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Ultrasound-assisted extraction of lipids from *Mortierella isabellina*

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ABSTRACT

This work is focused on the optimization of process conditions of the ultrasound-assited extraction that promote high yields of lipids from freeze-dried cells of *Mortierella isabellina* produced by submerged fermentation. The influence of ultrasound intensity (26.89 W cm^{-2} - 85.00 W cm^{-2}) and pulse cycle (0.57–1.0) using chloroform:methanol:water (2:1:0.8, v/v/v) mixture and ethanol was evaluated on the yields fatty acids and it was compared with extractions without ultrasound and conventional Soxhlet method. Ultrasound intensity of 75.11 W cm^{-2} and pulse factor of 0.93 were the optimized conditions in the central composite design, yielding 14.46 wt% and 19.49 wt% of fatty acids using ethanol and chloroform:methanol:water as solvents, respectively. The fatty acids profile was similar for all extractions. The results show the lipids yields were higher when ultrasound was applied in biomass from fungus *Mortierella isabellina.*

1. Introduction

Several microorganisms have been used for the production of lipids, mainly the fungus *Mortierella isabellina* for the production of polyunsaturated fatty acids (PUFA) (Demir et al., 2013). PUFA belong to the omega-3 or omega-6 family, depending on the position of the first double bond, counting from the methyl group located at the end of the fatty acid molecule (Giudetti and Cagnazzo, 2012). These fatty acids are identified as potential food additives or pharmaceuticals because of their biological activities (Jang et al., 2005). Many researches have demonstrated the importance of fatty acids in the health, as a positive influence on the brain system functions (Bradbury, 2011), cardiovascular system (Jump et al., 2012) and some types of cancer (Jump et al., 2012). PUFA (n-3 and n-6) are considered essential fatty acids. Therefore, they are necessary in human health while they are not synthesized in the body. Consequently, PUFA could be obtained from dietary sources (Dyal and Narine, 2005).

The oleaginous fungus *Mortierella isabellina* has been extensively studied for single cell oil production because it is able of accumulating lipids (Chatzifragkou et al., 2010). However, lipids produced from *Mortierella isabellina* are intracellular. In such case, it is necessary an extraction to remove the lipids from fungi cells. Several solvents have been intensively studied for lipids extraction, such as, hexane and diethylether (Fakas et al., 2009), and methanol and chloroform (Harde et al., 2016). Furthermore, the Bligh & Dyer method with a modified methanol:chloroform:water ratio of 2:1:0.8 (v/v/v), hexane and isopropanol has been tested (Halim et al., 2012). Other solvents have been also evaluated, as dichloromethane, methanol, hexane and petroleum ether (Hussain et al., 2014; Mackėla et al., 2017; Xing et al., 2012).

Regarding extractions, supercritical CO_2 and compressed liquefied petroleum gas (Sallet et al., 2017), and ultrasound-assisted extraction (Zhang et al., 2014; Zhou et al., 2013) are some methods with crescent interest in the scientific community. Some other treatments are more economical, but most of them are neither effective nor easy to scale up (Geciova et al., 2002). A combination of chemical and mechanical methods was reported to enhance the efficiency of lipid extraction. Bligh and Dyer method which uses chloroform–methanol ratio of (1:2) is one practical procedure for lipid extraction (Breil et al., 2017).

More recently, ultrasound-assisted extraction is one of the disruption methods that has being receiving attention in the literature for lipid extraction (Moraes et al., 2013). Ultrasound is able to disrupt cells with less energy loss compared with high-shear force methods (Chemat et al., 2011; Wang et al., 2014), and also intensifies the extraction process due to a cavitation phenomenon. Ultrasonic waves produce bubbles in the solvent. The bubbles burst near the cell walls, which make shock waves and cause the release of lipid in the solvent (Wei

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et al., 2008). Furthemore, applications of ultrasound generally involve processes that can increase rates, improve quality and/or safety, and reduce processing time (Li et al., 2018; Silva et al., 2015).

Considering the ultrasound extraction of lipids from *Mortierella isabellina*, only the work of Zhou et al. (2013) could be found in the literature up to now that evaluated ultrasound-assisted extraction using hydrochloric acid in extraction of single cell oil. As far as we now, no other study using ultrasound-assisted extraction from cells of the *Mortierella isabellina* has been reported. Therefore, the novelty of this study stands for applying ultrasound extraction using different solvents for improving the lipids yield and decreasing the extraction time.

In this context, the aim of this study was to evaluate ultrasound extraction of lipids from *Mortierella isabellina* produced by submerged fermentation. The influence of solvent (ethanol and mixture of chloroform:methanol:water) and ultrasound (power intensity and pulse cycle) on yields and composition was evaluated. In order to provide the more suitable condition, the extraction yields and fatty acids composition were taken into account as results.

2. Material and methods

2.1. Materials

Mortierella isabellina was purchased from Tropical Culture Collection André Tosello (Campinas, Brazil). The culture was maintained in potato dextrose agar (PDA) at 4 °C. Ethanol (99.8%) was purchased from Alphatec (Brazil), chloroform (P.A) and methanol (P.A) were purchased from Dinâmica Contemporary Dynamics Chemistry Ltda (Brazil), and hexane was purchased from Sigma-Aldrich (Brazil).

2.2. Fermentation

Fatty acids were produced by submerged fermentation in an orbital shaker. Erlenmeyer flasks (500 mL) were used for loading the medium and the inoculum. The temperature, reaction time, composition of the medium and all the procedure followed the methodology described in the previous work (Sallet et al., 2017).

2.3. Ultrasound-assisted extraction

The experimental apparatus for ultrasound-assisted extraction (Fig. 1) was composed of a jacketed reactor (250 mL of capacity) connected to a thermostatic water bath (temperature accuracy of ± 1.0 °C) for temperature control, a high-intensity ultrasound processor of 400 W and frequency of 24 kHz (Hielscher, Model UP 400S, Germany). The ultrasound was equipped with a titanium probe (Model H22, Tip 22), presenting a maximum ultrasound intensity of 85 W cm^{-2}. The solvents (ethanol and mixture chloroform:methanol:water) were defined after preliminary tests. Initially, hexane was used; however, the yields were small. The mixture of chloroform:methanol:water was used because chloroform and methanol mixture is currently employed for lipid extraction from microorganisms and is found to be efficient (Zhang et al., 2014; Keris-Sen et al., 2014). The same proportion of solvents from Bligh Dyer method (Bligh and Dyer, 1959) was used in the assays.

For the extractions, the ultrasonic probe was placed at the center of the jacketed reactor containing 2.5 g of cells and 100 mL of ethanol. In the extractions using chloroform:methanol:water, the ultrasonic probe was placed at the center of the jacketed reactor containing 2.5 g of cells and 26 mL of chloroform, 53 mL of methanol and 21 mL of water (2:1:0.8, v/v/v). Afterward, temperature was adjusted to 10 °C ± 2 °C by circulating water through the jacket. All extractions were carried out for 30 min (defined according to preliminary experimental assays, data not shown) at constant ultrasound power and pulse cycle (related to the time that ultrasound is on). After this, the samples were centrifuged at 4500 rpm for 5 min. The liquid phase was carefully collected and the solvents were evaporated at 40 °C under vacuum. For a control sample,

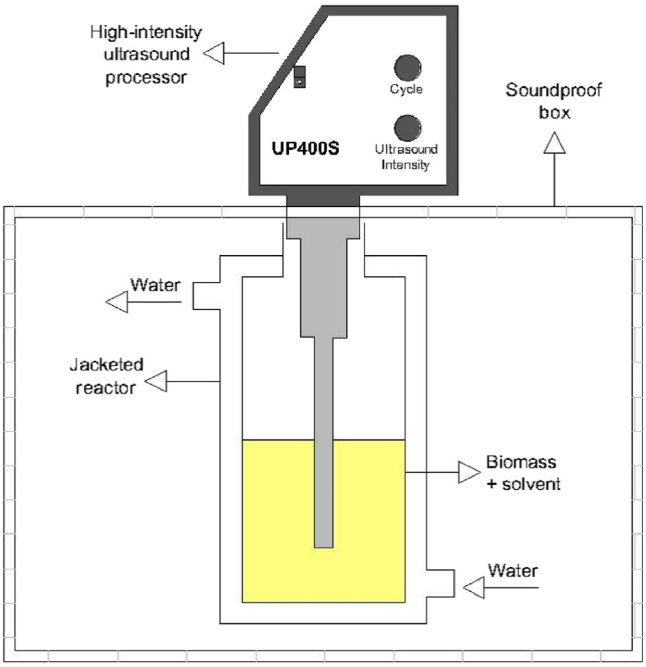

Fig. 1. Diagram of experimental apparatus for ultrasound-assisted extraction.

extractions without ultrasound were done for each solvent. The same conditions (biomass, amount of solvent and time) were used.

The effects of ultrasound intensity (17–85 W cm^{-2}) and pulse cycle (0.5–1.0) on extraction yields of cells were evaluated through a Central Composite Rotational Design (CCRD – Table 1). After analysing the results of CCRD, three additional assays were carried out to validate the results. The responses evaluated were the yields of lipids (wt.%) (lipids mass/biomass) and fatty acids profile.

2.4. Conventional extraction

Soxhlet extraction was performed according to the Association of Official Analytical Chemists (AOAC, 1997). Freeze-dried cells (1 g) were wrapped in a piece of filter paper and then placed in a Soxhlet apparatus connected to a solvent flask with 150 mL of *n*-hexane. The system was refluxed for 150 min. The solvent was removed from the extracted mixture at room conditions (25 °C and 0.1 MPa). The mass of oil was determined by an analytical balance (Marte, AY-220, São Paulo, Brazil). The assays were performed in triplicate and the responses were

Table 1
Yields of lipids obtained from *Mortierella isabellina* by ultrasound-assisted extraction through a CCRD experiments.

Run	Ultrasound intensity (W.cm^{-2})	Pulse cycle (−)	Yield[a] (wt. %)	Yield[b] (wt.%)
1	26.89 (−1)	0.57 (−1)	2.92	15.38
2	75.11 (1)	0.57 (−1)	13.04	19.08
3	26.89 (−1)	0.93 (1)	6.90	16.40
4	75.11 (1)	0.93 (1)	14.47	19.49
5	17 (−1.41)	0.75 (0)	3.70	14.08
6	85 (1.41)	0.75 (0)	14.43	18.90
7	51 (0)	0.50 (−1.41)	8.44	17.36
8	51 (0)	1.0 (1.41)	11.40	18.65
9	51 (0)	0.75 (0)	9.95	17.99
10	51 (0)	0.75 (0)	8.91	18.16
11	51 (0)	0.75 (0)	10.31	18.13
12[c]	0	0	1.45	13.92

[a] Extraction using ethanol.
[b] Extraction using chloroform:methanol:water (2:1:0.8, v/v/v).
[c] Extraction without ultrasound (sample control).