ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2019, Vol. 45, No. 1, pp. 34–41. © Pleiades Publishing, Ltd., 2019.
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# Initiated Oxidation of Phosphatidylcholine Liposomes with Some Functional Nutraceuticals

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Received May 3, 2018; revised May 28, 2018; accepted June 16, 2018

**Abstract**—Design and research of liposome structures on the basis of soy phosphatidylcholine (PC) as nanocontainers for delivery of various functional nutraceuticals in tissues and cells of organisms, as well as models for studying biochemical processes in cell membranes, has been of undoubted interest recently. As liposomes are oxidized and destroyed under the influence of light and high temperatures, there are various ways to protect them. In the present work, the effect of temperature on the soy PC liposome oxidation initiated by a water-soluble azo-initiator AAPH was investigated; also the influence of ω-3 α-linolenic fatty acid (ALA) supplement in the liposomes on the process was studied. Inhibition of liposome oxidation by essential oil of clove buds (EOC) (*Eugenia caryophyllata* Thumb) or liposome encapsulation in sodium caseinate was studied. The possibility to efficiently inhibit oxidation of PC liposomes at $T = 37°C$ by phenolic compounds (eugenol) of EOC was demonstrated; however, at an elevated temperature (60°C) the oxidation rate increased 3–4 times and the rate of inhibition of conjugated diene formation significantly decreased. Addition of ALA to liposomes led to considerable intensification of oxidation. Encapsulation of liposomes in sodium caseinate reduced liposome oxidation by 70%, and the combined effect of sodium caseinate encapsulation and EOC, by 90%. With a temperature increase, the effectiveness of protection by the caseinate envelope significantly decreased, even in the presence of clove oil.

*Keywords*: liposomes, phosphatidylcholine, eugenol, sodium caseinate, initiated oxidation

**DOI:** 10.1134/S1068162019010138

## INTRODUCTION

Today, works on development of liposomal structures based on soy phosphatidylcholine (PC) as containers for delivery of various functional nutraceuticals (substances that are of both nutritive and pharmaceutical value), including essential polyunsaturated ω-3 and ω-6 fatty acids (PUFAs), antioxidants, vitamins, and organic acids, in tissues and cells of living organisms are of considerable interest [1–3]. Phosphatidylcholine liposomes are also used as models to study biochemical processes in membranes of living human and animal cells, particularly, lipid peroxidation (LP) and effect of various substances in the process [4–7].

Owing to the presence of polyunsaturated fatty acids (PUFAs) in soy phosphatidylcholine, liposomes prepared thereof are easily oxidized in air and destroyed under the effect of light and high temperature; therefore, they require protection from oxidation and degradation for prolonged storage under various extreme conditions, e.g., during production and processing of food products [2, 3].

First of all, to prevent oxidation, various antioxidants are introduced into the liposomes [8–11], among which fat-soluble phenolic compounds comprising essential oils of aromatic plants look promising [10]. These compounds include natural plant mono- and polyphenols: eugenol, thymol, carvacrol, phenolic acids, flavonoids, carotenoids, coumarins, anthocyanidins, tannins, alkaloids, etc. Study of inhibition of autooxidation of PUFA methyl esters isolated from flax-seed oil showed that essential oil of clove buds (EOC) was the most efficient [10]. In addition to antioxidants, to protect PC liposomes from oxidation, encapsulation into various biopolymers is often used; not only does it prevent liposomes from oxidation, but it also improves their functionality and solubility in an aqueous medium, which is rather important to increase the bioavailability of nutraceuticals therein [12–14].

The aim of the work was to study the kinetics of soy PC liposome oxidation initiated by 2,2'-azobis(amidinopropane) dihydrochloride (AAPH) at various temperatures and the effect of α-linolenic (ALA) acid

Abbreviations: AAPH, 2,2'-azobis(amidinopropane) dihydrochloride; PC, phosphatidylcholine; ALA, alpha-linolenic acid; DPPH, diphenylpicrylhydrazine; AO, antioxidative; AR, antiradical; Cas-Na, sodium caseinate; ROS, reactive oxygen species; DC, diene conjugates; KD, ketodienes; PUFA, polyunsaturated fatty acids; LP, lipid peroxidation; EOC, essential oil of clove buds.

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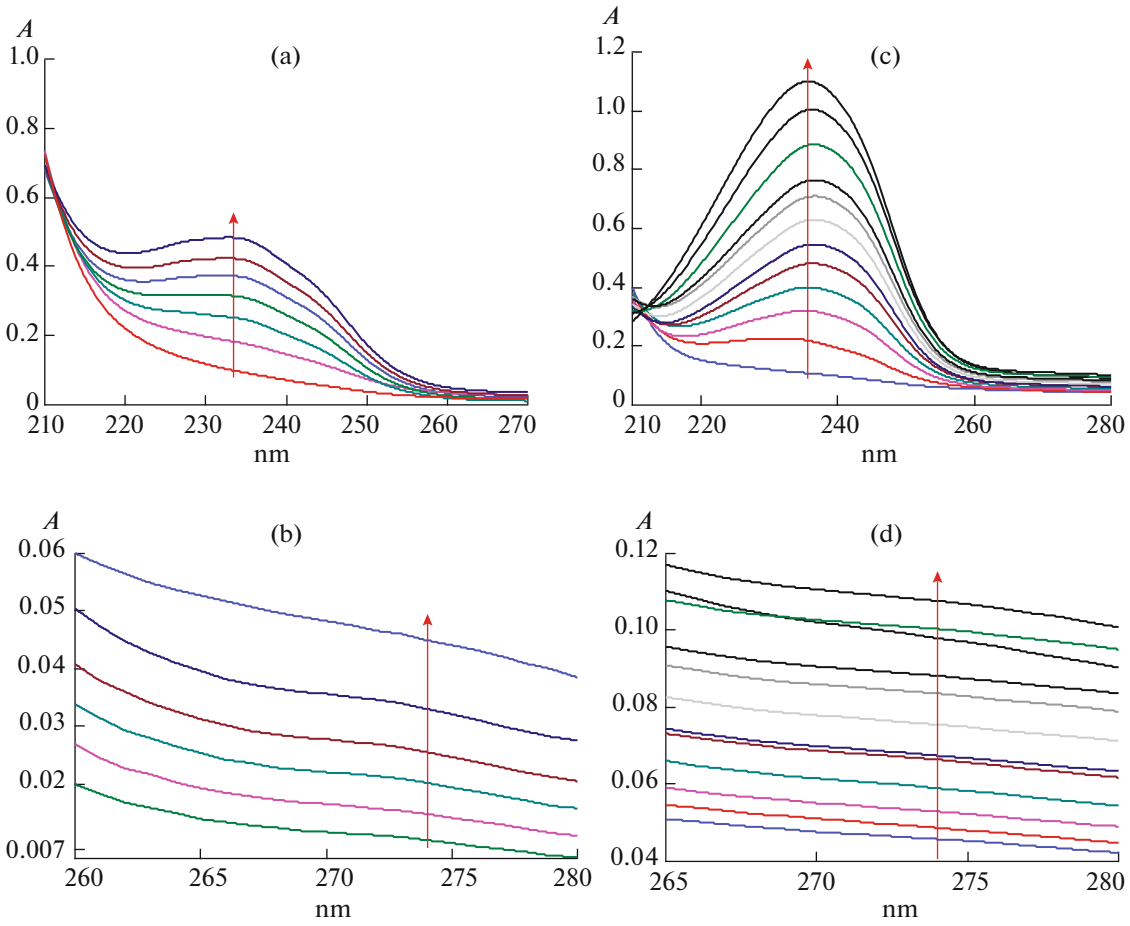


**Fig. 1.** Absorption spectra of PC liposomes (1 mg/mL) in the process of initiated oxidation at 37 (a, b) and 60°C (c, d) for wavelength ranges from 210 to 270 nm (a, c) and from 260 to 280 nm (b, d). Arrows indicate time interval of oxidation (from starting point $t_0 = 0$ to $t_{end} = 400–500$ min). Measurement error of optical absorption did not exceed 3%.

addition to liposomes on initiated oxidation, as well as inhibition of oxidation of the liposomes by addition of EOC and encapsulation of liposomes in sodium caseinate.

## RESULTS AND DISCUSSION

At the first stage, we studied soy PC liposome oxidation processes as a function of temperature. Figure 1 presents examples of PC liposome absorption spectra at various time points upon initiation of oxidation at two temperature values (37 and 60°C). At either temperature, there were absorption maxima at λ 233 nm typical of oxidation products, namely diene conjugates (DC) (panels a and c), while no pronounced maxima at λ 274 nm, typical of ketodienes (KD), was observed (panels b and d). Over the whole period of observation, the accumulation of KD less than 10% of the formation of DC. Therefore, further study of oxidation was performed by following only the DC concentration.

Figure 2 presents the dynamics of DC absorption for liposomes prepared of pure PC ("pure" liposomes) in the process of their auto (curves *1* and *2*) and AAPH-initiated (curves *3* and *4*) oxidation at temperatures 37 (curves *1* and *3*) and 60°C (curves *2* and *4*). At 37°C, absorption at λ 233 nm of PC liposomes in the course of their autoxidation developed very slowly (induction period is about 5 h) and till up to 10 h only an insignificant increase in DC content could be noted (curve *1*). Temperature increase accelerated the process, decreasing the induction period in the DC accumulation curve to 30 min (curve *3*). Taking into account the magnitude of molar extinction coefficient of DC at λ 233 nm of 274000 $M^{-1}$ $cm^{-1}$ [9], the rate of DC generation upon autoxidation can be estimated. At 37°C it is $0.84 \times 10^{-7}$ M $min^{-1}$ during 300 min (300 through 600 min). When the temperature increased to 60°C, the autoxidation rate increased by almost an order of magnitude ($8.14 \times 10^{-7}$ M $min^{-1}$ over the curve *2* fragment from 40 to 200 min).

AAPH-initiated oxidation at 37°C (curve *3*) yielded a nonmonotonic curve of DC accumulation, that is, alteration of accelerating and slowing down of oxidation. Apparently, this is in connection with the

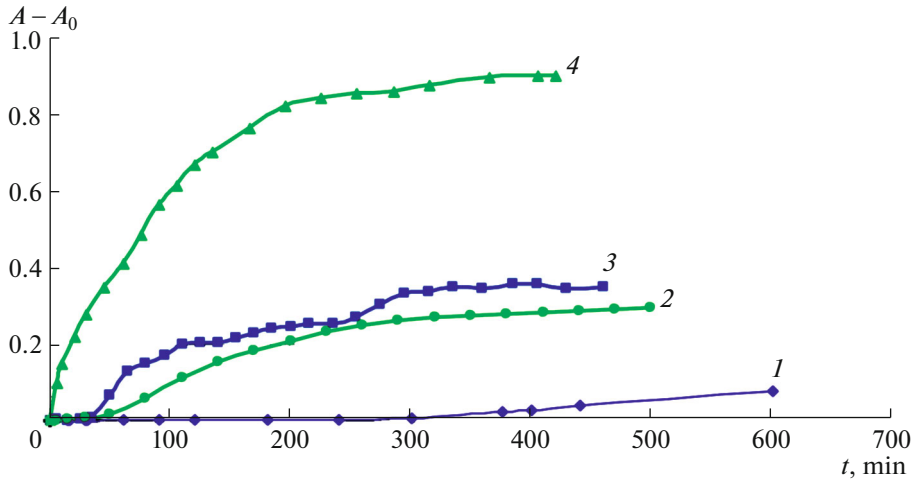


**Fig. 2.** Accumulation of DC in the process of auto (*1* and *2*) and initiated (*3* and *4*) oxidation of PC liposomes (1 mg/mL) at 37 (*1*, *3*) and 60°C (*2*, *4*). $A_0$, optical absorption at $t = 0$. Error of $A - A_0$ determination for three measurements did not exceed 10%.

presence of three major unsaturated fatty acids (PUFAs) in the Lipoid S100 soybean PC (Lipoid GmbH, Germany): oleic, linoleic, and linolenic (13 : 60 : 7 percent ratio to total FA). Probably, they get involved in the process of oxidation in turns with regard to their concentration and oxidation rate constant ratios (1 : 10 : 15) [15]. At higher temperature (curve *4*) the effect was barely observed. The small (30 min) induction period, which is present at 37°C and is caused not only by kinetics of DC accumulation [16, 17], but also small amount of α-tocopherol (0.15–0.25 g/100 g) present in the Lipoid S100 PC, also disappeared. The process of oxidation product accumulation may be slowed down by the presence of saturated fatty acids (approximately 20%) in the Lipoid S100 PC as well [18, 19].

In a liposome dispersion, under a constant rate of the AAPH initiator radical generation upon thermal decomposition of the latter, chain radical oxidation of FA (termed LH in equations below) in PC liposomes rises. It proceeds according to the following simplified process [5, 9, 11]:

$$R{-}N{=}N{-}R \rightarrow 2eR^{\bullet} + N_2, \quad (1)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}, \quad (2)$$

$$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet}, \quad (3)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}, \quad (4)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}, \quad (5)$$

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{nonradical products}, \quad (6)$$

$$LOO^{\bullet} + InH \rightarrow LOOH + In^{\bullet}. \quad (7)$$

Reactions (1)–(3) describe thermal dissociation of AAPH (1), rapid ($k_2 \approx 10^9 \times M^{-1} s^{-1}$) formation of initiator peroxide radicals $ROO^{\bullet}$ (2), and alkyl radical $L^{\bullet}$ (3) ($k_3 \approx 6 \times 10^1\ M^{-1} s^{-1}$); (4), (5), oxidation chain reaction ($k_4 = 10^9 \times M^{-1} s^{-1}$, $k_5 = 6 \times 10^1\ M^{-1} s^{-1}$); reactions (6, 7) represent chain interruption due to recombination (6) and effect of an inhibitor (InH) (7). In the Lipoid S100 PC, the linoleic fraction is the largest, therefore, the constant values reported are for the linoleate [17].

AAPH initiates radicals in equation (1) at a rate of $W_i = k_i$ [AAPH], where $k_i = 1.3 \times 10^{13}\ e^{-112600/RT}$ is the rate constant of the reaction [20]. At 37°C $k_{i37} = 1.36 \times 10^{-6}\ s^{-1}$ and at 60°C $k_{i60} = 27.6 \times 10^{-6}\ s^{-1}$, that is, 20 times higher. According to laws of fluid-phase oxidation of hydrocarbons and lipids by molecular oxygen that proceeds according to the scheme described in (1)–(7) with a second-order chain interruption, the $O_2$ consumption rate is $W_{O_2} = k_p/(2k_t)^{0.5} \times [LH] \times W_i^{0.5}$, where $k_p$ and $k_t$ are efficient rate constants of chain oxidation prolongation and interruption (4)–(7) [21, 22].

Comparison of curves *3* and *4* in Fig. 2 shows that increase in temperature leads to an approximately threefold increase in the rate of DC accumulation till it reaches a plateau. The average rate of PC liposome oxidation increased 4.5–5.0 times (from $0.33 \times 10^{-5}$ M/min to $1.61 \times 10^{-5}$ M/min) in the first 20–30 min of oxidation (DC accumulation). This mainly occurred due to a considerable increase in the rate ($W_i$) of thermal dissociation of AAPH and accumulation of oxidation-initiating radicals. With allowance for the DC accumulation rate corresponding to the rate of oxygen consumption $W_{O_2}$, one can estimate the effect of temperature on the fatty acid oxidation rate (LH) in PC liposomes and compare it with measured values. At the same value of [AAPH], the ratio of initiation rates at different temperatures $W_{i60}/W_{i37} = k_{i60}/k_{i37} = 20$. Using the equation for $W_{O_2}$ and taking into account

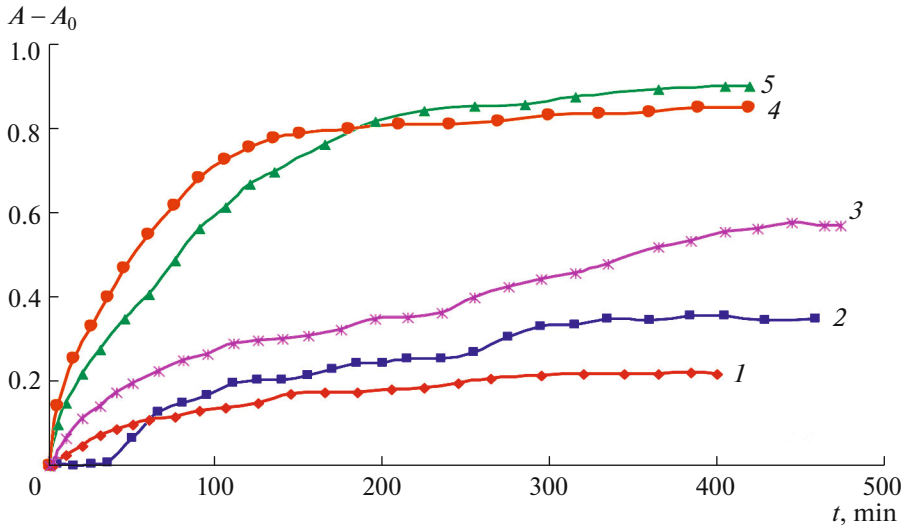


**Fig. 3.** Initiated oxidation of "pure" PC liposomes (*2*, *5*), [PC] = 1 mg/mL, at 37 (*2*) and 60°C (*5*), as well as ALA-supplemented PC liposomes (*1*, *3*, *4*), PC : ALA, 1 : 0.59, at 37 (*1*, *3*) and 60°C (*4*). [PC + ALA] = 1 mg/mL (curves *1*, *4*) and 1.59 (*3*). Error of $A - A_0$ determination for three measurements did not exceed 10%.

the equality of [LH], $k_p$, and $k_t$, (60°C)/(37°C) = $(W_{i60}/W_{i37})^{0.5} = (20)^{0.5} \approx 4.5$, which corresponds to the experimental ratio of DC accumulation rates.

As indicated above, PC liposomes can be used as carriers for delivery of essential ω-3 and ω-6 PUFAs in the organism. In this connection, the effect of PUFA on oxidation of these liposomes is to be elucidated. For this purpose, we used PC liposomes with ω-3 α-linolenic acid (ALA) incorporated in the bilayer (Fig. 3).

First of all, as follows from curves *1*, *3* versus curve *2* in Fig. 3, ALA accelerated liposome oxidation at the initial stage of the process (till 60 min), excluding the induction period, even in the case of lower PC concentration compared to "pure" liposomes (curve *2*). In the case of equal amounts of PC in the control (curve *2*) and ALA-supplemented liposomes (curve *3*), the rate of oxidation in the presence of ALA increased considerably, while DC add-on was 1.5 times higher than the value in the control. At 60°C, introduction of ALA in liposomes considerably (1.5–2 times) accelerated liposome oxidation (curve *4*) compared to control (curve *5*) till about 200 min; however, later on DC accumulation curves nearly coincide, which can be explained apparently by complete spending of ALA introduced in PC liposomes.

Phosphatidylcholine liposomes present a convenient model to study antioxidant (AO) activity of natural compounds, particularly, if they are of a fat-soluble nature. As mentioned above, these compounds comprise phenolic compounds of essential oils, the most active of which is the essential oil of clove buds (EOC) [10]. EOC exhibits antiradical (AR) activity in a model system with diphenylpicrylhydrazine (DPPH) and acts as iron chelating agent in reaction with hydroxide radicals inhibiting oxidation of linoleic acid [23]. Therefore, it seemed reasonable to check its AO effect in the model of initiated oxidation of PC liposomes.

Figure 4 demonstrates the dynamics of LP at 37°C for PC liposomes (curve *1*) and inhibition of oxidation due to incorporation of various concentrations of EOC in liposomes. Control curve *1* has an induction period of approximately 30 min, while EOC slows down liposome oxidation considerably, with linear dependence of induction period on EOC concentration.

EOC comprises (by mass) eugenol (70 to 85%), eugenol acetate (13%), caryophyllene (10%), eugenyl acetate (5%), α-bergamote (3%), cyneon (3%), linalool, terpenes, and other compounds at concentrations below 1% [10]. Eugenol (4-allyl-2-methoxyphenol, M 164.2 g/mol) is a very potent monophenolic antioxidant. It is the main determinant of inhibition of PUFA oxidation in PC liposomes.

In addition to the above-indicated papers [10, 23], a number of works [24–26] reports studies of AO and AR properties of EOC and eugenol. Using gas chromatography and autoxidation of *trans*-2-hexanal, AO properties of eugenol in EOC were evaluated; even at low EOC concentrations (1%), eugenol exhibited high AO activity, while eugenol was preserved in EOC at this concentration at room temperature for approximately 100 days [24].

In work [25], luminol-dependent chemiluminescence was used to study the inhibiting effect of pure eugenol on the formation of reactive oxygen species (ROS) in the course of stimulated respiratory burst in human neutrophils at 37°C. The decrease in formation of ROS depended linearly on the concentration of

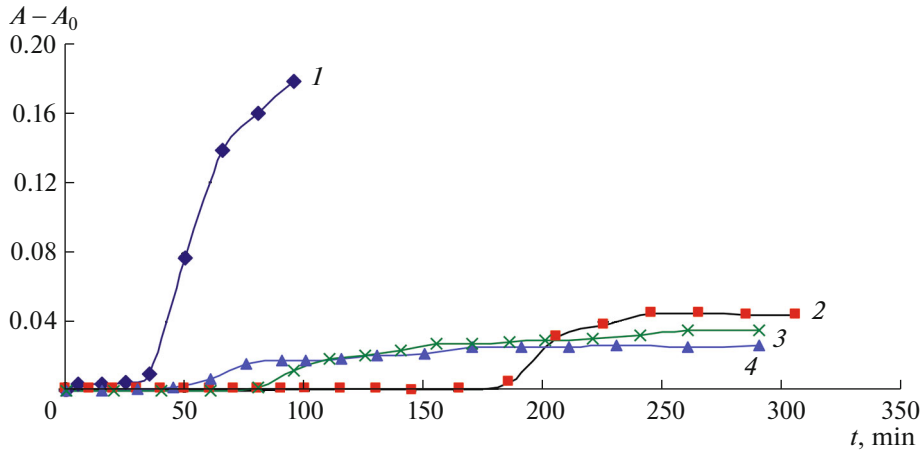


**Fig. 4.** Oxidation of PC liposomes (curve *1* is a fragment of curve *3* in Fig. 2) and inhibition of oxidation by EOC at a concentration of 4.0 (*2*), 2.0 (*3*), and 1.5% (*4*) in liposomes (37°C, [PC] = 1 mg/mL). Error of $A - A_0$ determination for three measurements did not exceed 10%.

eugenol and was 86% at a concentration of the latter of 22 μg/mL, which evidences high AR activity of eugenol. This finding is supported by another study [26], in which AR activity of EOC was measured in the reaction of DPPH radical reduction and AO activity, in the inhibition of β-carotene bleaching in a system of methyllinoleate autoxidation, where the efficiency of inhibition was 80%.

As mentioned above [10], along with eugenol, EOC contains other active antioxidants, i.e., unsaturated aliphatic and cyclic compounds, many of which compete for radicals with PUFAs, while the mechanism of the reaction with radicals differs from the mechanism of reaction of phenols. Therefore, the efficiency of EOC is always higher than that of individual antioxidants due to the mutual effect of the essential oil components. The AO effect of essential oils is prolonged. In this connection, it is important to note that in our experiments on oxidation of EOC-containing liposomes (4% by weight) at 37°C for 180 min, PC oxidation was completely inhibited, while at the moment of maximum DC accumulation its concentration in the presence of EOC was approximately eight times lower (by 87.5%) (Fig. 5a, curves *1* and *4*). These EOC properties (synergistic effect of its components and prolonged inhibition of oxidation) are rather important in the storage of food products.

A temperature increase to 60°C (Fig. 5b) decreased the time of complete inhibition of PC oxidation by EOC from 180 min to 10 min (curves *4* in Figs. 5a, 5b). DC concentration decreased on average 1.5 times (by 37.5%) compared to "pure" liposomes (Fig. 5b, curves *1* and *4*) over the whole period of oxidation. Despite the rigid oxidation conditions, EOC retained its AO properties.

The efficiency of PC + ALA mixture oxidation inhibition by EOC (Fig. 5a, curves *2*, *3*) is somewhat different: at 37°C, EOC decreased DC accumulation only four times (by 73%) and induction period, two times, probably, due to very active oxidation of ALA. Upon a temperature increase (Fig. 5b), the induction period almost disappeared, while concentration of DC during EOC inhibition changed unevenly (from 30 to 5%) (curves *2*, *3*). The decrease in inhibiting activity of EOC at this temperature is associated with decomposition of some of its components, including eugenol, and loss of AL and AR activity.

As mentioned in the Introduction, one of the ways to prevent PC liposomes from oxidation is their encapsulation in various hydrophilic biopolymers acting as nanocontainers improving PC liposome solubilization in aqueous media while preserving their functionality [27, 28]. In the work, we used a natural biopolymer sodium caseinate (Cas-Na) [27–29]. It is a micelle-like sodium form of a basic milk protein combining such individual proteins as $\alpha_{S1}$-, $\alpha_{S2}$-, β-, and κ-caseins, therefore, its advantages as a nanocontainer for soybean PC liposomes include not only prevention of PC oxidation, but also increased nutritive value [27–29]. To study the effect of Cas-Na on PC liposome oxidation, the dynamics of oxidation inhibition in pure liposomes and in the presence of EOC by Cas-Na was studied at 37 and 60°C (Fig. 6).

At 37°C, Cas-Na decreased the maximum level of DC approximately 3.5-fold (by 70%) (Fig. 6a, curves *1* and *2*), and together with EOC, 10-fold (by 90%) (curve *4*). Notably, Cas-Na barely influenced the duration of the induction periods of PC (Fig. 6a, curves *1* and *2*) and EOC–PC liposome oxidation (see curves *3*, *4*). Based on the data on structural parameters of the complex particles measured by multiangle light scattering, that is, molar mass, size, shape, and density of particles [13, 29], Cas-Na complexes with

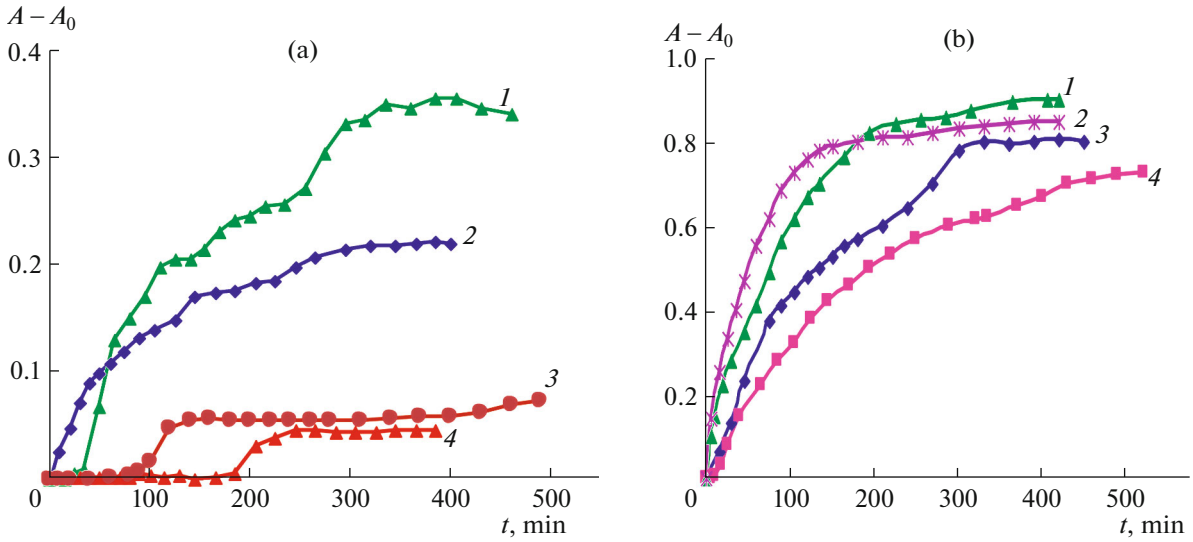


**Fig. 5.** Oxidation at 37 (a) and 60°C (b) of "pure" (control) PC liposomes (curve *1*), ALA-supplemented PC liposomes (*2*), in the presence of 4% EOC (*4*) and in the presence of both ALA and EOC (*3*). Concentration of all mixtures was 1 mg/mL, ALA content was 37% or 0.37 mg/mL. Error of $A - A_0$ determination for three measurements did not exceed 10%.

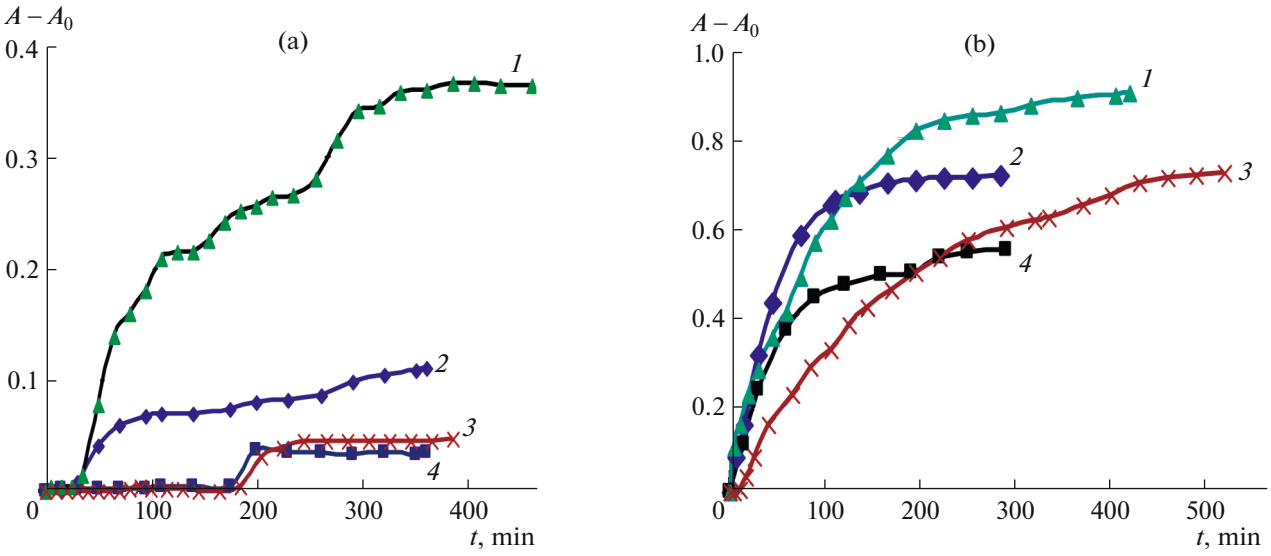


**Fig. 6.** Inhibiting effect of encapsulation of PC liposomes in sodium caseinate (Cas-Na) (curves *2* in both panels) and combined effect of EOC (curve *4*) at 37 (a) and 60°C (b). Control PC liposomes (curve *1*) and PC + EOC (curve *3*). [PC + EOC] = 1 mg/mL, Cas-Na–PC–EOC, 10 : 1 : 0.04. Error of $A - A_0$ determination for three measurements did not exceed 10%.

studied PC liposomes are supramolecular particles with a dense structure.

Formation of such a complex probably prevents oxygen diffusion to unsaturated hydrocarbon bonds in PUFAs of liposomes thus promoting their protection from oxidation. The increase in temperature leads to a significantly lower inhibiting effect of Cas-Na, while induction periods almost disappeared (Fig. 6b, curves *2*, *4*). Encapsulation of PC liposomes into Cas-Na leads to only a 1.3-fold (by 20%) decrease in the maximum level of DC, while the joint effect of Cas-Na and EOC increased the effect to 1.7-fold (by 40%). The decrease in the inhibiting effect of Cas-Na upon heating is probably associated with structural changes in the complexes and considerable loss of protective functions with respect to PC oxidation.

## EXPERIMENTAL

Lipoid S100 (Lipoid GmbH, Germany) soybean PC in phosphate buffer (pH 7.2, ionic strength 1 mM, which is justified further on) was used to prepare liposome dispersion. Composition of the Lipoid S100 soy-

bean PC according to the manufacturer is as follows, g/100 g: PC, 94; *N*-acylphosphatidylethanolamine, 0.5; phosphatidylethanolamine, 0.1; phoshatidylinositol, 0.1; lysophosphatidylcholine, 3; nonpolar lipids, g/100 g: triglycerides, 2.0; free fatty acids, 0.5; α-tocopherol, 0.15. Fatty acid content in percent to total amount thereof: palmitic acid, 12–17; stearic, 2–5; oleic, 11–15; linoleic, 59–70; and linolenic, 3–7. The suspension was prepared from a weighted amount of PC in the buffer volume needed to prepare 1 mg/mL suspension. The mixture was stirred for 20 min on a shaker; if needed, various volumes of EOC (*Eugenia carophyllata* Thumb., Plant Lipids Ltd., India) (1.5, 2.0, and 4.0% by weight to PC weight) dissolved in 100 μL ethanol (buffer–ethanol ratio 50 : 1) were added. α-Linolenic acid (Sigma Chemicals) was added to the suspension in the same ethanol volume PC–ALA, 1 : 0.59, to reach the ratio of ω-3 and ω-6 PUFAs in liposomes of 1 : 1, taking into account fatty acid composition of the Lipoid S100 PC.

For ALA-supplemented PC liposomes, suspensions of total lipid concentration of 1 and 1.59 mg/mL were prepared. Then, they were homogenized mechanically in a homogenizer (Heidolph, Germany). Liposomes were formed using a VCX-130 (Sonics & Materials, United States) ultrasonic homogenizer in the 15 s : 15 s mode at 50% (to maximum) power for 15 min. To prevent liposomes from heating and oxidation in the course of sonication, suspension-containing vessel was placed in the mixture of water and ice. Then, the liposomes were passed through membrane filter with pore diameter of 100 nm using extruder (Avanti Polar Lipids, United States).

Cas-Na (Sigma, New Zealand) for encapsulation of liposomes therein, was dissolved in the same phosphate buffer (pH 7.2, ionic strength 1 mM) by stirring the mixture with a magnetic stirrer, then the solution was centrifuged (30 min, 4000 rpm) to remove admixtures. Low ionic strength buffer was chosen to facilitate electrostatic interactions between oppositely charged functional groups in lipids and proteins in the course of PC liposomes encapsulation in Cas-Na. In the work [30], calorimetry of mixing showed that electrostatic interactions between Cas-Na and PC liposomes contributed the most to the formation of particles at neutral pH values. Protein concentration in the solution was determined by differential refractometry using a known value of the protein refractive index increment. Protein solutions were stored at 7°C. Liposome encapsulation in Cas-Na was performed according to a technique described in [13, 14] using the following weight ratio of components: Cas-Na–PC, 10 : 1, and Cas-Na–PC–EOC, 10 : 1 : 0.04 at PC concentration of 1 mg/mL in liposomes. Cas-Na and EOC–PC liposome solutions in phosphate buffer were mixed in a GFL 3032 (Germany) shaker for 1 h at 40°C.

To initiate liposome oxidation, water-soluble azoinitiator 2,2'-azobis(amidinopropane) dihydrochloride (AAPH; Fluke, Germany), was used at final concentration of 2 mM in liposome suspension. Liposome autoxidation and initiated oxidation were performed in tubes with temperature control at physiological (37°C) or elevated (60°C) temperatures. The rate of LP progression was controlled by spectrophotometry registering UV absorption spectra (from 200 to 300 nm) over time on a Lambda-25 (PerkinElmer, Germany) spectrophotometer. LP products—diene conjugates (DC) and ketodienes (KD)—were determined at absorption maxima at λ 233 and 274 nm, respectively. Statistical processing of the experiment results was performed using the Microsoft® Office Excel 2003 software. Measurements were performed at least in triplicate. Errors in the determination of optical absorption (*A*) in oxidized samples over three measurements did not exceed 10%.

## CONCLUSIONS

In the work, it was shown that AAPH-initiated oxidation of soybean PC liposomes at physiological temperature (37°C) could be rather efficiently (by 80–90%) inhibited by phenolic compounds of EOC. Upon a temperature increase to 60°C, the oxidation rate increases considerably (3–4 times) and inhibition of liposome oxidation products becomes significantly less efficient. Addition of ω-3 ALA to PC liposomes leads to appreciable acceleration of oxidation compared to "pure" PC liposomes, and the effect is enhanced with an increase in temperature. Encapsulation of liposomes into Cas-Na decreases oxidation by 70%, while the combined effect of Cas-Na and EOC, by 90%. Temperature increase results in a much less efficient protective effect of casein encapsulation, even in the presence of EOC.

The results can be used to develop both modern systems based on PC liposomes for delivery of various nutraceuticals and technologies for their storage and processing.

## COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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*Translated by N. Onishchenko*