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Lipid Transformations under Mechanochemical Destruction of Therapeutic Muds

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Abstract

Effect of the mechanochemical activation of therapeutic mud in the planetary mill AGO-2 on the degree of dispersing, the yield of free and bound lipids and the content of β-carotene and antioxidants in them was studied. It was shown that the amount of extractable free lipids increases substantially after mechanochemical activation of the organomineral raw material in the planetary mill in the presence of orthophosphoric acid due to the release of a part of bound lipids. An increase in the content of β-carotene and antioxidants in lipids was established.

Key words: therapeutic muds, mechanochemical activation, lipids, carotenoids, antioxidants

INTRODUCTION

The organic matter of therapeutic mud formed as a result of transformations of phyto- and zooplankton contains lipids. In modern sediments, lipids are present in the free form and in the bound state. The latter is represented by the lipids sorbed by the mineral matrix (carbonate, aluminosilicate lipids) and those chemically bound with protokerogen (hydrolyzed) [1–4]. The lipids may comprise alcohols, fatty acids, esters, nitrogen bases, saturated and unsaturated hydrocarbons, chlorophyll, carotenoids, phosphoric acid *etc*. Separate classes and groups of lipids (phospholipids, glycerolipids, carotenoids, hydrocarbons) possess high biological and antioxidant activity [5–9], promoting the removal of toxic radical products of peroxidation and thus preventing the development of pathologies in living organisms. Bound lipids that do not undergo biochemical transformations at the early stages of sediment formation serve as additional sources of valuable biologically active compounds. Various alkaline and acidic reagents are used to extract them. These reagents have a substantial effect on the chemical composition and antioxidant properties of lipids. In this connection, the search for new efficient methods of the extraction of bound lipids is urgent.

The development of high-energy mechanical activators allowed one perform grinding of the organomineral raw material to micro- and nanosized particles thus destroying the mineral matrix and releasing the intracellular structures. Mechanochemical activation promotes an increase in the effective contact surface between the components of dispersed systems, a decrease in diffusion hindrance due to the deterioration of the morphology of the raw material, and it also creates the conditions for active chemical transformation of the target substances into the forms readily soluble in water or another solvent, which simplifies extraction [10, 11]. The high efficiency of physical action allows one to govern transformations within a broad range and to obtain the products with required properties [12, 13]. Investigation of the changes in the composition and properties of lipids is of independent interest from the view-

point of using lipids as the source of biologically active substances and developing the physiologically active preparations based on lipids [14, 15].

The goal of the present work was to study the chemical transformations of lipids under mechanochemical activation of therapeutic mud.

EXPERIMENTAL

The therapeutic mud from Lake Tukhloye (Novosibirsk Region) was chosen as the object of investigation. It contains carbonates 20 % and clay 80 %. The organomineral raw material was dried preliminarily to the humidity of 5–8 % and crushed to the particle size of 1–3 mm. Mechanical activation (MA) was carried out in the mill of the planetary type AGO-2 (developed at the ISSCM, SB RAS, Novosibirsk) with the acceleration of milling bodies g equal to 200 and 400 m/s^2. The mass of the substance under investigation loaded into the mill was 50 g, ball mass was 250 g, the diameter of balls was 8 mm. The treatment of mud in the planetary mill was carried out in the presence of quartz sand to prevent particle adhesion during the release of water. Orthophosphoric acid (1–5 mass %) was used as the hydrolyzing agent during the MA of therapeutic mud.

Electron microscopic analysis was carried out using the transmission electron microscope LIBRA 120 (Zeiss, Germany) with the accelerating voltage of 80–120 000 V. The size of particles was measured with the help of soft non-destroying ablation under the action of the terahertz radiation of the free electron laser using the diffusion spectrometer of aerosol [16].

Lipids were isolated from therapeutic mud by means of extraction with a mixture of alcohol and chloroform at the ratio of 1 : 1 under mixing and heating on the water bath to the temperature of 30 °C [17]. After that, the mud sample was treated with the aqueous solution of hydrochloric acid (1 : 1). Lipids bound with the mineral matrix were isolated from the dried precipitate according to the procedure described in [18].

The concentration of β-carotene in lipids from the organomineral raw material was determined on the basis of the optical density with the spectrophotometer at the wavelength of λ = 450 nm in the cells with the layer thickness of 10 mm using the calibration with standard β-carotene [19].

The amount of carboxylic groups contained in the free and bound lipids was determined by means of potentiometric titration [17].

The concentration of antioxidants (AO) in the free and bound lipids was determined using the kinetic method on the basis of the model reaction of the initiated oxidation of cumene [20].

RESULTS AND DISCUSSION

Using soft non-destroying ablation we studied the effect of the time of MA of organomineral raw material on particle size. It was established that after the treatment for 1–5 min the size of particles of therapeutic mud change from 150 to 25 nm. The presence of nanosized particles was confirmed by electron microscopy.

The data on the effect of MA duration on the amount of free and bound lipids extractable from the organomineral raw material are shown in Fig. 1. One can see that 0.22 mass % of free lipids is extracted with the help of organic solvents from the therapeutic mud ground to the particle size of 1 mm; after the treatment with hydrochloric acid 0.26 % of bound lipids are extracted. With an increase in MA time from 1 to 5 min the amount of free lipids increases by a factor of 4, which is connected with a decrease in the size of particles of the raw material to 25–37 nm. The yield of lipids bound with the mineral matrix remains almost independent of the time of MA of therapeutic mud.

For comparative evaluation of the effect of mechanochemical acid treatment on the degree

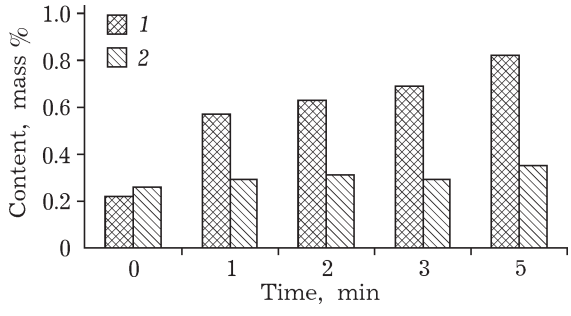

Fig. 1. Effect of the time of mechanical activation of the organomineral raw material in the presence of quartz sand on the content of free (*1*) and bound (*2*) lipids.

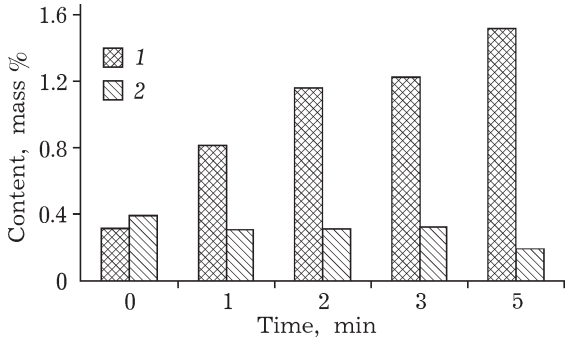

Fig. 2. Effect of the time of mechanical activation of the organomineral raw material in the presence of quartz sand and orthophosphoric acid on the content of free (*1*) and bound (*2*) lipids.

of lipid extraction from the organomineral raw material, we carried out their preliminary treatment with orthophosphoric acid. It was established that the yield of free lipids increases as a result of the preliminary treatment to 0.32 mass %, the yield of bound lipids increases to 0.39 mass % (Fig. 2). It should be noted that the amount of extractable free lipids increases substantially after the MA of therapeutic mud in the presence of orthophosphoric acid. With an increase in treatment time, their yield increases by a factor of several units. In mechanically activated samples with the high free lipid content, the amount of bound lipids transformed intot he free form due to the rupture of chemical bonds, including ester bonds, with the mineral components decreases. We determined the content of carboxylic groups in lipids (Table 1). One can see that the amount of the compounds having the acidic character increases within the free lipids. The obtained results agree with the data of model experiments [21] that confirm the tendency of carbonate to adsorb mainly fatty acids. As a consequence, the compounds of acidic character prevail in bound lipids.

TABLE 1

Effect of the time of mechanical activation (MA) of the organomineral raw material on the content of COOH groups in free and bound lipids

Treatment conditions	Content, mg-eq/g in lipids	
	free	bound
Without MA	6.2	11.3
Without MA + H_3PO_4	7.9	13.5
MA, 5 min	10.8	14.9
MA + H_3PO_4, 5 min	12.5	17.8

The lipids of lake sediments contain carotenoids, xanthophylls, tetrapyrrhol pigments (up to 4 mass %) that determine the biological activity of the lipids of therapeutic mud [7]. Due to the unsaturated nature of molecules, these compounds are unstable and undergo profound biochemical transformations when buried in sediments. The lipid components bound with the protokerogen matrix are less prone to the external action (chemical, physical or microbiological). We studied the effect of MA on the amount of extractable carotenoids. The data on the content of β-carotene in free lipids after MA of the organomineral raw material are presented in Fig. 3. One can see that the MA of the raw material for 2–3 min in the presence of orthophosphoric acid causes a maximal (by a factor or 3–5) increase in the amount of β-carotene in free lipids. Further treatment of the raw material (up to 5 min) causes a decrease in the content of β-carotene, which may be connected with its destruction.

It is known that the lipids of many living organisms comprise inhibitors of radical chain oxidation processes; these inhibitors are inherited by the sedimentary material and transformed at different stages of the formation of organomineral sediments [20]. The AO terminating the oxidation chains due to the interaction with peroxy radicals include the compounds with functional groups containing a mobile hydrogen atom (–OH, –NH, –SH).

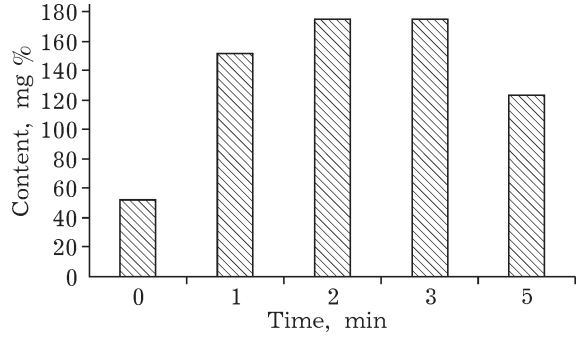

Fig. 3. Effect of the time of mechanical activation of the organomineral raw material in the presence of quartz sand and orthophosphoric acid on the content of β-carotene in lipids.

TABLE 2

Effect of mechanical activation (MA) of therapeutic mud on the content of antioxidants in free and bound lipids

Treatment conditions	Content, mol/kg, in lipids	
	free	bound
Without MA	0.18	0.05
Without MA + H_3PO_4	0.21	0.06
MA, 1 min	0.34	0.10
MA, 3 min	0.31	0.12
MA, 5 min	0.27	0.10
MA + H_3PO_4, 3 min	0.35	0.14

In free lipids isolated from therapeutic mud, the concentration of AO is 0.18 mol/kg (Table 2). The acid treatment of the raw material without MA has almost no effect on the content of AO, while dispersing for 1–3 min causes nearly 2-fold increase in their amount. The maximal content of AO in free and bound lipids is detected after MA of therapeutic mud in the presence of orthophosphoric acid.

Evidently, MA results in the rupture of hydrogen bonds between separate components in lipid molecules and the liberation of –OH and –NH bonds. An increase in the duration of dispersing treatment of the raw material to 5 min leads to a decrease in the amount of AO, perhaps due to the interaction of AO with active radicals.

CONCLUSION

It was established that the size of the particles of ground organomineral raw material reaches several tens nanometres, depending on the conditions of mechanochemical activation. The yield of free lipids from the extraction of mechanically activated organomineral raw material in the presence of quartz sand and orthophosphoric acid increases by a factor of 3–6 and depends on treatment time. The comparative analysis of the results of acid and mechanical treatment of therapeutic mud allows us to assume that an increase in the concentration of bound lipids occurs due to the rupture of ester bonds and the release of them from the protokerogen matrix in the form of the compounds of acidic character. The concentration of β-carotene and antioxidants in lipids increases correspondingly.

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