YEAST LIPIDS EXTRACTION BY SUPERCRITICAL CARBON DIOXIDE AND COSOLVENTS

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Saccharomyces cervisiae which is one of the most studied and industrially exploited yeast, is a non-oleaginous yeast whose lipids are mainly phospholipids. In the present work we studied the extraction of yeast lipids by supercritical carbon dioxide ($SCCO_2$) and ethanol as a co-solvent. In particular our attention was focused on the selectivity toward triacylglycerols, and in a subsequent extraction of the phospholipids present in the yeast. Indeed CO_2 is a non-polar solvent and by itself is not an efficient solvent for the extraction of phospholipids. However, $SCCO_2$ can be used to extract neutral lipids, as triacylglycerols, and the addition of polar co-solvents like ethanol, at different concentrations, could allow a more efficient extraction of triacylglycerols and also an extraction-fractionation of phospholipids. In this work $SCCO_2$ extractions of *S. cervisiae* and a membrane complex of this yeast, subjected to different pretreatments, were carried out at 200 bar and temperatures in a range of 40 to 60°C, using different concentrations of ethanol as a co-solvent. The kinetics of the extraction were successfully represented by Sovova's model. The extraction and fractionation of special lipids with SCO_2 and co-solvents from *S. cervisiae* was studied regarding it as a promising process to the recovery of valuable by-products from yeasts and such kind of process could then be extended to different types of oleaginous yeasts.

1. Introduction

The term lipid is commonly used for triacylglycerols; however, oilseeds and microorganisms contain other lipids like phospholipids, sterols, polyphenols, sphingolipids, glycolipids, etc. Lipids from yeast, for example, generally contain high quantities of phospholipids. These secondary lipids are potentially valuable by-products which could be recovered to economic advantage. Phospholipids are potential multifunctional additive for food, pharmaceutical and industrial applications. The extraction process of these lipids must maintain the structure and the quality of phospholipids. The supercritical CO_2 extraction of lipids from oil seeds has been one of the most important and studied applications of this technology since pioneering works upon supercritical fluids were published [1]. In particular, the extraction and fractionation of specialty lipids is very attractive when considering their essential role in many functions of the human body. More recently, lipids have been considered as a source of energy in the production of bio-fuels. In this last trend, the fractionation of lipids and derivatives plays an important role in obtaining secondary high-value bio-products [2].
Microorganisms have often been considered for the production of oils and fats as an alternative to agricultural and animal resources. The yeasts considered as oleaginous species accumulate more than 20 % by weight fraction of their biomass. Oleaginous yeasts, such as *Cryptococcus albidus* and *Rhodotorula glutinis*, were reported to grow and accumulate very significant amounts of lipids (60 to 70 % by weight fraction). The main lipids composition of these oleaginous species are triacylglycerols or triglycerides, and in minor quantities

phospholipids and sterols. However, most microorganisms even under the most propitious conditions could have only a small fraction of their biomass as lipid. For example, *Saccharomyces cereviciae* exhibit between 5-10 % by weight fraction of dry mass, and phospholipids are the more important fraction of these lipids. They are mainly located in cell membranes [3-4].

The lipid extraction from yeasts with different conventional organic solvents, such as chloroform and methanol, hexane and/or petroleum ether and their application even at industrial scale have been reported [4]. However, 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 [5]. CO_2 is an inert, inexpensive, easily available, odorless, tasteless environment-friendly, and GRAS solvent. The supercritical technology is a green sustainable process in which the solvent power and selectivity can be tuned according to the operating conditions.

Extraction of lipids from yeast using $SCCO_2$ has been scarcely considered in the literature.

Supercritical studies have been reported for the extraction of high added-value products from yeast, such as astaxanthin and squalene [6-7]. Few references can be found in the literature about the detailed yeast lipid extraction with CO_2 and co-solvents and the kinetic analysis of the process taking into account the pretreatment of the process which has a marked influence in the extraction efficiency. Because of its similarity, it is of interest to consider work about oil extraction from seeds. So, the use of $SCCO_2$ and co-solvents for the selective extraction of triglycerides has been studied, for example, for the extraction of canola, soybean lecithin and sunflower oil [8-11]. Ethanol as a co-solvent increases the solvent power of $SCCO_2$ at given operating conditions. For example, Cocero and Calvo [8] have reported a value of sunflower oil solubility in CO_2 around 3.5 g/kg at 200 bar and 42°C, value which increases up to nearly 25 g/kg when 10 % of ethanol by weight fraction is used as a co-solvent. The addition of polar co-solvents, enhance the solubility of phospholipids which are insoluble in pure CO_2. Dunford and Temelli [9] have studied the SC-CO_2 extraction triglycerides and phospholipids from canola flakes. They observed that the selectivity of CO_2 towards the extraction of triglycerides was not affected by the addition of ethanol (8 % by weight fraction) and phospholipids contents lower than 200 ppm were found in the oil extracted at 55.2 MPa and 70°C. However, they reported an increasing amount of phospholipids detected when canola flakes of reduced oil content were extracted at the same operating conditions. Teberikler et al. [10] studied the extraction and fractionation of phospholipids with $SCCO_2$ and ethanol as a co-solvent from deoiled soybean lecithin. They worked at 200 bar and temperatures of 60 - 80 °C, observing that there was not significant amounts of phospholipids in the first part of the extraction process due to the triglycerides initially present in the lecithin. Teberikler et al. [10] reported selectivity towards phosphatidylcholine at 200 bar and 60 °C with a global solubility near 0.15 g/kg in the $SCCO_2$ with 10 % of ethanol as a co-solvent.

2. Materials and Methods

2.1 Extraction Substrates

S. Cerevisiae yeast and a *membrane complex of S. Cerevisiae* (termed here as C1) of high lipid content were provided by Lesaffre Group.

S. Cerevisiae material was dried in a drying drum. It was milled in a knife grinder and size-classified in a sieve shaker (-0,5 to +1,8 mm). The solid and global densities of the material were found to be 1200 and 830 kg/m^3, respectively.
The membrane complex C1 has higher lipid content, nearly 20 % by weight fraction of dry material. Different pretreatment studies were tested in this work. Initially, the material was heated and dried by atomization at 60 °C, with a mean particle diameter of 70 µm. The solid and bulk densities of this material were 1130 and 550 kg/m3. This material was then subjected to a cooking pretreatment during 30 minutes.
Alternatively, the yeast complex C1 with 80 % by weight fraction of water was also dried in an air oven at 60 °C, overnight. Then, the dried material was milled in a knife grinder and size-classified with a mean particle diameter of 300 µm.

2.2 Soxhlet Extractions

Lipid content of the yeasts was gravimetrically determined by Soxhlet extraction to exhaustion (8 hours) with solvents of technical grade and solvent mixtures of different polarities (hexane, ethanol and chloroform-methanol in different volume ratios). The solvents were recovered in a rota-evaporator which was operated with a vacuum pump.
The crude lipidic extracts obtained from Soxhlet extractions contain carbohydrates, proteins, and other unwanted materials which were separated by a method based on previous works [12].
Phospholipids present in the total lipids (1 to 3 g) obtained by Soxhlet extraction were roughly separated and quantified by dispersion in cold acetone (90 ml). The solvent containing neutral lipids was decanted into a separated beaker. The process was repeated two times, the second time the solvent was color free. This method has been employed to enrich phospholipids content in commercial Soybean Lecithin [13].
Table 1 shows the total lipids content after separation of non-lipids material according to the Soxhlet extraction by different solvents. **Table 2** shows the fraction of lipids insoluble in acetone found in different Soxhlet extractions, assumed hereafter in this work as the phospholipids content (PL). Soluble lipids in acetone in turn are assumed to be triacylglycerols (TG).

Table 1. Soxhlet lipid extractions by organic solvents. Total lipid content in substrates.

	Hexane	Ethanol	Chloroform Methanol (2:1)	Chloroform Methanol (1:2)
S. Cerevisiae	0.003	-	0.021	0.055
Memb. Complex (C1)	0.001	0.22	0.212	0.23

Table 2. Phospholipids content by weight fraction in the yeast obtained by dispersion in cold acetone of the total lipids extracted by Soxhlet

	Hexane	Ethanol	Chloroform Methanol (2:1)	Chloroform Methanol (1:2)
S. Cerevisiae	0.003	-	0.016	0.048
Memb. Complex (C1)	0.003	0.066	0.062	0.069

2.3 Supercritical Extractions

Supercritical extractions were carried out in a pilot plant from Separex Chimie Fine, France (type SF 300) represented in **figure 1**, having an extraction capacity of 300 mL, two cyclone separators in series and a maximum CO_2 flow rate of 6 kg/hr. The experimental procedure was already described in previous papers [14-15]. However, in this work, a co-solvent (ethanol 9 % by weight fraction) was used to increase the solvent power of CO_2 and to allow better mechanical recovery of the extract in the separators. Briefly, the heating system was set at the desired temperature (40-60 °C); the extraction cylinder (reduced at one -third of its capacity: 41.5 mm i.d., 165 mm height) was loaded with a given amount of substrates (ca. 33 g) and placed into the extractor vessel. Carbon dioxide was pumped at the desired temperature at the extractor up to reach the operating pressure (200-230 bar); a given amount of ethanol was pumped to the vessel up to obtain the desired fraction by weight percent of ethanol as co-solvent. A static extraction period of 15 minutes was initiated to attain the desired process conditions and solvent and co-solvent were subsequently pumped at the desired flow-rates. The extraction pressure was maintained by a back pressure regulator (BPR). The outlet line of the BPR was connected to the first separator regulated at 70 bar by another BPR valve whose outlet line was connected to the last separator operated at 40-50 bar.
Unequally spaced samples (12-18) were taken in all cases during the total extraction time (120 – 180 min.). The samples from the bottom of separators were collected along the time in 15 cm^3 capacity glass vials. Then ethanol was recovered at 40°C in a rota-evaporator operated with a vacuum pump.

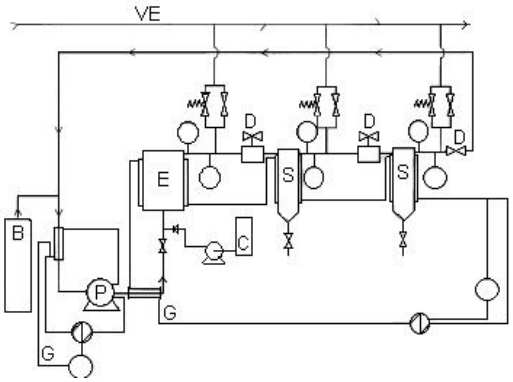

Figure 1. Simplified flowsheet of the pilot: B, CO_2 bottle; E, extractor; S, Separators; P, pump; G, Cooling and heating group; D, Back pressure regulator valves; C, Co-solvent; VE, venting system.

3. Modeling of the process

3.1 Mathematical Model

The mathematical model adopted in this work, equations (1) to (3), is the model of Broken and Intact Cells (BIC), a general approach proposed by Sovová [16]. This model assumes that the solute is homogeneously distributed in the untreated solid particles placed in the extractor. The particles contain broken cells near the surface and intact cells in the core. The easily accessible solute from broken cells is transferred directly to the fluid-phase, while the solute from intact cells diffuses first to broken cells and then to the fluid-phase. The BIC model does not account for concentration gradients within the substrate (normally assumed as spherical

particles) and the differential mass balance for the solid is written as a function of the average mass fraction of residual oil. The model assumes also constant physical properties of the SC-CO_2 and substrate, and negligible pressure drop and temperature gradient in the bed. The differential mass balance equations in the solvent phase (1) and solid particles (2 and 3) according to Sovová model are:

$$\rho_f \varepsilon \left(\frac{\partial y}{\partial t} + U \frac{\partial y}{\partial z} \right) = j_f \tag{1}$$

$$r\rho_s (-\varepsilon) \frac{\partial x_1}{\partial t} = j_s - j_f \tag{2}$$

$$(-r)\rho_s (-\varepsilon) \frac{\partial x_2}{\partial t} = -j_s \tag{3}$$

In Eqs. (1) to (3): y, x_1 and x_2 are the concentration of oil in the solvent phase, in the broken cells and in the intact cells, respectively; *t* is the extraction time; *z*, is the axial coordinate, *U*, is the interstitial fluid velocity; *r*, is the fraction of broken cells or non-bound lipids; j_f and j_s are the fluxes of solute from the broken cells to the solvent and from the intact cells to the broken cells, respectively; ρ_f and ρ_s are the densities of $SCCO_2$ and solid particles, respectively; ε is the void volume fraction of the bed.

Different mass transfer fluxes were adopted as proposed by Sovová. The flux j_f of solute from broken cells to the solvent is given by Eq. (4), while the internal flux from intact cells to the broken cells is described by Eq. (5):

$$j_f = k_f a_0 \rho_f (y^* - y) \text{ for } x_1 < x_t,\ y < Kx_t,\ y^* = Kx_1 \tag{4}$$

$$j_s = k_s a_s (x_2 - x_1) \tag{5}$$

a_0 is the specific interfacial surface ($a_0 = 6(-\varepsilon)/dp$ where *dp* is the average particle diameter) and k_f is the external mass transfer coefficient. k_s is the internal mass transfer coefficient, which globally accounts for the internal diffusional resistance (linear driving force model). An important feature of the BIC model proposed by Sovová is that it considers different types of pseudo-equilibria in the first extraction period: independent on matrix (solubility equilibrium) and adsorbed on the matrix (partition coefficient). Sovová proposed four types of extraction curves according to the type of equilibria and the initial mass balance for the solute in broken cells and in the solvent. From an examination of our extraction curves we decided to work with a "type D" extraction curve of the BIC model. Type D extraction curves are characteristic of a strong matrix-solute interaction and the pseudo-equilibrium present in the system is governed by $y^*=Kx_1$ from the beginning of the extraction, where *K* is a partition coefficient. According to Eq. 4 the maximum concentration of solute in the solvent is proportional to the solute concentration in the broken cells by the partition coefficient *K*. The initial concentration y_0 corresponding to the first part of the extraction curve in this case could be much lower than the thermodynamic solubility of the solute in the supercritical solvent.

The value of *K* in this case can be determined from the slope of the first part of the extraction curve and the fraction of broken cells so as the physical properties of the material by Eq. (6):

$$y_0 = Kx_{1,0},\ x_{1,0} = \frac{rx_u}{r+\gamma K}, \gamma = \frac{\rho_f \varepsilon}{\rho_s (1-\varepsilon)},\ x_u - x_t \le \frac{\gamma}{r} K x_t \qquad (6)$$

In eq. (6) y_0 is the initial solubility of solute in the solvent, $x_{1,0}$ is the initial concentration of oil in the broken cells, x_u is the initial concentration of solute in the solid material.

3.2 Model parameters

The density of pure $SCCO_2$ was estimated according to NIST [17]. The viscosity of pure CO_2 was estimated as a function of its reduced temperature ($T_r = T/T_c$, T_c = 304.1 K), reduced density ($\rho_r = \rho/\rho_c$, ρ_c = 0.4687 g/cm^3) and low-pressure (gaseous) viscosity (μ°) using the method of Jossi et al.[18]. Value of μ°, in turn, was estimated using Lucas' method for non-polar gases (1.59 10-5 Pa.S). The density and viscosity of liquid ethanol are from Perry's Handbook [19]. Mixture viscosity was estimated according to the method of Grunberg and Nissan [18]. Finally, the binary diffusion coefficient for triglycerides in CO_2 (D_{12}) was estimated as a function of T_r, ρ_r and the molecular weight (885.4 g/mol) and critical volume (3200 cm^3/mol) of the solute using the equation of Catchpole and King [20]. The values of ρ, μ and D_{12} are summarized in table 3.
The external mass transfer coefficient k_f was estimated by the correlation of Tan et al. [21].
The value of the Peclet number, based on the height of the packed bed, *Pe*, was calculated by the correlation of Catchpole [22] to estimate the degree of axial dispersion in the extractor vessel. According to Goto et al. [23] the effect of axial dispersion in the extractor is small when $10<Pe<100$ and negligible for Pe > 100.

Table 3. Physical properties and parameters used in the BIC model for the $SCCO_2$ extraction of lipids from *S. cerevisiae* and the membrane complex. k_s and *r* are obtained from numerical fiiting of the experimental curves

ρ(kg m^{-3})	μ (Pa.S)	D_{12} (m^2 s^{-1})
840	7.72 10^{-5}	5.5 10^{-9}

Material	d_p(mm)	k_f(m s^{-1})	*Pe*	k_s(m s^{-1})	*r*
S. Cerevisiae	0.878	1.54 10^{-5}	6	2.014 10^{-08}	0.58
M. complex (1.0)	0.073	3.37 10^{-5}	166	3.930 10^{-11}	0.043
M. complex (1.1)	"	"	"	7.397 10^{-11}	0.050
M. complex (1.2)	"	"	"	7.397 10^{-11}	0.064
M. complex (2.0)	0.309	2.64 10^{-5}	20	2.064 10^{-09}	0.33

(1.0) Membrane complex dried by atomization
(2.0) Membrane complex heated and dried by atomization and cooked at 90 °C
(3.0) Membrane complex heated and dried by atomization and cooked at 120 °C
(4.0) Membrane complex dried at 60 °C overnight and milled in a knife grinder

3.3 Numerical resolution and fitting of experimental data

Eqs. 1-3 were expressed as dimensionless concentration, axial position and time as proposed by Sovová [16]. The continuous extraction bed was represented by a series of 100 stages of homogeneous composition. In this way equations 1-3 were transformed into a set of ordinary differential equations in function of time which were integrated numerically using Runge-Kutta-Felhberg method. The total amount of extracted solute was estimated by integration over time of the solute concentration in the supercritical fluid flow at the extractor outlet. Integrals were assessed numerically using Simpson's method. The fraction of broken cells or non-bound lipids, r, and the internal mass transfer parameter, k_s, were left as fitting parameters. Conventionnally experimental cumulative extraction curves are expressed in terms of yield (g of solute extracted / kg of free solute material) versus the specific solvent mass ratio, q, (kg of CO_2 /kg of free solute material). Here the reference solutes were triglycerides.

4. Results and discussion

4.1 Supercritical CO_2 extractions of S. cerevisiae

The extraction of lipids from *S. cerevisiae* with pure CO_2 shows low yields, similar to the Soxhlet extraction with hexane, and as a consequence it was not possible to study the kinetics of the extraction process. The yield when working with 30 g/min of $SCCO_2$ at 313 K and 200 bar was 3 g/kg (lipids extracted / treated yeast) after 150 minutes of extraction.
The addition of ethanol as co-solvent (9 % by weight fraction) increased the yield at 6-7 g/kg after 150 minutes of extraction at the same CO_2 flow. The co-solvent in this case also acts as a cleaning agent of the separators and allows collecting at the bottom of the separators all the lipids extracted. **Figure 2** shows the effect of the mass flow rate of SC-CO_2 on the cumulative extraction yield of the milled yeast (average dp = 878 μm) as a function of the specific solvent mass ratio.

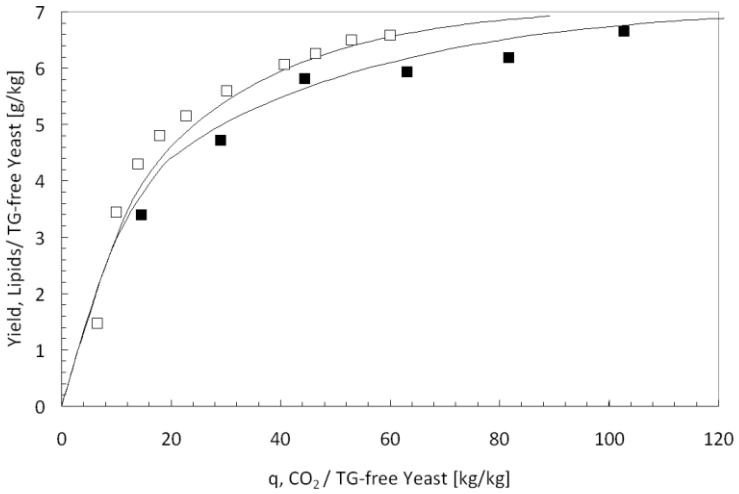

Figure 2. Extraction of lipids from *S. cerevisiae* with $SCCO_2$ (313 K and 200 bar) and 9 % by weight fraction of ethanol as co-solvent. Lines: BIC model. Symbols: experimental data (■ 30 g/min., □ 20 g/min.)

The cumulative extraction curves converge to a single line for a specific solvent mass ratio lower than 6. As expected, the cumulative extraction yield for any given specific solvent mass ratio, q, above the aforementioned limits increased as the interstitial solvent velocity decreased due to increased contact time between the phases. Best-fit lines obtained by the BIC model are also included in **figure 2**, and values of best-fitting parameters which were used to adjust simultaneously the curves in **figure 2** are reported in table 3.
The slope of the cumulative extraction curves for the first extraction period is 0.33 g of lipids / kg of CO_2. The solubility value obtained is one order of magnitude lower than the vegetable oil solubility in pure CO_2 (3.4 g/kg according to Del Valle Aguilera empirical equation [24]) and up to two orders of magnitude lower than the solubility of sunflower oil in CO_2 + ethanol at 10 % (25 g/kg according to Cocero and Calvo [8]). Soluble lipids seem to have a strong interaction with the matrix from the start of the extraction process.
Figure 2 shows that $SCCO_2$ + ethanol extracts 6 – 7 g of lipids / kg of yeast. This yield is in the same order as the lipids soluble in acetone obtained by Soxhlet extractions with chloroform/methanol 1:2 (table 1-2). The results show selective extraction towards non-polar lipids. According to the mathematical model the fraction of broken cells in the extracted material is 60 % if the maximum amounts of extractable lipids are the acetone soluble lipids fraction or triacylglycerols. The internal mass transfer coefficient was found to be around 1.86 10^{-8} m s^{-1} and is in the same order of magnitude as those reported by Reverchon and Marrone [25] for oil-containing seeds.

4.2 $SCCO_2$ extractions of the Wall Membrane Complex

As in $SCCO_2$ extraction of *S. cerevisiae*, the extraction of Complex C1 with pure CO_2 (operating conditions: 200 bar, 313.15 K and 30 g/min.) produce low yields (<3 g/kg) after 150 minutes (lipids found after cleaning the extraction plant) and it was not possible to follow the extraction kinetic.
Figure 3 shows the results of the $SCCO_2$ extraction of the wall membrane complex C1 using 9 % by weight fraction of ethanol with a flow of 32 g/min at 200 bars and 313 K. The results are expressed in this case as the lipids extracted over the total lipids obtained in Soxhlet studies, in function of the solvent mass (kg of CO_2) used in the experience. As it can be observed the maximum yield obtained is near 10 %.
A study of the solvent power and selectivity of the mixture CO_2-ethanol was realized on the lipidic material extracted in Soxhlet studies with methanol + chloroform mixtures. In this study, glass beads (1.5 mm – 86,474 g) were impregnated with yeast lipids (3.626 g) from the Soxhlet. The impregnated quantity corresponds to the quantity of lipids contained in 17.7 g of yeast. Following the same extraction procedure and operating conditions, **Figure 3** shows also the results obtained in this case and the comparison with results of the direct extraction of the wall membrane complex *C1*. A significant solvent power can be observed, the initial solubility in this study being 5.6 g/kg, more than one order of magnitude greater than the initial solubility obtained in the direct extraction of the yeast. It can be deduced from this experiment that a pretreatment could be very useful to lower the affinity existing between the lipids and the matrix and develop the lipid extraction in more efficient conditions. **Figure 3** also allows seeing the selectivity of the supercritical solvent: 69 % of the total lipids are extracted in the process while the rest of the lipids remains in the residual material in the extraction vessel. The non-extracted lipids in this case are acetone insoluble, in agreement with the results obtained in the Soxhlet studies. An estimated specific solvent mass ratio, q, of 55 allows extracting near the 90 percent of triacylglycerols. This selectivity was observed in

oilseeds extraction. For example, Cocero and Calvo [6], at similar operating conditions obtained only 0.06 g of phosphorous/kg oil extracted. Montanari et al.[24] obtained 0.7 g of phospholipids / kg of "defatted" soybean flakes, near 13 % of total phospholipids, at 23 MPa and 60 °C.

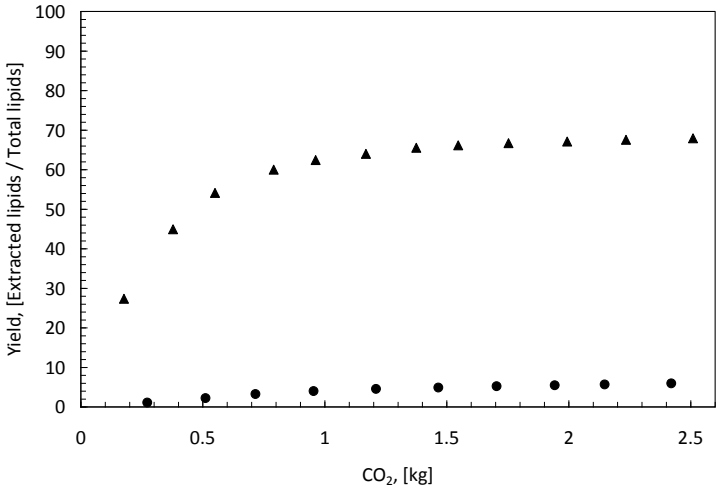

Figure 3. Lipid extraction from *Membrane complex C1* dried by atomization at 60°C with $SCCO_2$ (30 g/min. 313 K and 200 bar) and 9 % by weight fraction of ethanol as co-solvent. Symbols: experimental data (▲) direct extraction of the lipids obtained by Soxhlet, (●) extraction from the material *C1* heated and dried by atomization.

4.3 $SCCO_2$ extractions of the Wall Membrane Complex after different pretreatments

Different pretreatments were employed to perform the supercritical CO_2 extraction in order to increase the yield. Thermal pretreatments are normally applied in the industry of edible oils to coagulate proteins, decrease oil affinity for the solid surfaces and agglomerate the oil into larger droplets [3]. The material complex C1 previously heated and dried by atomization was cooked at 90 °C and 120 °C for 30 min. in a convection oven. **Figure 4** shows the cumulative extraction curves as a function of the specific solvent mass ratio, q, for different thermal pretreatments of the material complex C1 dried by atomization. It also includes the best fit lines obtained with the BIC model for the parameters reported in table 3. A good agreement between the model and experimental data can be observed.

The extraction of the complex C1 shows that soluble lipids still have a strong interaction with the matrix from the start of the extraction process, whatever the pretreatment. The solubility in the first extraction period is 0.33 g of lipids / kg of CO_2, as explained before, one order of magnitude lower than the vegetable oil solubility in pure CO_2. The partition coefficient, K = 0.0019, calculated from the slope of the cumulative extraction curves shows a low extraction efficiency when compared to the value corresponding to non bound solute, which is K = 0.033.

Figure 4 shows that the first extraction period is constant and independent of the thermal pretreatment. However, the fraction of broken cells of non-bound lipids increased from 4.3 % in the original material to 6.4 % with the thermal pretreatment at 120 °C. Also, the original material presented a value of internal mass transfer coefficient (table 3) lower than in the case of thermal pre-treatment, which appears to lower the resistance of material diffusion from the intact to the broken cells. Both temperatures of thermal conditioning show similar internal

mass transfer coefficients. However, the values remain in the same order of magnitude, indicating persistence of mass transfer limitations.

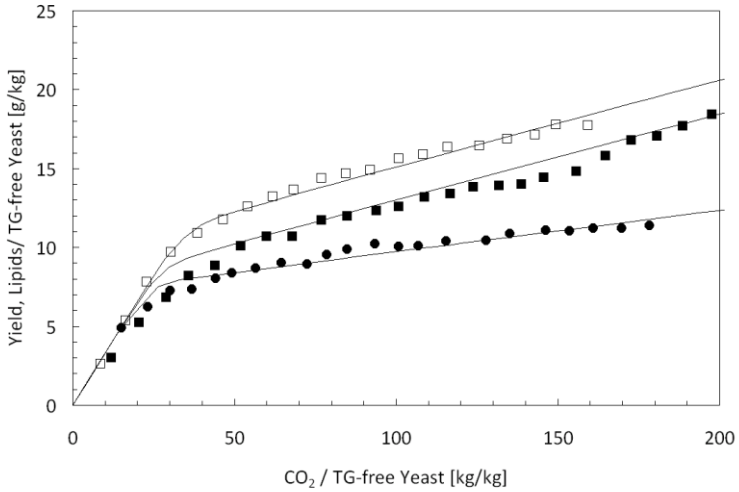

Figure 4. Lipid extraction from *Membrane complex C1* dried by atomization and subjected to cooking pretreatments with $SCCO_2$ (30 g/min. 313 K and 200 bar) and 9 % by weight fraction of ethanol as co-solvent. Symbols: experimental data (●) No-thermal pretreatment, (■) Cooking at 90 °C, (□) Cooking at 120 °C. Lines: BIC model.

To study another general pretreatment, the original wet material was filtrated and dried in a oven at 60 °C overnight and then ground. **Figure 5** shows the yield in function of the specific solvent mass ratio obtained in this last case and, in order to compare the results, also shows the yield of lipids extracted from the complex dried by atomization. There is clearly a great improvement in the extraction yield with the pretreatment. From an examination of the extraction curve it is possible to deduce an initial solubility y_0 = 3.2 g/kg and one third of the lipids extracted by Soxhlet with chloroform/methanol 2:1 soluble in acetone are extracted in the first part of the extraction with a specific solvent mass ratio of 30.

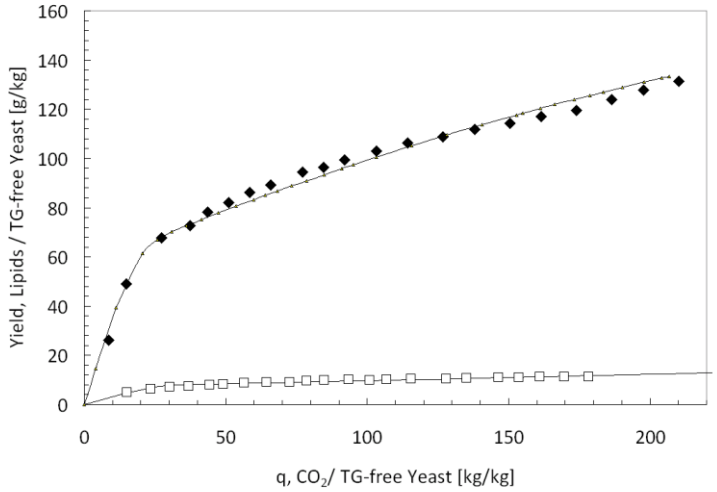

Figure 5. Lipid extraction from *Membrane complex C1* with $SCCO_2$ (30 g/min. 313 K and 200 bar) and 9 % by weight fraction of ethanol as co-solvent. Symbols: experimental data (♦) Dried in an air oven at 60 °C overnight, (□) Dried by atomization at 60 °C. Lines: BIC model.

The BIC model which fits properly the extraction curve shows a fraction of non-bound lipids r = 0.33 and an internal mass transfer coefficient k_s = 2.06 10^{-9} m/s, a value more in agreement with the results usually obtained for oilseed extraction [25] and two orders of magnitude than the one obtained when the material is heated and then dried by atomization.

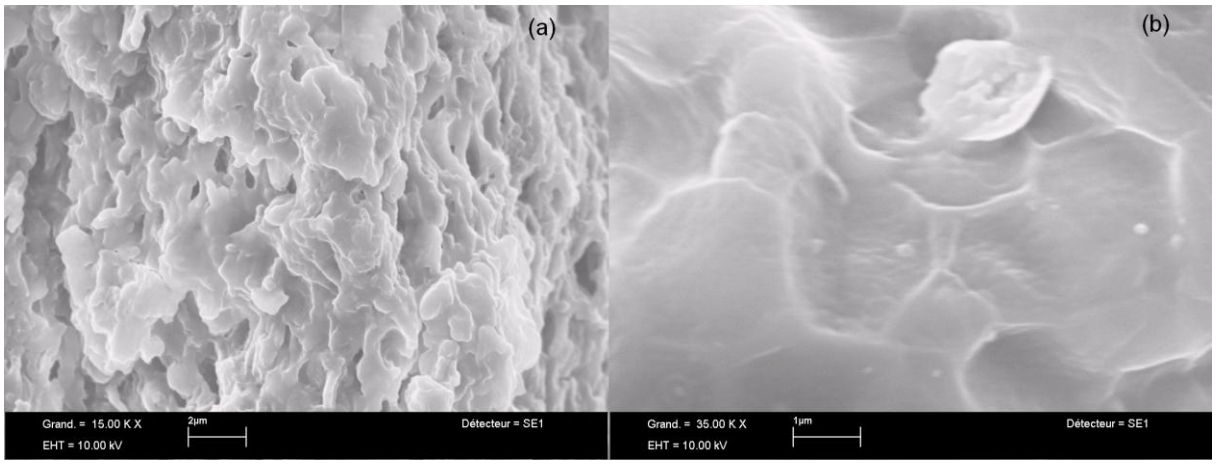

Figure 6. SEM images of a section of (a) material dried at 60 °C overnight and (b) the material dried by atomization.

A scanning electron microscope (SEM) analysis of the material was carried out to observe the difference in the solid material structure after different pretreatments. **Figure 6** shows that there is a great impact of the pretreatment upon the structure. When the material is dried at 60 °C a porous material is obtained after the grinding process and the solvent could interact more easily with the material and the lipids to extract. These observations agree with the greater fraction of broken cells and also the lower mass transfer coefficient obtained by the BIC model.

5. Conclusions

Lipid extraction from *S. cerevisiae* and a membrane complex of this yeast with $SCCO_2$ + ethanol as co-solvent has been carried out at 200 bar and 313 K and with conventional organic solvent Soxhlet extractions. Soxhlet extractions have indicated the presence of an important amount of non-soluble in acetone lipids, in both the yeast and the membrane complex. First results with *S. cerevisiae* have revealed strong interaction between the lipids and the matrix. The extraction of triacylglycerols with $SCCO_2$ + 9% of ethanol was performed with a specific solvent mass ratio 60 to 100 at solvent flow rates of 20 to 30 g/min., respectively. However, a very low amount of triacylglycerols was found in this material, whatever the extraction technique, supercritical or conventional. On the other hand, the extraction of the membrane complex with chloroform/methanol solvents showed a lipid content of 21 to 23 % by weight fraction of material, with a 30 % of these lipids being insoluble in acetone. When the lipids obtained by Soxhlet extraction were subjected to $SCCO_2$ + ethanol (9 %) a selective extraction of triacylglycerols was obtained as expected according to the low solubility of phospholipids at these operating conditions. The $SCCO_2$ extraction showed an important and markedly interaction between the lipids to extract and the raw material. Besides, as it was observed, the performance of the $SCCO_2$ extraction of the membrane complex depends

markedly on the pretreatment of the material and drying process determined the success of triacylglycerol extraction.
$SCCO_2$ extraction of triacylglycerols from yeast can be performed selectively with a 9 % of ethanol as co-solvent at 200 bar and 313 K which yielded a residual material rich in phospholipids, which can be extracted after this first stage with an increment of pressure or an increase of the ethanol concentration.

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