Fatty Acids Profile Analysis in Fresh Suspension of Japonochytrium Sp.

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FATTY ACIDS PROFILE ANALYSIS IN FRESH SUSPENSION OF *JAPONOCHYTRIUM SP.*

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Abstract

Japonochytrium sp. represents the unicellular marine fungi, which doesn't contain chlorophylls in its biomass. It accumulates high proportion of lipids, containing a significant amount of omega-3 polyunsaturated fatty acids, especially DHA (docosahexaenoic acid, C22:6n3). These valued components should be isolated and applied as components in food supplements, cosmetics or pharmaceutical products.
The study is focused on concentration of a lipid content and determination of the extractable part oriented on included fatty acids in separated phases of centrifuged wet biomass. The main attention was focused on the DHA concentration from the upper lipid layer, containing obviously concentrated proportion of fatty acids. Water fungi suspension was submitted to centrifugation with subsequent separation of individual liquid/solid fractions and extracted by two solvent systems. Dichloromethane/methanol 1/1 and 1-hexane/ethanol 2/3 was applied. Both solvent systems afforded comparable fatty acids profiles. Dominant acids C16:0 (in the range of 43-47%) and DHA C22:6n3 (43-47%) were detected.
Biomass residue can be applied e. g. as an animal food supplement due to high protein content. Also level of pigments with antioxidant effect, particularly carotenoids, can be dietary significant.

Introduction

Japonochytrium sp. belongs to the unicellular marine fungi, which is characterized by lipid content with a relatively high proportion of omega-3 unsaturated fatty acid triglycerides[1]. They have a high nutritional potential with the perspective application in the food industry as a valued dietary supplement. This microorganism is not investigated in detail, yet. The existing studies were focused on morphology and ultrastructure definition[2] of a *Japonochytrium sp.*, optimal cultivation conditions[3], suitable nutrition[4,5] and/or proper strain selection or modification for DHA yield maximisation. The aim of study was focused on optimisation of operating conditions for extractive separation of lipids from fresh biomass suspension of *Japonochytrium sp.* (produced by the Ecofuel Labs. Ltd. company). The extraction experiments were conducted with regard to the selection of extraction system for maximization of fatty acid production.

Materials and methods

Sample preparation and processing

A sample of the *Japonochytrium sp.* water suspension (dry matter 16 $g.l^{-1}$) was centrifuged to separate three phases: a - an upper lipid layer, b - bottom centrifuged biomass and c- middle aqueous solution. Individual phases were processed as demonstrated in the diagram in figure 1. Biomass was not dried in order to avoid undesirable losses of unstable substances.
Two extraction systems were chosen for the extraction experiments. The proportions of components in the extraction solvents were as follows: dichloromethane/methanol 1/1 (v/v), 1-hexane/ethanol 2/3 (v/v). The presence of alcohol was necessary for the processing of wet biomass to limit the possible formation of emulsions or foams.
All extraction experiments were carried out in a sealed stirred Erlenmeyer flask protected from light under an inert nitrogen atmosphere at ambient temperature of 23 °C for 4 h. This procedure was suitable for the separation of thermally unstable materials susceptible to oxidation by atmospheric oxygen. Extraction of both, the upper lipid layer and the lower sediment biomass, was performed with the ratio of a raw material:solvent 1:15, whereas in the case of the aqueous phase, the ratio 1:5 was selected. Original suspension of fungi *Japonochytrium sp.* was separated into individual fractions to identify the phase with concentrated lipid proportion.

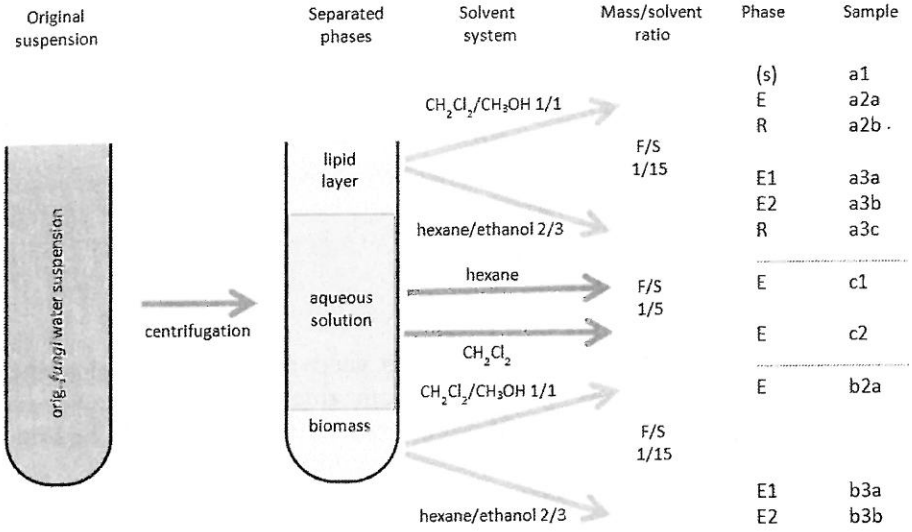

Figure 1. Scheme of three phases of *Japonochytrium sp.* centrifuged suspension processing

Analysis of fatty acids

The profile of fatty acids contained in lipids was analysed at the Department of Food Analysis and Nutrition of the University of Chemistry and Technology Prague. The obtained extracts were transformed applying saponification to fatty acids methyl esters; and, based on accredited ISO 17025 method, they were analysed using gas chromatography. The presence of fatty acids in the range of C4 to C24 string lengths was examined.

Results and discussion

From the mass balance and microscopic observation of the individual phases was noticeable, that centrifugation of 1,000 grams (about 1 litre) of fresh culture *Japonochytrium sp.* arose three phases in the following proportions: 19.4 g of the upper lipid phase, 941.9 g of aqueous phase and 38.7 g of moist bottom biomass.

Extraction of upper lipid layer

In this study, the main attention was focused on the extraction of the upper lipid layer, containing obviously the largest proportion of fatty acids. It consists of wet biomass of cells and lipid bodies. Figure 2 shows the weight proportional representation of fatty acids in the lipid layer and in homogeneous extract and/or raffinate after extraction with a mixture of dichloromethane/methanol.

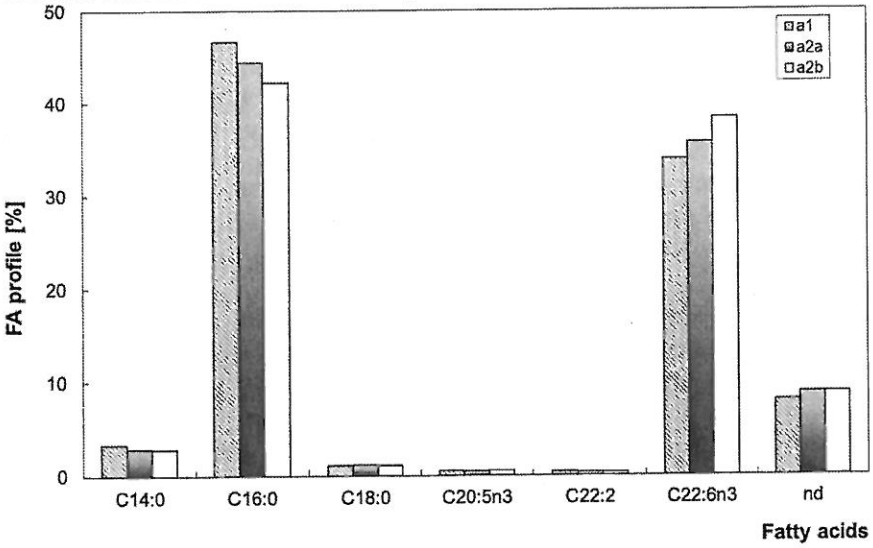

Figure 2. Proportion of selected fatty acids in lipid layer. Dichloromethane/methanol extraction system was used. a1 – original lipid layer, a2a – organic extract, a2b aqueous raffinate

It is evident that the lipid layer and extraction products contain in addition to the dominant saturated palmitic acid (C16:0) also omega-3 unsaturated docosahexaenoic acid (C22: 6n3), referred to as DHA. The second valued omega-3 eicosapentaenoic acid (C20:5n3), referred to as EPA, is contained in lipid layer in an insignificant amount (0.5%). In the lipid layer there were further identified 3% of myristic acid (C14: 0), 7-8% of unknown long chain acid and other unidentified components (<1%).
Extraction of the lipid layer with hexane/ethanol system provided a similar result on the fatty acids profile (see Figure 3) as in the case of dichloromethane/methanol. However, the obtained extract was heterogeneous, containing an organic and an aqueous phase, the composition of which are depicted in the Figure 3 as the extract 1 and 2. This situation is caused by the content of 4% water in the used ethanol azeotrope. The composition of the raffinate using both extraction solvent mixtures is practically the same.

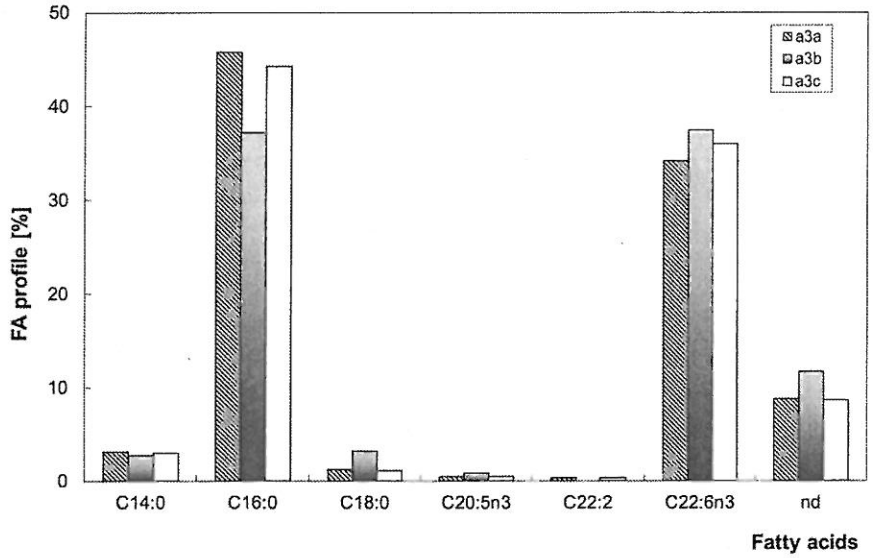

Figure 3. Proportion of selected fatty acids in lipid layer. Hexane/ethanol solvent system was used. a3a – extract 1, a3b – extract 2, a3c - raffinate

Comparison of weight proportion of fatty acids, expressed in grams per kilogram of lipid layer and dried raffinates after extraction in both solvent mixtures - dichloromethane/methanol and hexane/ethanol are shown in Table I.

Table I
Quantification of selected fatty acids in lipid layer and/or its raffinates

Fatty acids [$g.kg^{-1}$] /sample	a1	a2b	a3c
C12:0	0.18	0.00	0.29
C14:0	6.37	2.77	10.37
C15:0	0.25	0.13	0.43
C16:0	90.01	42.17	155.19
C18:0	2.13	1.12	3.90
C18:1n11c	0.26	0.00	0.34
C20:0	0.18	0.14	0.36
C20:2	0.21	0.11	0.40
C20:3n3	0.27	0.12	0.41
C20:3n6	0.15	0.12	0.42
C20:4n6	0.23	0.11	0.31
C20:5n3	1.00	0.55	1.65
C22:2	0.77	0.35	1.08
C22:5n3	0.21	0.00	0.39
C22:6n3	66.21	38.79	127.91
C24:0	0.18	0.00	0.25
C24:1n9	0.19	0.00	0.33

It is evident from the comparison of the remaining fraction composition after suction of the organic extracts that the solvent system dichloromethane/methanol is more effective. The higher proportion of monitored acids from the solid residue was removed.

Extraction of bottom centrifuged biomass

Biomass sediment is primarily composed of particles with a cellular structure of microorganisms (cell fragments and undamaged fungi cells) after lipid releasing by centrifugation of *Japonochytrium sp.* suspension. Comparison of the fatty acid content, represented in the extract using dichloromethane/methanol and hexane/ethanol mixtures, is shown in Figure 4. In the case of the second solvent system, the extract was again heterogeneous and it was divided into organic and aqueous phase, which is shown in the picture as the extract 1 and extract 2. The composition of the originated extracts differs, due to different polarity of solvent and perhaps by the presence of unidentified impurities.

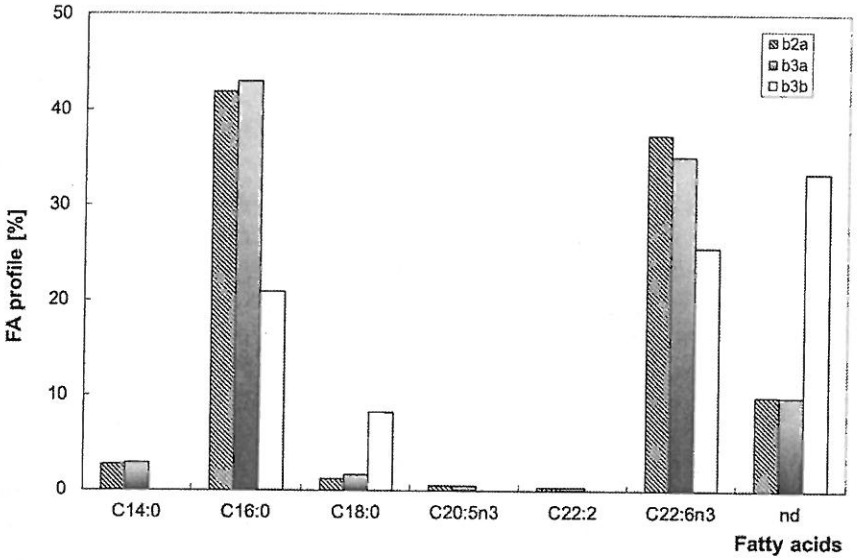

Figure 4. Fatty acids profile in the bottom biomass layer - the sediment. b2a - dichloromethane/methanol, b3a - hexane/ethanol – extract 1, b3b - hexane/ethanol – extract 2

Extraction of the aqueous phase

The middle aqueous phase appeared practically clear, free of cells and lipids, at a given mode of centrifugation. The analyses showed that middle aqueous phase is virtually free of fatty acids. They were concentrated mainly in the upper lipid layer and the bottom sediment phase.

Summary

The performed laboratory tests indicate that *Japonochytrium sp.* can be certainly used as a promising source of omega-3 DHA docosahexaenoic acid. Moreover, optimal conditions were found for extractive separation of fatty acids from FA-rich biomass. Based on the mass balance obtained by laboratory extraction from wet fungi slurry it can be expected the yield of DHA in the extract of about 13-79% of the original content in the suspension depending on the solvent system and processed separated phase. Processing of the wet biomass seems as promising solution. The challenge for future research is to optimise conditions for extraction and development of the separation apparatus for treatment of the *Japonochytrium sp.* – based biomass.
Fungi biomass residue can be after partial or complete removal of lipids applied as an animal food supplement due to high protein content. Also level of pigments with antioxidant effect, particularly carotenoids, can be dietary significant.

Acknowledgement

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