Ultrasonics Sonochemistry 37 (2017) 216–221

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Short communication

Manothermosonication as a useful tool for lipid extraction from oleaginous microorganisms

A. Meullemiestre, C. Breil, M. Abert-Vian, F. Chemat *

Université d'Avignon et des Pays de Vaucluse, INRA, UMR408, Sécurité et Qualité des Produits d'Origine Végétale, 84000 Avignon, France

ARTICLE INFO

Article history:
Received 16 November 2016
Received in revised form 1 January 2017
Accepted 11 January 2017
Available online 12 January 2017

Keywords:
Manothermosonication (MTS)
Ultrasound
Oleaginous microorganism
Lipid
Extraction

ABSTRACT

Manothermonication is a recognized and efficient method used for sterilization in food preservation. The synergistic effect of sonication combined with pressure and temperature allows enhancing the cavitation activity. Never employed for extraction, this study is about the transposition of this process as a tool of extraction. In this study, *Rhodosporidium toruloides* yeast was submitted to extraction by four modes of sonication, with a temperature ranged from 20 to 55 °C and a pressure between 1 to 2 bars. The lipids extraction yields were compared to the conventional maceration. Microbial oils obtained from both processes were analyzed and quantified by HPTLC (High Performance Thin-Layer Chromatography) and GC-FID (Gas Chromatography with flame ionization detector) after transesterification of lipids. Manothermosonication (30 min, 2 bars, 55 °C) permits to enhance of approximately 20% the extraction yield of lipids to compared to conventional maceration. The fatty acid profiles of each pretreatment and extraction by US, MS, TS and MTS do not affect the fatty acid profiles of yeast (majority of oleic acid (C18:1n9), linoleic acid (C18:2n6) and palmitic acid (C16:0)). Manothermosonication technique shows a great potential for lipid extraction from oleaginous microorganisms.

1. Introduction

Manothermosonication (MTS-process) (Spanish Patent Number 9200686) is a combined process including heat and ultrasonication under pressure [1]. MTS-process was developed for food preservation to improve inactivation efficacy. The idea to use ultrasound, heat and pressure combination came from the resistance of certain bacteria to ultrasound technology in food preservation as sterilization method, because of its limited lethal effect on bacterial spores [2]. Consequently, some studies of the combination of ultrasound with other treatments such as pH [3], chemical treatment [4], heat [5], or ionizing radiation [6] have been investigated and have shown the increasing lethal effect of ultrasound on vegetative cells, but the inactivation of bacterial spores was still low to compare to traditional preservation technique. When low pressure is introduced into a thermosonication system to achieve MTS, the level of inactivation of enzymes and/or microorganisms is increased [7]. From these studies, two hypotheses stand up to enhance cavitation activity: (i) increase the number of cavitation bubbles, by lowering cavitation threshold or using multiple frequency techniques, and/or (ii) increase the power of bubble implosion, by an addition of external static pressure during sonication or increasing low acoustic area.

Rhodosporidium toruloides, previously called *Rhodotorula glutinis* is known as one the archetypal oleaginous microorganisms able to accumulate a large lipid content (up to 20% of dry cell weights) and is consequently a promising source of feedstock for biodiesel production [8,9]. The most common used methods for determining total lipid content in micro-organisms are the Soxhlet extraction with hexane for dry samples [10] and those using a mixture of chloroform and methanol described by Folch et al. [11] and Bligh and Dyer [12]. These methods remain the reference in academic and industrial research laboratories, but are limited. The Bligh and Dyer extraction method is used for analytical extraction in laboratory scale. The major problem in the lipids extraction from yeasts is the lack of miscibility of must fermentation with hexane, thus a drying step is required before extraction. This drying step involves high energy consumption, loss of time, and is an important cost for industrial, and to respect the six principles of eco-extraction the reduction of number of unit operations is primordial [13]. Furthermore, most of yeast strain own ultra-rigid wall cells, thus intracellular extraction of lipids are difficult and organic solvent extraction become not enough efficient to free lipid bodies [14,15].

* Corresponding author.
E-mail address: farid.chemat@univ-avignon.fr (F. Chemat).

http://dx.doi.org/10.1016/j.ultsonch.2017.01.014
1350-4177/

Our idea has been to transpose the MTS-process, usually used in food preservation, as a tool to improve lipid extraction from oleaginous microorganisms. In this study, power of ultrasound has been used in four modes: ultrasonication (US), thermosonication (TS), manosonication (MS), and manothermosonication (MTS) and compared in term of extraction yield and lipid profiles to conventional maceration.

2. Material and methods

2.1. Strain, culture, and harvesting conditions

Oleaginous yeast *Rhodosporidium toruloides* CECT 1137, previously called *Rhodotorula glutinis* was grown during 16 h in a shake flask containing 100 mL of YEPD Broth using a shaking incubator at 200 rpm, 30 °C. The fermentation was then carried out in a 3600 mL fermenter (Labfors 4, Infors-HT) stirred with two Rushton Impellers. The oleaginous microorganism was grown in batch mode in 1 L of an industrial substrate containing hydrolyzed starch diluted to an initial concentration in glucose of 60 mg/mL at 30 °C and adjusted to pH 5.6. After consumption of the glucose, the fed-batch mode was started with a solution of dextrose syrup (Sirodex, Tereos Starch and Sweeteners) and a solution of NH_4OH as nitrogen source. The fermentation was carried over during 72 h before harvesting the broth. The industrial substrate (by-product from a starch plant) was obtained from Tereos Starch and Sweeteners. The hydrolysis of starch to glucose was carried out during 24 h at 55 °C and pH 4.5 using a commercial amyloglucosidase (Spirizyme Fuel HS, Novozymes) at a concentration of 120 μL/L.

2.2. Conventional lipid extraction method

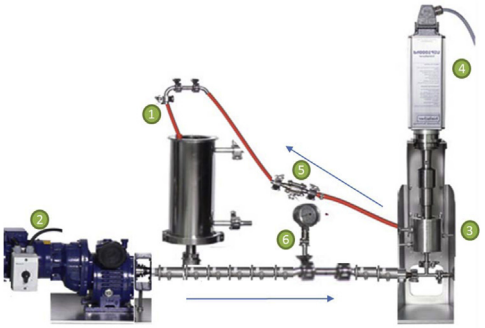
The standard solvent mixture methanol:chloroform approved for extraction of lipids from yeast [11,12] was employed to determine and compare the lipid yield of yeast. A quantity of 3 g of fresh yeast biomass was mixed with 10 mL of solvent mixture of chloroform and methanol (1:2, v/v) and subjected for 30 min at room temperature with a constant stirring. The mixture was then centrifuged at 5000 rpm for 5 min. The lower phase was recovered and transferred in a vial and conserved in freezer at - 4 °C for the further analysis. The experiment was performed in triplicate.

2.3. Bead milling extraction

Extraction by bead milling was performed using ULTRATURRAX® Tube Drive (UTTD, Ika, Germany) operating in a 20 mL tube with 20 g of ceramic beads. 3 g of fresh biomass was mixed with 10 mL of standard solvent mixture of chloroform and methanol (1:2, v/v) and submitted in drive tube operating at 4000 rpm during 3 hours. After extraction, the beads were filtered before solid/liquid separation by centrifugation.

2.4. Ultrasonic apparatus

Sonication, thermosonication, manosonication and manothermosonication were performed in a continuous ultrasonic apparatus built in our own laboratory (Fig. A.1). The different treatments were carried out using an ultrasonic device operating at low frequencies (20 kHz) with a 1000 W ultrasonic processor (UIP1000hd, Hielscher Ultrasonics, GmbH, Germany). A stainless steel cylindrical feed reactor of 1 L (1) was linked to a SEEPEX pump (2) to insure the flow of yeast and connected to a stainless steel cylindrical treatment chamber (3) to which a sonication horn (4) (BS2d34 standard titanium sonotrode, Hielscher Ultrasonics, GmbH, Germany) was attached. The treatment chamber and the feed reactor were surrounded by a double layer thermostated by a water recirculated chiller/heater module system (Huber, GmbH, Germany) in order to control the medium temperature. A manual valve (4) was connected between the output of the ultrasonic reactor and the feed reactor to maintain the desired pressure due to the perilstatic pump. A manometer (5) was connected to the output of the pump to controlled the level of the pressure. Temperature was monitored in the circuit and immediately after the exit by means of thermocouples. Treatment time was regulated by adjusting the speed of the perilstatic SEEPEX pump to reach a certain (and measured) volume flow rate through the treatment circuit.



(1) Feed reactor; (2) Perilstatic SEEPEX pump; (3) Sonication reactor; (4) Ultrasonic probe; (5) Manual valve; (6) Manometer.

Fig. A.1. Experimental setup. (1) Feed reactor; (2) Perilstatic SEEPEX pump; (3) Sonication reactor; (4) Ultrasonic probe; (5) Manual valve; (6) Manometer.

Considering the actual input, power from the device is converted to heat which dissipated in the medium. Calorimetric measurements were performed to assess actual ultrasound power P (W), calculated by Eq. (A.1) [16].

$$P = m \cdot Cp \cdot \frac{dT}{dt} \quad (A.1)$$

where Cp is the heat capacity of the solvent at constant pressure ($J\ g^{-1}\ C^{-1}$), m is the mass of solvent (g) and dT/dt is temperature rise per second ($°C\ s^{-1}$). Then, the applied ultrasonic intensity (UI) was determined using the calculated power as shown in the Eq. (A.2) [17].

$$UI = \frac{4P}{\pi D^2} \quad (A.2)$$

where UI is the ultrasonic intensity (W/cm^2), P is the ultrasound power (W) as calculated by Eq. (A.1) and D is the internal diameter (cm) of the ultrasonic probe.

2.5. Sonication treatment

The different treatments (sonication, manosonication, thermosonication, and manothermosonication), whether performed in direct extraction or in pretreatment were compared to conventional method of lipid extraction. Each experiment was performed in triplicate to calculate the standard deviation (SD).

2.5.1. Sonication, MS, TS, and MTS pretreatment

800 ml of fresh biomass at 5% dry basis was placed in the feed reactor with a rate flow of 0.9 L/min, then subjected for 30 min of irradiation time with amplitude of 100% and ultrasonic intensity of 25 W/cm^2, for each treatments applied. For MS treatment, the pressure was fixed at 2 ± 0.2 bars and the temperature was monitored at 20 ± 2 °C. For TS treatment, the temperature was fixed and