiologically functional ingredients (fish oil, vitamin D3, essential oil of clove buds) in encapsulated form allows meeting the daily requirement of EPA+DHA by 70%, and vitamin D3 by 470%, respectively, as well as claiming the product as a "source" of omega-3 fatty acids and "a product with a high content of vitamin D3" in accordance with the legislation of the Russian Federation. When simulating the digestion of the studied meat product, it was revealed that the use of physiologically functional ingredients in encapsulated form had a positive effect on the bioavailability of EPA and DHA. At the same time, the use of heat treatment did not affect their bioavailability, as well as sensory parameters and oxidative stability of the meat product.

Thus, the use of functional ingredients in encapsulated form for meat product fortification helps improving their lipid profile.

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