EXTRACTION OF LIPIDS FROM *YARROWIA LIPOLYTICA*

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ABSTRACT

A major sector of lipid biotechnology focuses on the ability of oleaginous microorganisms to convert various natural substances into reserve lipids. These lipids are mainly triacylglycerols and steryl esters. The yeast *Yarrowia lipolytica* is a high lipid-content yeast. In the present work, supercritical carbon dioxide ($SCCO_2$) lipid extractions from *Y. lipolytica*, using ethanol as a co-solvent, were performed. To better assess the supercritical extraction yield, high pressure phase boundaries in the systems 'CO_2 + yeast oil' and 'CO_2 + ethanol + yeast oil' were determined. It was found that a maximum concentration of 10 g of oil / kg of solvent could be dissolved with pure CO_2. The addition of ethanol to the solvent mixture increased this value. It is shown that the oil solubility increases with the pressure at a given temperature. $SCCO_2$ can be used to extract neutral lipids, as triacylglycerols. The addition of the ethanol also allows a more efficient extraction of triacylglycerols and also an extraction-fractionation of other lipids as phospholipids. Different pretreatments are necessary to obtain good extraction yields. The best performance was obtained for the ethanol macerated yeast. The ethanol maceration of the yeast was shown to modify the matrix, allowing the extraction of bonded lipids.

INTRODUCTION

The yeast *Yarrowia lipolytica (Y. lipolytica)*, also referred to as *Candida lipolytica*, is a high lipid content yeast. Several technologies, including various fermentation configurations, have already been used for single cell oil (SCO) production by strains of *Y. lipolytica* grown on various agro-industrial by-products or wastes [1]. The potential applications of these processes include the production of reserve lipids with particular structures (e.g., oils enriched in essential polyunsaturated fatty acids) and the production of nonspecific oils for use as renewable starting materials for the synthesis of bio-fuels [2]. According to the Principles of Green Chemistry, the use of these organic solvents at pilot and industrial scale has to be substituted in the near future by non-flammable, less toxic and more benign solvents in order to obtain sustainable processes [3]. Carbon dioxide (CO_2) is an inert, inexpensive, easily available, odorless, tasteless environment-friendly, and GRAS (Generally Recognized As Safe) solvent. Supercritical CO_2 has been successfully utilized to extract lipids from oil seeds and this has been one of the most important and studied applications of this technology, since pioneering works upon this topic were published [4-5]. To estimate the maximum oil concentration in the solvent that can be expected in the output effluent of a supercritical extractor, oil solubility measurements (phase boundaries) are very helpful.

The goal of this work is to estimate from solubility measurements the maximum oil concentration that can be expected at the output of the supercritical extractor and to evaluate the $SCCO_2$ lipid extraction from yeast, using ethanol as a co-solvent. Supercritical 'CO_2 + ethanol' extraction conditions were selected in order to obtain selectivity towards

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triacylglycerols (TAGs). The raw material used in this work was a strain of *Y. lipolytica* with high lipid content. It was provided by an European major industrial yeast producer. Different pretreatments were applied in order to increase yield and selectivity.

MATERIALS AND METHODS

Yeast materials. The yeast *Y. lipolytica* was provided by Lesaffre Group®, after harvesting and preconditioning. According to the provider, the lipidic content of the raw material was 17.7% w/w. This lipid content includes the phospholipidic (PL) and the TAG fractions. The original raw materials were subjected to different pretreatments:

1. Drying during 15 hours at 60°C and then milled and sieved.
2. Cell disruption by rapid decompression after exposure to $SCCO_2$ (6 hours at 200 bar and 40 ºC). After that, the yeast was dried at 60°C during 15 hours, milled and sieved.
3. Maceration in ethanol for 20 hours, dried at 60°C during 15 hours and milled.
4. Maceration in methanol for 2 hours, dried at 60°C during 20 hours and milled.
5. Atomization (done by the provider)

Phase equilibrium measurements. The experimental determination of the phase equilibrium for the 'yeast oil + CO_2' binary system and the 'yeast oil + CO_2 + ethanol' ternary system was performed in a high-pressure variable-volume equilibrium cell. The final aim of these experiments was the estimation of the maximum concentration of oil in the supercritical solvent which can be expected in the output effluent of the extraction, using $SCCO_2$ or '$SCCO_2$ + ethanol' solvent mixture. The phase transitions, resulting from pressure variations, are observed by direct visualization. To determine the solubility of oil in the solvent, a gravimetrically determined amount of yeast oil is placed inside the cell. Then, a given amount of CO_2 is loaded into the cell. For the ternary system, a gravimetrically determined amount of ethanol is loaded, after the oil loading. At the end of the loading process, there is a mixture of known composition inside the cell. Next, the system is set under conditions of temperature and pressure such that a homogenous fluid could be observed through the cell window. Then, the pressure was slowly reduced, at constant temperature, until the fluid system becomes unclear.

Supercritical extractions. Supercritical extractions were carried out in a SF 300 pilot plant from Separex Chimie Fine (France) with a series of two cyclone separators after the extractor. Subcooled liquid CO_2 was pumped by a volumetric membrane pump, then heated to the desired temperature and continuously introduced into the extractor. Ethanol (9% w/w) was used to increase the solvent power of CO_2 and to facilitate the mechanical recovery of the extract in the separators. Briefly, once the temperature was set (40°C); the extraction cylinder was filled with a given amount of substrate (around 20 g in all experiments) and placed inside the extractor vessel. Once the high pressure extractor vessel was closed, CO_2 was pumped (at the desired temperature) into the extractor until the operating pressure (200 bar) was reached. Then, a given flow-rate of ethanol was pumped into the vessel to obtain the desired solvent mixture. The solvent (CO_2) and co-solvent (ethanol) started to flow through the fixed bed at the desired flow-rate. The extractor pressure was regulated by a back pressure regulator (BPR). The outlet line of the BPR was connected to the first separator, at 60 bar. The last separator was operated at 20 bar.

Lipids + ethanol samples were taken from the separators every 5 or 10 min during the total extraction time (120 – 180 min). The samples from the bottom of separators were collected in

100 cm^3 glass flasks. Then the ethanol was separated from the lipids in a rota-evaporator at 60°C operated with a vacuum pump. For each sample the amount of extracted lipids was gravimetrically quantified after the separation.

RESULTS

Determination of yeast oil solubility in CO_2 and in CO_2 + ethanol. In order to determine the maximum amount of oil that can be dissolved by the pure CO_2 or CO_2 + ethanol mixture, the yeast oil solubility was measured as a function of pressure, at three different temperatures.
Figure 1 shows the solubility data obtained in the present work as a function of pressure for the system 'yeast oil + CO_2' at 40, 50 and 60°C. The dashed lines correspond to smoothed data and have been added to facilitate the visualization of the trends. The solubility is increased with the increase of pressure at a given temperature. On the other hand, the solubility is reduced with the increase of temperature at constant pressure. This phenomenon is called retrograde solubility [6]. Also, Figure 1 shows that almost 10 g of oil per kg of pure CO_2 can be dissolved at 40°C and 200 bar.

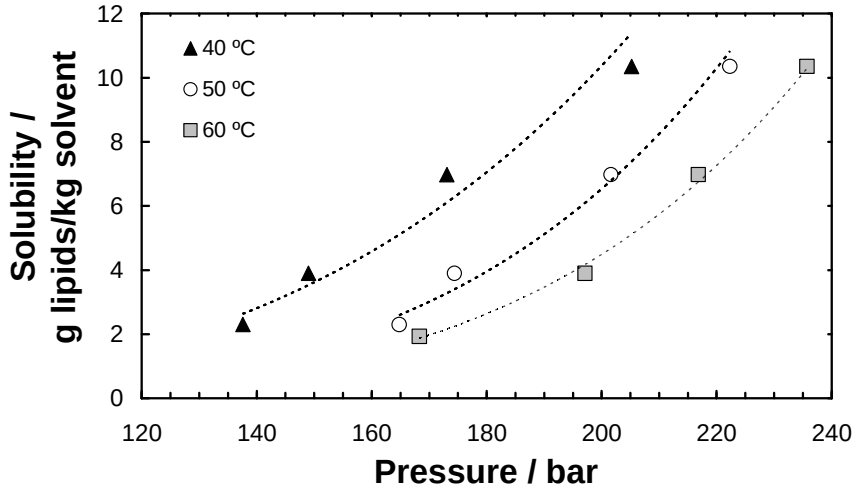

Figure 1. Experimental solubility values as a function of pressure the system 'yeast oil + CO_2' at three different temperatures. Filled triangles: 40 ºC. Empty circles: 50 ºC. Grey squares: 60 ºC. Triangles, circles and squares: raw experimental data (this work). Dashed lines: smoothed data.

Figure 2 displays the experimental solubility data for the 'yeast oil + CO_2 + ethanol' system using a solvent mixture with 10% w/w of ethanol. The trends are similar to those presented for the 'yeast oil + CO_2' system. The solubility is increased with the increase of pressure at a given temperature and is reduced with the increase of temperature at constant pressure. The addition of ethanol to the solvent mixture increases the amount of oil that can be dissolved. According to Figure 2, more than 15 g of yeast oil per kg of CO_2 can be dissolved at 200 bar and 40 ºC.

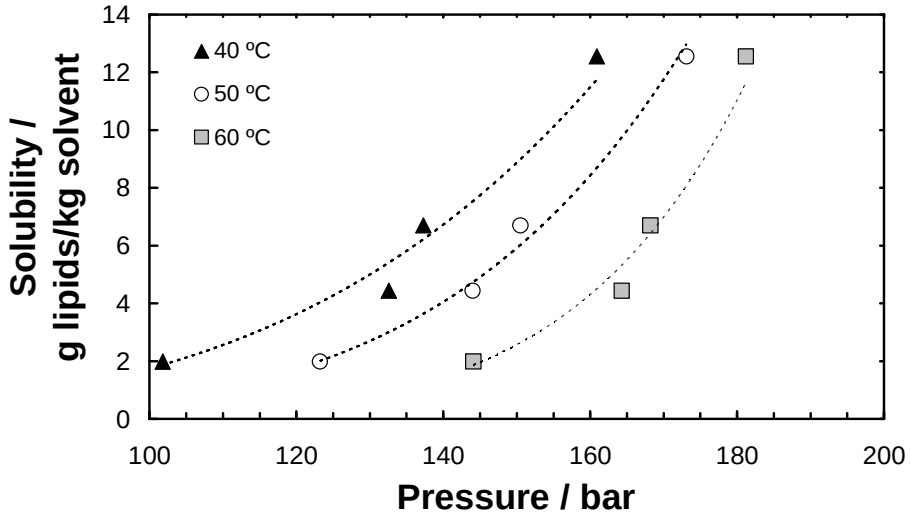

Figure 2. Experimental solubility values as a function of pressure the system 'yeast oil + CO_2 + ethanol' with a solvent mixture of 90% w/w of CO_2 and 10% w/w of ethanol at three different temperatures. Filled triangles: 40 ºC. Empty circles: 50 ºC. Grey squares: 60 ºC. Triangles, circles and squares: raw experimental data (this work). Dashed lines: smoothed data.

Supercritical CO_2 + ethanol extraction. The extraction studies were done using $SCCO_2$ and ethanol as a co-solvent. The operating conditions were: 200 bar in the extractor, 60 bar in the first separator and 20 bar in the second separator, a temperature of 40°C for the extractor and the separators, a flow-rate of 30 ± 2 g/min of CO_2 and a flow rate of 3 ± 0.2 g/min of ethanol. The experiments were carried out during 120 – 150 minutes. Figure 3 shows the results of the extraction for the different pretreated yeasts.

In the first part of the extraction curves, generally, the solute concentration in the fluid phase is high, as can be seen from high values of the slope of the curves. The different pretreatments can affect the interactions between the solute and other biomolecules. The accessible free solute can easily be removed by the solvent in this period. The amount of free solute depends on the efficiency of the pretreatment. High lipid concentration in the output fluid phase can be found in this extraction step. In the second period of the extraction curves the solute concentration in the output fluid phase decreases markedly. This is a diffusion-controlled extraction stage characterized by a strong solute–matrix interaction.

The results in Figure 3 show that the best performance was obtained for the ethanol macerated yeast. In this case, the solute concentration in the fluid phase reaches a value of 4.3 g of oil/kg of solvent in the first part of the extraction curve. Due to hydrodynamic or mass transfer problems, this value is far from the solubility values obtained from our solubility measurements. The total extraction yield at the end of the extraction is 10.8% w/w. For the atomized yeast the total extract yield reaches almost 8% w/w but the shape of the extraction curve is different. The initial solute concentration is very low (0.25 g of oil/kg of solvent) and increases along the extraction. The highest observed value is around 0.5 g of oil/kg of solvent. In the final part of the extraction curve, the solute concentration decreases again. For the supercritical disrupted yeast the initial slope is 2.4 g of oil/kg of solvent. In the second period of the extraction curve, a relatively high oil concentration (0.25 g of oil/kg of solvent) is observed for this extraction stage, evidencing incomplete solute extraction caused by a strong matrix-lipid interaction. The yield at the end of the extraction reaches 6.4% w/w. The

methanol macerated yeast and the dried and milled yeast shows low initial concentrations, less than 2 g of oil/kg of solvent, and also low total extract yield at the end of the extraction, less than 5% w/w.

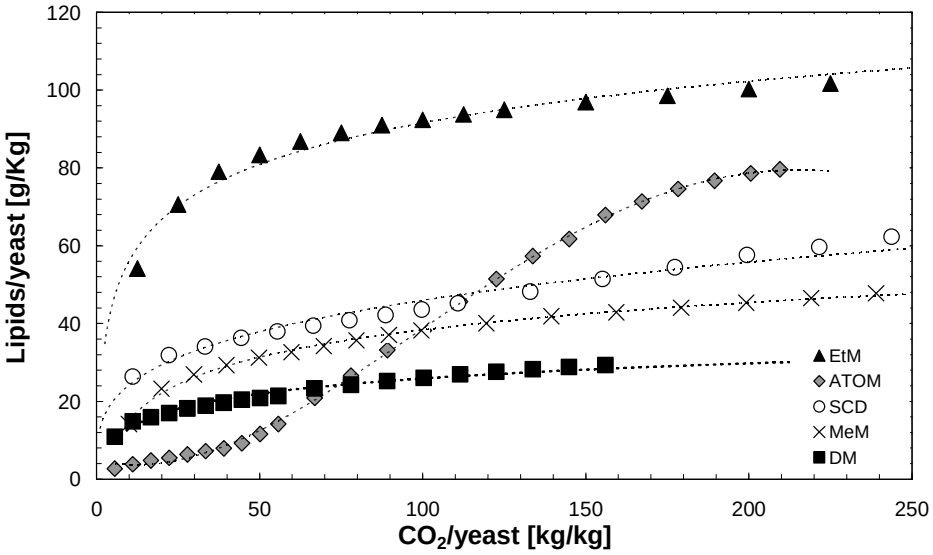

Figure 3. $SCCO_2$ + cosolvent experimental extraction curves for different pretreated *Y. lipolytica* yeast samples. Filled squares: Dried and milled yeast (DM). X's: Methanol macerated yeast (MeM). Empty circles: Supercritical disrupted yeast (SCD). Grey rhombuses: Atomized yeast (ATOM). Black triangles: Ethanol macerated yeast (EtM).

CONCLUSION

Yeast oil solubility in pure CO_2 and in CO_2 + ethanol was measured in a high pressure cell in order to determine the maximum possible solute content at the output of the supercritical extractor. The oil solubility is increased with the increase of pressure at a given temperature. Also a retrograde solubility phenomenon was pointed out in the range of tested pressures. At the conditions of the supercritical extractor (40°C and 200 bar), a maximum concentration of 10 g of oil per kg of solvent can be expected in pure CO_2 and addition of ethanol increases this value (above 15 g of yeast oil per kg of solvent). Also, the extraction of lipids from this non conventional matrix, has been carried out using $SCCO_2$ + ethanol high pressure extraction. The '$SCCO_2$ + ethanol' supercritical extraction curves revealed a description of the extraction mechanism. In the first part of the extraction curves, generally, the solute is not bound with other biomolecules and its concentration in the fluid phase is high. The second part of the extraction curves corresponds to an adsorption-diffusion controlled extraction stage, where the solute concentration in the fluid phase decreases significantly. The best extraction performance was obtained for the ethanol macerated yeast. The atomized yeast showed a peculiar extraction curve, with low initial oil concentration and high yield at the end of the extraction. In all studied cases a strong solute–matrix interaction was observed and the performance of the extraction was markedly dependent on the pretreatment of the material.

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