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Optimizing the Technological Process of Lipid Recovery from the Silt Sulphide Therapeutic Mud of the Tukhloye Lake

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Abstract

An effect of the major technological parameters of lipid recovery from therapeutic mud was studied. The optimal conditions of lipid recovery from the silt sulphide mud of the Tukhloye Lake (Novosibirsk Region) have been determined.

Key words: silt sulphide therapeutic mud, lipids, antioxidants, β-carotene, extraction, recovery

INTRODUCTION

The lipid fraction of therapeutic mud is manifold with respect to the chemical composition of organic compounds and includes fats and fat-like substances such as lipoids (phosphatides, glycolipids), isoprene derivatives (steroids and carotenoids), *etc*. those exhibit a significant biological effect. Carotenoids, unsaturated fatty acids, phospho- and sulpholipids, prostaglandines, whose composition and properties have been studied in detail by the authors of [1] belong to some of basic biologically active substances such as lipids from therapeutic mud.

The lipids of silt sulphide mud exhibit hepatoprotective and antioxidative properties, high reparation activity with no contraindication [1, 2], as well as they are employed as biologically active substances (BAS) to prepare medicines for external and internal use [3, 4].

The authors of [3] have demonstrated that the Tukhloye Lake (Novosibirsk Region) represents a prospective source of the mud for lipid extraction. In order to isolate lipids from the mud one uses various solvents and their mixtures. The authors of [5] have proposed to use the solution of chloroform and ethyl alcohol as an extracting agent. In this case, ethyl alcohol provides the extraction of more polar lipids, whereas less polar ones are extracted with chloroform. So, under the extraction of native mud from the Tukhloye Lake by equivolume ethyl alcohol-chloroform mixture polar and non-polar lipids are isolated on the basis of those a hepatoprotective preparation "Eplir" (Extract of Polar Lipids from Rocks) has been developed and patented. A high efficiency of this preparation [3, 6] is proved.

The method for extracting lipids by an ethyl alcohol–chloroform mixture proposed in [5] has been realized by the authors with the use of a specially made laboratory-scale installation, however any detailed investigation of the influence of technological parameters for obtaining lipids on the level of their extraction and the qualitative composition was not carried out.

For satisfying an increasing market demand concerning the preparations based on lipids from therapeutic mud with the minimal expenses for their extraction, one should study and optimize, first of all, the technological parameters of the mud preparation and extraction.

The goal of this work consisted in studying the influence of key technological parameters of obtaining lipids from the mud (raw material humidity, drying conditions of, the tempera-

ture of extraction, average particle size, extraction time, the ratio between raw material and the extracting agent, the extraction ratio) upon the extraction level and qualitative composition.

EXPERIMENTAL

Determining the qualitative and quantitative characteristics of lipids in the therapeutic mud

As a subject of inquiry we used modern sediments of the mineralized Tukhloye Lake with the content of water-soluble substances amounting to 50–60 g/kg as calculated for absolutely dry mud. The sediments were collected under reducing conditions of weak hydrosulphuric infection. The role of the basic suppliers of organic substance in the lake is played by phyto-, zooplankton and photosynthesizing bacteria [2].

In order to determine the influence of technological parameters of lipid extraction process we performed a comparative analysis the yield and qualitative composition of lipids resulting from mud extraction at concrete technological parameters with the maximal content of lipids therein.

The maximal content of lipids in the native mud with the humidity value $W = 30$ % was determined employing the technique described in [5]. The extraction of lipids was carried out at a room temperature using an ethyl alcohol-chloroform equivolume mixture ($V_{eth} : V_{chl} = \alpha = 1 : 1$), at the mass ratio raw material : extracting agent (γ) equal to 1 : 10, the extraction ratio $\lambda = 4$ and intensive stirring (500–1000 min^{-1}) in a laboratory mixer, during 60 min.

The extract obtained was reduced to a constant mass using a vacuum rotary evaporator at the temperature less than 50 °C. The lipid concentrate (LC) formed as the result contains insignificant amount of elemental sulphur and water-soluble compounds. In order to separate the latter the LC were diluted with chloroform and washed then with water using a separating funnel. After separating the solution of lipids was dried by anhydrous sodium sulphate, filtered and dried to gain the constant mass characterizing the yield of lipids. All the operations were carried out in a darkened room.

The qualitative composition of lipids was estimated from the content of β-carotene (C_β) therein and from the total content of antioxidants (C_{AO}). The content of β-carotene in lipids was determined from the optical density (absorbance) for the solution of lipids in chloroform over the range of wavelength values within 450–460 nm employing SF-201 spectrophotometer (Russia). As the reference solution we used chloroform. The content of antioxidants in lipids was determined from oxygen absorption rate in the process of cumene oxidation according to the technique described in [6].

The yield of lipids (f_l) resulting from the extraction can be defined by expression

$$f_l = [m_l 100/(m_w(100 - W))] \cdot 100\ \% \quad (1)$$

Here m_l is the mass of absolutely dry lipids; m_w is the mass of mud with the humidity level W.

The extraction level for lipids (F_l) and for β-carotene (F_β) were calculated as

$$F_l = (f_l/f_{l\,max}) \cdot 100\ \% \quad (2)$$

$$F_\beta = (C_\beta/C_{\beta\,max}) \cdot 100\ \% \quad (3)$$

where $f_{l\,max}$ is the maximal yield lipids; $C_{\beta\,max}$ is the maximal content of β-carotene in lipids isolated from native mud.

The procedure of mud drying was carried out using convective and vacuum shelf-type dryers. Preliminary grinded mud was put in a thin layer (0.5–2.0 cm) on the shelves of dryers. Heated air was used as the drying agent in the convective drying apparatus. In the vacuum drying case, mud was heated through the heat-transferring surface of chromium-plated shelves. The duration of drying amounted to 1–3 h.

The granulometric composition was specified by sifting the dried grinded mud through a sieve with the size of square cells d_1 and d_2. Mud particle size was varied within the range of d_1–d_2 values amounting to 0.5–1.5, 4–6, 8–10 and 12–14 mm. The average particle size of mud under extraction we determined as

$$d_{av} = (d_1 + d_2)/2 \quad (4)$$

It is experimentally proved that at a mass ratio raw material : extracting agent $\gamma = 1 : 10$, and a single-stage extraction procedure under the conditions of approaching the dynamic equilibrium in the system, the extraction level of the substances (lipids) under extraction from the mud amounts to about 90 %. For growing this value one should increase to a considerable extent the ratio of γ. So, at $F_l = 95$ % the γ value amounts to about 1 : 20, which would complicate to a considerable extent the process of obtaining lipids. In this connection, all the

experiments concerning the studies on the influence of technological parameters upon the yield and quality of lipids were performed at $\gamma = 1 : 10$ and $\lambda = 1$. Studying the yield of lipids depending on time as well as the γ and λ parameters we performed a re-maceration process (stepwise insisting), *i. e.* the raw material was treated by an extracting agent in parts, successively, rather than by all the volume of the extracting agent.

The validity of experimental results was estimated using the Student's *t*-criterion.

Criteria of optimizing the technological parameters

The optimization of a great number of the interconnected parameters represents a complicated problem, those solving requires, first of all, establishing the ranges of varying the values of parameters. In turn, they are determined by technological potentialities, by the quality of end product and the expenses for obtaining the product.

So, the extraction of mud with the humidity level $W > 20$ % results in "swelling" clay particles, which restricts the throughput efficiency of the mud. Moreover, at the mentioned humidity level the "watering" of the extracting agent occurs, which results in the loss of the extracting agent, an increase in the temperature and time of evaporation, and, hence, in worsening the quality and in increasing the cost price of the lipid concentrate [5, 8].

The maximal extraction temperature (T_e) is restricted by the boiling point of an extracting agent.

The bottom boundary level of the mud particle size (d_1) is determined by the extract ability of passing through the layer of the mud and the time of its filtering, whereas the top boundary level (d_2) is determined by the extraction time and the completeness of extraction dependent on the time of extraction. So, obtaining a "pure" (not containing particles of mud) extract of lipids from dried mud with $d_1 < 0.5$ mm results in a considerable increase in the time of filtering the extract which implies additional expenses.

The minimal ratio $\gamma = 1 : 2$ was chosen basing on the condition that carrying out the process of extraction the extracting agent should cover the surface of mud completely.

With the increase in both/either parameter γ and/or parameter λ the yield of lipids f_l exhibits an increase, and, hence, the energy consumption for the evaporation of solvent tends to grow.

The choice of the volume ratio between solvents (α) in the process of mud extraction is of important significance since the cost of chloroform is 5–6 times higher than the cost of ethyl alcohol. In this connection, we have also investigated the influence of parameter α upon the yield and quality of lipids.

The temperature of drying and extraction, the size of dried mud particles and the mud humidity level, the volume ratio between solvents affect not only the process of extraction, but also the quality of lipids.

In studying and optimizing each technological parameter determining the yield and quality of lipids, one should take into account the cost price of the end-product (C_e) which is defined as it follows:

$$C_e = (P_m + \Phi)/m \quad (5)$$

Here P_m is the price of mud under extraction; Φ the expenses for obtaining dry LC with mass m.

Studying and optimizing the parameters of the technological process for obtaining LC were performed according to the conditions of obtaining LC with regulated quality taking into account the cost price C_e.

RESULTS AND DISCUSSION

The maximal yield of lipids in the native mud amounted to 0.46 %, the content of β-carotene in the extracts and the total content of antioxidants were equal to 3.48 and 0.38 mol/kg, respectively.

The influence of mud humidity up on the yield and qualitative composition of lipids were studied using mud samples with the humidity value amounting to 5, 10, 15, 20 and 30 %. The extraction of lipids was carried out at T_e = 25 °C, $\alpha = 1 : 1$, $\gamma = 1 : 10$. The time of extraction (t_e) was equal to 60 min. The average particle size dried mud was equal to 1.0 mm. The mud was dried via a convection method at the temperature of 40 °C.

The results of the studies concerning the yield of lipids, the content of β-carotene and the total content of antioxidants depending on

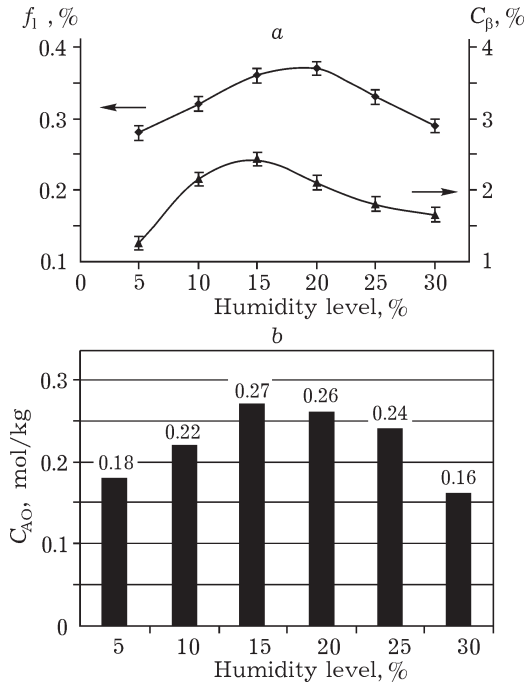

Fig. 1. Yield of lipids (f_l), β-carotene content C_β (*a*) and the total content of antioxidants C_{AO} (*b*) depending on the mud humidity level. T_d = 40 °C, γ = 1 : 10, α = 1 : 1, T_e = 25 °C, t_e = 60 min, d_{av} = 1.0 mm, λ = 1.

the mud humidity level are presented in Fig. 1. It can be seen that the greatest yield of lipids from the mud with the maximal content of antioxidants has been obtained when the mud humidity level W = 15–20 %. In this case, the maximal content of β-carotene is inherent in the lipids isolated from the mud with the humidity level amounting to 15 %. When the W value is diminished down to 5 % the yield of lipids becomes more than by 20 % lower, in this case the content of β-carotene decreases twice, whereas the content of antioxidants decreases by 30–32 %. To all appearance, it is connected with the fact that lipid substances tend to be oxidized by atmospheric oxygen in the course of mud drying in the air environment.

Drying the raw material to gain the humidity level W = 5 % results in the formation of crushproof mud structure, which complicates the solvent diffusion into the raw material as well as the diffusion of substances under extraction into the liquid phase of the solvent and thus, as a consequence, results in the decrease of the total yield of lipids. At the mud humidity level equal to 20 % the total yield of lipids and the content of active substances decrease to a considerable extent. So, the content of в-carotene and the total content of antioxidants in lipids, isolated from raw material with W = 30 % exhibit a more than 30 % decrease. It could be caused by the fact that water passes into the phase of solvent making its polarity to increase and reducing the solvent ability to extract the substances with non-polar nature.

The presence of residual mud humidity plays a considerable role in preservation the quality of lipids preventing the substances of lipid nature from the oxidation by atmospheric oxygen. However, the presence of moisture in the mud complicates the process of obtaining lipids. In the case of extracting the mud with W > 20 %, stagnant zones due to the agglutination of particles are formed, which results in the under extraction of lipids. It should be noted that the removal of moisture from the mud results in substantial and irreversible changes in a more labile and, as a rule, a more biologically active part of lipids (see Fig. 1).

One should note also that the extraction of the mud with W > 20 % results in an increase in the specific loss of the solvent, in time and in the temperatures of solvent removal.

Thus, in order to obtain the greatest yield of lipids with a high content of β-carotene and antioxidants one should use mud with the humidity level of 15–20 %. However, the estimation of C_e value has demonstrated that the optimum value of the mud humidity is equal to 15 %.

When preparing the mud for extraction it is important to determine the influence of atmospheric oxygen and of raw material drying temperature range upon the yield of lipids and the latter properties.

The influence of drying conditions upon the yield and qualitative composition of lipids was studied with the use of mud samples with d_{av} = 1.0 mm dried *via* convective and a vacuum method at T_d = 40 °C to obtain the humidity level equal to 15 %. The extraction of lipids was carried out at T_e = 25 °C, α = 1 : 1, γ = 1 : 10, t_e = 60 min.

From data presented in Fig. 2 it can be seen that the yield of lipids insignificantly depends on the temperature of mud drying (T_d) within the range of 30–60 °C. The temperature exerts a stronger effect on the qualitative composition of lipids, especially in the process of convective mud drying. So, the increase in T_d from 40 to

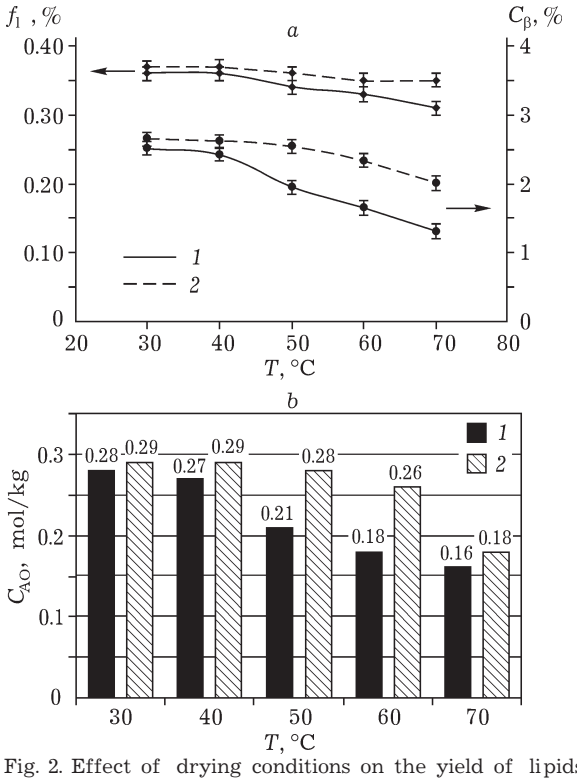

Fig. 2. Effect of drying conditions on the yield of lipids (f_l), the content of β-carotene C_β (*a*) and the total content of antioxidants C_{AO} (*b*) (T_d = 40 °C, W = 15 %, γ = 1 : 10, α = 1 : 1, T_e = 25 °C, t_e = 60 min, d_{av} = 1.0 mm, λ = 1): *1* – convective method of drying, *2* – vacuum method of drying.

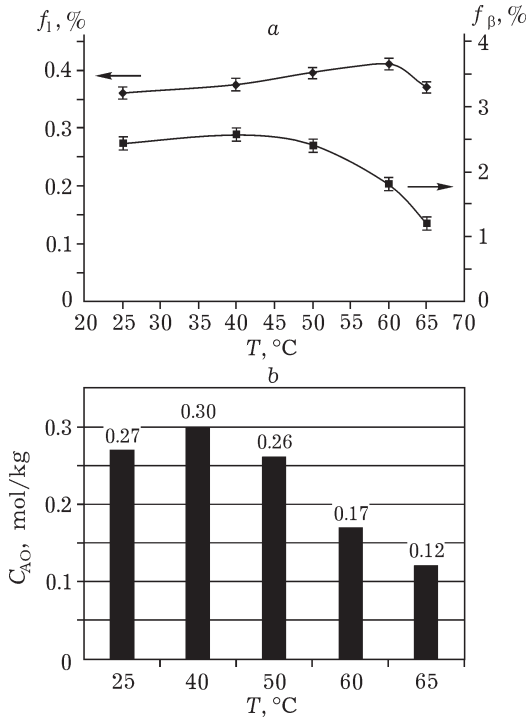

Fig. 3. Yields of lipids f_l and of β-carotene f_β (*a*), the total content of antioxidants C_{AO} (*b*) depending on the temperature of extraction. T_d = 40 °C, W = 15 %, γ = 1 : 10, α = 1 : 1, t_e = 60 min, d_{av} = 1.0 mm, λ = 1.

70 °C results in a 44–46 %, decrease of β-carotene content in lipids as well as in 36–39 %. Decrease in the total content of antioxidants.

We also used the samples of mud dried up by convective and vacuum methods up to obtaining the humidity level W = 5 % in order to compare the conditions of drying.

Drying the mud under vacuum and atmospheric pressure up to obtaining the humidity level equal to 15 % does no result in any considerable differences observed in the yield of lipids and the content of active substances. On the contrary, drying the mud under vacuum up to obtaining the humidity level equal to 5 % resulted in the yield lipids appeared 21 % higher, the content of β-carotene – two times higher and the total content of antioxidants on 40 % higher as compared to the convective drying procedure (Table 1). It should be noted that storage of dried mud under normal atmospheric conditions during 4–5 days results in a significant decrease of all the extractive substances irrespective the method of drying (see Table 1).

TABLE 1

Influence of a mud drying method upon the yield and qualitative composition of lipids

Parameters	Duration of mud sample storage			
	2–4 h		4–5 days	
	Mud humidity level, %			
	15	5	15	5
f_l, %	0.36/0.37	0.28/0.34	0.27/0.29	0.21/0.28
f_β, %	2.43/2.61	1.05/2.09	1.75/1.89	0.84/1.46
C_{AO}, mol/kg	0.27/0.29	0.18/0.25	0.13/0.18	0.12/0.15

Note. The first value is referred to the convective drying method; the second value is referred to the vacuum drying method.

Thus, drying the mud at atmospheric pressure up to obtaining the humidity level $W = 15$ % could be considered an optimum.

The influence of the extraction temperature upon the yield and qualitative composition of lipids was studied with the use of mud dried by the convective method up to $W = 15$ % at $T_d = 40$ °C. The extraction of lipids was carried out at α =1 : 1, $\gamma = 1 : 10$, $t_e = 60$ min, d = 1.0 mm and temperatures equal to 25, 40, 50, 60 and 65 °C (the solvent boiling point at atmospheric pressure).

Increasing the extraction temperature from 25 up to 60 °C results in 13–15 % increase in the yield of lipids (Fig. 3). To all appearance, this could be connected with the fact that with rising the temperature the viscosity of the extracting agent is reduced, thus the process of diffusion is accelerated, which, correspondingly, results in the acceleration of the extraction process. At the same time, the increase in T_e results in the destruction of thermo-labile substances of the lipid nature as well as in the reduction of C_β and C_{AO} values. So, with increasing T_e from 40 to 65 °C the content of β-carotene decreases more than by 50 %, whereas the total content of antioxidants exhibits a 2.5-fold decrease. Thus, the extraction of mud should be carried out at the temperature not higher than 40 °C.

The curves demonstrating the yield of lipids depending on the extraction time significantly differ from each other for different mud particle size (Fig. 4). Lipids were isolated from the samples of mud dried by the convective method at

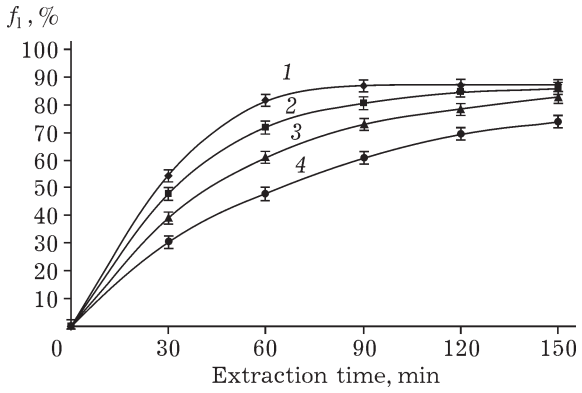

Fig. 4. Lipid extraction kinetics at different mud particle size, mm: 1 (*1*), 5 (*2*), 9 (*3*), 13 (*4*). $T_d = 40$ °C, $W = 15$ %, $\gamma = 1 : 10$, $\alpha = 1 : 1$, $T_e = 40$ °C, $t_e = 60$ min, $\lambda = 1$.

$T_d = 40$ °C up to W =15 % with the particle size d_{av} = 1, 5, 9 and 13 mm *via* one-stage extraction at $T_e = 40$ °C, α =1 : 1, $\gamma = 1 : 10$.

The results have demonstrated that under extracting the lipids from the mud with d_{av} = 1 mm during 60 min the extraction level amounts to 82–84 %. The increase in the extraction time up to 90 min resulted in a less than 3–4 % increase in the extraction level. The further increase in the extraction time almost did not influence upon the yield of lipids.

The yield of lipids depending on the mud extraction time, the raw material to extracting agent ratio as well as the extraction ratio values are presented in Table 2 and in Fig. 5. The extraction of lipids was performed using mud with $d_{av} = 1$ mm.

It can be seen that when the raw material is extracted by the identical amount of solvent the yield of substances extracted with multiple extraction is higher than the yield after a

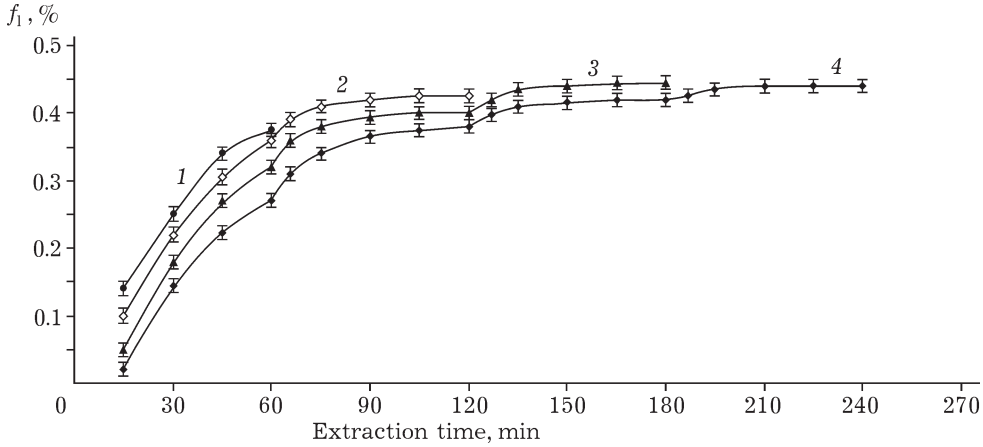

Fig. 5. Lipid extraction kinetics depending on γ and λ values (T_d= 40 °C, $W = 15$ %, $\alpha = 1 : 1$, T_e = 40 °C): *1* – $\gamma = 1 : 10$, $\lambda = 1$; *2* – $\gamma = 1 : 5$, $\lambda = 2$; *3* – $\gamma = 1 : 3$, $\lambda = 3$; *4* – $\gamma = 1 : 2$, $\lambda = 4$.

TABLE 2

Yield of lipids depending on the extraction ratio (λ), time of each extraction stage (t_e), total extraction time (t_{tot}) and the mass ratio raw material/extracting agent (γ)

λ	t_e, min	t_{tot}, min	f_l (F_l), %, at the ratio γ equal to:			
			1 : 10	1 : 5	1 : 3	1 : 2
1	30	30	0.25 (54)	0.22 (48)	0.18 (39)	0.15 (33)
	45	45	0.34 (74)	0.31 (67)	0.27 (59)	0.22 (48)
	60	60	0.38 (83)	0.36 (78)	0.32 (70)	0.27 (59)
2	15	75	–	0.41 (89)	0.38 (83)	0.34 (74)
	30	90	–	0.42 (91)*	0.39 (85)*	0.36 (78)
	45	105	–	0.425 (92)	0.395 (86)	0.38 (83)*
	60	120	–	0.425 (92)	0.40 (87)	0.38 (83)
3	15	135	–	–	0.435 (95)*	0.41 (89)
	30	150	–	–	0.44 (96)	0.415 (90)*
	45	165	–	–	0.44 (96)	0.42 (91)
	60	180	–	–	0.44 (96)	0.42 (91)
4	15	195	–	–	–	0.435 (95)*
	30	210	–	–	–	0.44 (96)
	45	225	–	–	–	0.44 (96)
	60	240	–	–	–	0.44 (96)
5	15	255	–	–	–	–
	30	270	–	–	–	–
	45	285	–	–	–	–
	60	300	–	–	–	0.44 (96)

*Optimum extraction time for each extraction ratio value.

one-stage extraction procedure. So, the extraction of mud at γ = 1 : 5 and λ = 2 allows one to extract up to 92 % of lipids, which is by 9 % higher than it is under one-stage extraction by all the volume (see Table 2).

However, it should be noted that at γ = 1 : 5, the increase in time for the second extraction equal to more than 30 min could result in slightly increasing the lipid extraction level, *i.e.* the further increase in the extraction time is pointless. The process of each extraction procedure should be finished till the moment of saturating the extraction agent by substances soluble therein. Thus, at γ = 1 : 5 the optimum time of mud extraction corresponds to the total duration time equal to 90 min.

The optimum time for mud extraction has been determined in a similar manner at γ = 1 : 3 and λ = 3 and at γ = 1 : 2 and λ = 5 which time amounted to 105 and 150 min, respectively. The extraction level for lipids in both cases is 93–95 % (see Table 2).

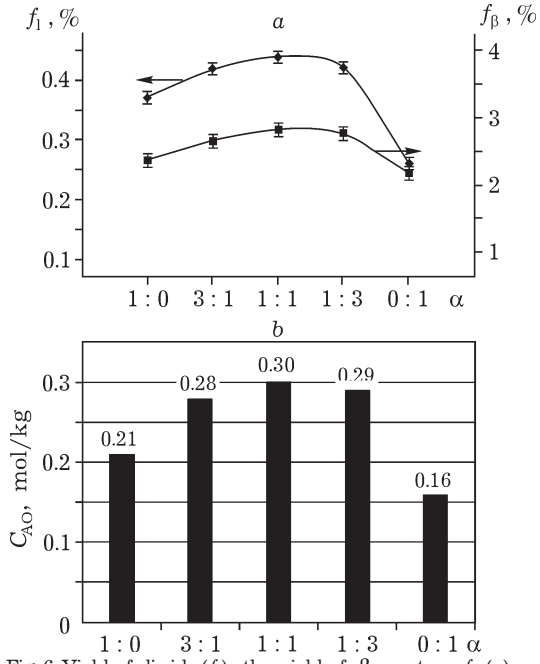

Fig. 6. Yield of lipids (f_l), the yield of β-carotene f_β (*a*) and the total content of antioxidants C_{AO} (*b*) depending on the volume ratio between the components of the extracting agent α. T_d = 40 °C, W = 15 %, γ = 1 : 3, t_e = 105 min, d_{av} = 1.0 mm, λ = 3.

TABLE 3

Extraction level for lipids (F_l), β-carotene (F_β) and the total content of antioxidants (C_{AO}) depending on the volume ratio of solvent components α . T_d = 40 °C, W = 15 %, γ = 1 : 3, λ = 3, T_e = 40 °C, t_{tot} = 105 min

Parameters	α				
	1 : 0	3 : 1	1 : 1	1 : 3	0 : 1
F_l, %	82	91	94	92	55
F_β, %	68	76	81	79	62
C_{AO}, mol/kg	0.21	0.28	0.30	0.29	0.16

The estimation of the expenses for extracting lipids has demonstrated that the mud extraction is appropriate for carrying out under the following conditions: the mass ratio raw material: extracting agent γ = 1 : 3, the extraction ratio λ = 3, the total extraction time being equal to 105 min, (the duration time of the first extraction stage being of 60 min, the duration time of the second extraction stage 30, that of the third stage being of 15 min). In this case, the extraction level for lipids amounted to 93–95 %.

It is obvious, that at various volume ratio values for extracting agents (α) the changes concern not only qualitative and quantitative composition of LC, but also the LC cost price owing to considerable difference in the cost of chloroform and ethyl alcohol.

Figure 6 presents the yield of lipids and its qualitative composition depending on volume ratio between the extraction agent components. It can be seen (see Fig. 6 and Table 3), that the maximal extraction level for active substances is reached with the use of the mixture of the solvents with the volume ratio between the components α = 1 : 1. Under extracting lipids by the mixture with α = 3 : 1 the extraction level for lipids is by 3 % lower, and for β-carotene this value is by 5 %, lower than in the case of employing the equal-volume mixture. In this case the expense for lipid extraction is 1.5 times less, therefore the optimum ratio for the extracting agent components is α = 3 : 1.

CONCLUSION

Optimum conditions have been determined for obtaining lipids from the therapeutic mud of the Tukhloye Lake (Novosibirsk Region): the average mud particle size being of 1 mm, convective drying method, the temperature of mud drying amounting to 40 °C, the humidity level the mud under extraction being of 15 %, the temperature extraction being of 40 °C, the volume ratio between ethyl alcohol and chloroform α = 3 : 1; the mass ratio raw material/ extracting agent = 1 : 3; the extraction ratio λ = 3; the total extraction time amounting to 105 min. In this case the extraction level for lipids amounts up to 91 %, for β-carotene this value is of 76 %, whereas the content of antioxidants is equal to 0.28 mol/kg.

Employing the mixture of solvents with α = 1 : 1 as an extracting agent allows extracting by 3–5 % more active substances; in this case the expense for their obtaining are 1.5 times increased.

The results could be used for designing and making an industrial-scale plant for obtaining the lipid concentrate.

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