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Pressurized fluid extraction of polyunsaturated fatty acids from the microalga *Nannochloropsis oculata*

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ABSTRACT

Microalgae are the primary producers of economically valuable polyunsaturated fatty acids (PUFAs) and can be used as a resource for biorefining. In this respect, these high-value products may support the economical viable energy recovery from microalgae. The extraction of PUFA-containing lipids from *Nannochloropsis oculata*, a marine species rich in eicosapentaenoic acid (EPA), was tested with a commercially available pressurized fluid extraction technique (PFE, traded as *Accelerated Solvent Extraction* (ASE®)) in our study. Solvents, which are suitable for an application in the food and pharmaceuticals industry, were used (*n*-hexane, *n*-hexane/propan-2-ol (2:1 vol.%), ethanol 96 vol.%) to test the quantitative effect of solvent polarity on the gravimetric extraction yield, *total* fatty acid and EPA yield. The highest extraction yield resulted from ethanol extraction (36 ± 4 mass %), compared to low yields from *n*-hexane extraction (6.1 ± 0.3 mass%). A maximum fatty acid yield of 16.7 ± 0.6 mass% was determined for the biomass extracted with the green solvent ethanol. The EPA yield of 3.7 ± 0.1 mass% with the use of ethanol indicates that EPA production from *N. oculata* is economically beneficial, referring to prognosis from literature. The remaining biomass may eventually be used for energy recovery and other applications within a biorefinery concept.

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1. Introduction

Microalgae are unicellular, eukaryotic organisms, and are very efficient in photosynthesis. In fact, they are more efficient than many land plants and are able to double their biomass within 24 h [1]. This provokes a research boom concerning their use as a renewable energy resource for biodiesel production from the lipid fraction, bioethanol production from carbohydrate-rich algae, and biomethane production via anaerobic digestion. However, although microalgae are promising due to their high biomass production rates, their use as an energy resource is challenged by the high biomass production costs [2,3].

In order to benefit from the high productivity of microalgae in terms of energy recovery, microalgae need to be addressed in a biorefinery approach, using high value products for economic viability of the process [4]. Fortunately, microalgae produce a variety of substances, which are of economic interest for an application in the food and fine chemicals industry [5,6]. The application of microalgae in the feed

Abbreviations: ASE®, *Accelerated Solvent Extraction*; CF, correction factor; DHA, docosahexaenoic acid; ECF, empirical correction factor; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl ester; FID, flame ionization detection; GC, gas chromatography; IS, internal standard; mass%, mass percentage; ME, methyl ester; MS, mass spectrometry; MUFA, monounsaturated fatty acid; PFE, pressurized fluid extraction; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TCF, theoretical correction factor; vol.%, volume percentage.

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industry (aquaculture) for the breeding of rotifers and fish is state-of-the-art [7–9].

A huge variety of microalgae strains exist, of which not all are yet well characterized concerning their composition and content of valuable chemicals. In addition, the composition can change with respect to environmental conditions such as temperature, light availability, CO_2 concentration or nitrogen limitation [10]. These conditions can be optimized in the commercial production process, which underlines the huge potential of microalgae as valuable source of different chemicals.

Economically interesting microalgal products are, among others, polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3, Fig. 1) [5].

PUFAs, particularly omega-3 (n-3) and omega-6 (n-6) PUFAs are essential for most animals. Omega-3 fatty acids support prevention of cardiovascular diseases, cancer, rheumatoid arthritis, inflammatory conditions (asthma, cystic fibrosis) or mental illness [11]. EPA and DHA are therefore used as pharmaceuticals and in food supplements due to their biochemical function. Currently, they are mainly produced from fish oil. Fish accumulate PUFAs by feeding microalgae, which are the primary producers of PUFAs in the trophic chain.

Recovery of EPA and DHA from fish oil has several disadvantages from a consumer perspective: the "fishy" odor is unpleasant, there are concerns about the contamination with heavy metals, and fish oil does not meet the needs of a vegetarian or vegan diet. This favors production of EPA and DHA from microalgae.

Microalgal PUFAs are primarily bound in the polar glycoglycerolipids of eukaryotic chloroplasts. They have potentially crucial functions in the photosynthetic system, and/or in the ability to adapt rapidly to external temperature changes, maintaining the membrane fluidity at lower temperatures [12–14].

For an industrial application of EPA and DHA from microalgae and other valuable chemicals in a biorefinery concept, the fatty acids (FA) need to be extracted from the biological matrix. Due to the predominance of PUFAs in the polar lipid fraction it is most likely that polar solvents are more favorable for extraction. The use of the conventional lipid extraction solvent mixture $CHCl_3$/MeOH is limited for an application in pharmaceuticals and food supplements, and for large scale use. Additionally, automated systems, which are easy and safe to handle, are required for a commercial application.

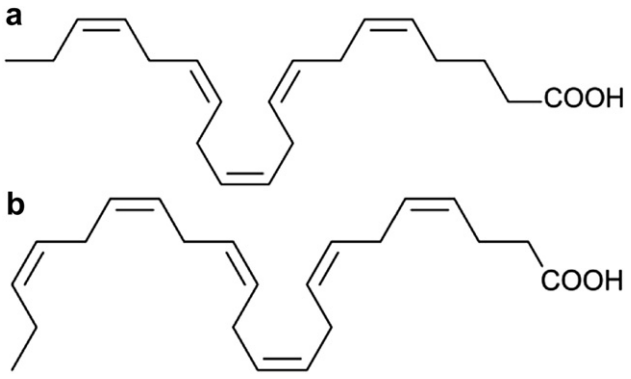

Fig. 1 – Chemical structures (skeletal formula) of polyunsaturated fatty acids. (a) all-(Z)-eicosa-5,8,11,14,17-pentaenoic acid (C20:5 n-3), (b) all-(Z)-docosa-4,7,10,13,16,19-hexaenoic acid (C22:6 n-3).

Extraction with supercritical CO_2 ($scCO_2$) is suggested by some researchers in analogy to production from fish oil. However, the achieved yields demonstrate clearly that $scCO_2$ is not suitable to extract microalgal lipids [15]. Supercritical CO_2 can only extract the neutral lipid fraction, which is a minor part of the total microalgal lipids. This is in contrast to fish, which contain the PUFAs linked to neutral lipids and can consequently be sufficiently extracted with $scCO_2$ techniques.

Alternative extraction techniques that could be combined with polar extraction solvents (e.g. microwave-assisted extraction, ultrasound-assisted extraction, extraction with pulsed electric field, bead-beating-assisted extraction, Soxhlet extraction, pressurized fluid extraction, and others) have been reported in the literature with their advantages and disadvantages [16–18].

Pressurized fluid extraction (PFE, traded as "*Accelerated Solvent Extraction*", ASE®)) is seen as one very promising alternative to $scCO_2$ extraction for microalgae. PFE has been reviewed as analytical solvent extraction technique previously [18,19]. The technique allows to perform efficient extractions, mainly due to the use of elevated temperatures, increasing compounds solubility. In contrast to extraction at room temperature (e.g. Soxhlet), the pressure keeps the solvent in its liquid state, even if temperatures above the boiling point are applied. Additionally, the pressure favors the penetration of the solvent into the biological matrix. This is specifically beneficial for microalgal cells with thick cell walls. Pressurized fluid extraction has been previously applied for the extraction of antioxidants from microalgae [20–22].

Experimental studies on lipid extraction from microalgae showed improved extraction yields when using ethanol as co-solvent in $scCO_2$ extraction or when applying pressurized "near critical dimethyl ether" (DME) [23,24]. These experiments demonstrate that pressurized or near critical fluid extraction systems have a potential for microalgal lipid extraction. However, safety issues for the use of DME need to be overcome, and other suitable solvents need to be tested.

Therefore, we tested an automated PFE technique (ASE®) in order to extract PUFA-containing lipids using alternative solvents, which are applicable in the food and pharmaceuticals industry (*n*-hexane, *n*-hexane/propan-2-ol (2:1 vol.%) and ethanol (96 vol.%)). The quantitative effect of solvent polarity on the *total* PUFA yield (i.e. PUFAs from FA-containing lipids) when applying PFE/ASE® on the marine microalga *N. oculata* was investigated. *N. oculata* was chosen because it is known as EPA-rich species with thick cell walls [4]. These quantitative data have not yet been described for *N. oculata*, but are required when evaluating the potential of this species for biorefinery concepts.

2. Material and methods

2.1. Biological samples

Microalgae samples of the marine species *N. oculata (Droop)*, which was identified and defined by D.J. Hibberd in 1981 [25],

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