Lipid Extraction from Algae Trachydiscus Minutus.

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LIPID EXTRACTION FROM ALGAE TRACHYDISCUS MINUTUS

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Introduction

It is characteristic for some species of algae that they contain lipids. Moreover, their structure contains unsaturated fatty acids. That is of particular importance mainly due to the presence of nutritionally valuable omega-3 fatty acids, which strongly supports the health condition of a person when consumed. An exceptional position belongs to eicosapentaenoic acid (EPA), so called 20:5n3 in physiological literature. EPA is an omega-3 fatty acid and it is sometimes called the timnodonic acid[1]. Its molecule contains 20 carbons with 5-cis-alkene bonds. The first double bond is located at the third carbon from the omega end. It is therefore polyunsaturated fatty acid (PUFA) and is a precursor for prostaglandin E3 and/or tromboxane synthesis with significant health support effects[2]. Another important place in human metabolism is occupied by alpha-linolenic acid (ALA), which is also an essential fatty acid. A human body converts this acid to eicosapentaneoic acid (EPA). This process significantly reduces diabetes and some types of patient's allergies[2].

Objective

The success of the biotechnological production of omega-3 fatty acids depends on appropriate types of microalgae, finding optimal conditions for their cultivation and last but not least developing a suitable technological procedure for isolating unsaturated fatty acids from them. The last mentioned aspect was the subject of current Research Centre BIORAF focusing on chemical-engineering issues of the feasibility and design of appropriate equipment for the separation of these valuable ingredients from selected microalgae. This work follows from previous study[3] aimed to extraction of fatty acids from *Chlorella vulgaris*.

Method

The initial step of microalgae processing was its separation from an aqueous solution of nutrients by flocculation. The following step was the batch extraction of lyophilized microalgae biomass using n-hexane/ethanol mixture (ratio 2/3) at the temperature of 23°C. The stirred batch extractor operated at an absence of light under an inert atmosphere for the period of 18 h. Individual phases (liquid organic extract and solid biomass) were separated from the obtained suspension by intensive centrifugation. The extraction was repeated twice, using a fresh extraction solvent mixture so that the process corresponded to the three-stage batch dispersed system extraction[4]. The main goal of the research was to define the number of extraction stages necessary for a highly effective extraction of lipids from algae *Trachydiscus minutus*.

Experimental results

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 through C24 string lengths was examined. The results provide a detailed picture of the representation of individual fatty acids and the content of these substances in processed biomass. Figure I summarizes concentrations of main selected fatty acids (in g/l). Data confirm the expected stepwise decrease of all fatty acids concentration in the extracts from subsequent extraction batches. Based on linear regression of these results (see Figure 2), a very good linearity between the fatty acids concentration and the batch number of the extraction process was confirmed in all cases. The intersection of the regression line with the horizontal coordinate enabled to define the number of extraction batches necessary for the isolation of all lipids from algae. The figures occur between 4 and 5; therefore, 5 extraction batches are recommendable for the quantitative isolation of lipids from the *Trachydiscus* algae.

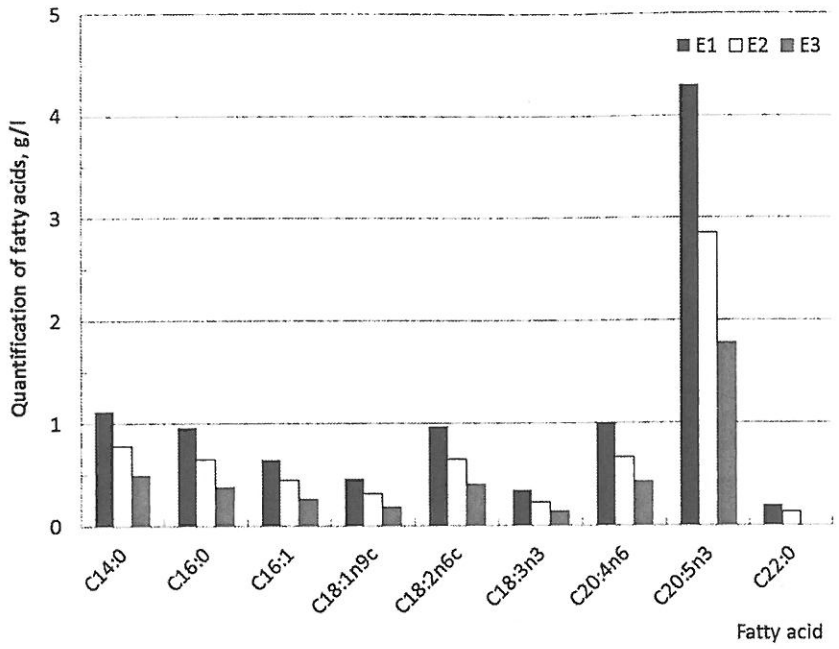

Figure 1. The concentration of fatty acids in extracts (g/l); batches E1, E2 and E3.

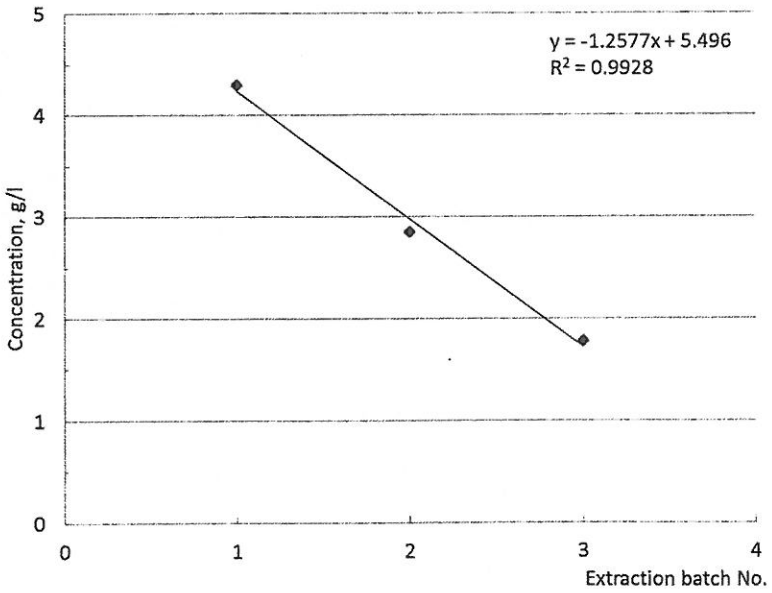

Figure 2. Linear regression of C20:5n3 acid concentration versus batches E1, E2, E3.

Table I expresses the profile of selected fatty acids largely represented in prepared extracts. Proportions of selected fatty acids in individual batches are almost uniform. It is evident high proportion of polyunsaturated EPA in comparison with other fatty acids due to gentle extraction treatment.

Table I
Profile of selected fatty acids in individual extracts - accuracy of GC-FID analysis (rel. %)

	Fatty acid	Extract 1	Extract 2	Extract 3
C14:0	myristic	9.41	9.79	10.24
C16:0	palmitic	8.08	8.08	7.82
C16:1	palmitoleic	5.41	5.55	5.53
C18:1n9c	oleic	3.80	3.92	3.83
C18:2n6c	linoleic	8.06	8.08	8.30
C18:3n3	α-linolenic	2.87	2.87	2.96
C20:4n6	arachidonic	8.32	8.25	8.85
C20:5n3	eicosapentaenoic	35.73	35.40	36.71
C22:00	docosanoic	1.61	1.59	0.88

Conclusion

It was found that the concentrations of all components in the extracts linearly fall with a number of extraction stages, as documented by the above figures. The overview of the results concerning selected analyses carried out is summarized in Table I. They indicate that algae *Trachydiscus* is rich in omega-3 eicosapentaenoic acid (EPA-C20:5n3), unsaturated fatty arachidonic acid (AA-C20:4n6), unsaturated fatty linoleic acid (LA-C18:2n6) and the following saturated acids - myristic acid (MA-C14:0) and palmitic acid (PA-C16:0). There is a group of acids also detected during the extraction, which is important to be mentioned here even though the figures representing them are considerably lower. These acids are: palmitooleic acid (C16:1), oleic acid (OA-C18:1n9), alpha-linolenic acid (ALA-C18:3n3) and docosanoic/behenic acid (C22:00). The extent of other fatty acids in the extracts was negligible, or they were not present at all.

Acknowledgement

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References

1. See: https://en.wikipedia.org/wiki/Eicosapentaenoic_acid; (23.1.2016).
2. See: http://www.vegetarian-dha-epa.co.uk/; (23.1.2016).
3. Rouskova M., Kohoutkova J., Vesely V., Kastanek F., Solcova O.: Extraction of fatty acids from microalgae. Proceedings of the 1st Internat. Conf. Chem. Technol., Mikulov, Czech Republic, 8-10 April 2013, pp. 123-128.
4. Hanika J.: Special separation processes (in Czech). Inst. Chem. Technol. Prague, 1995.