STUDY OF THE INFLUENCE OF PROCESS PARAMETERS ON LIQUID AND SUPERCRITICAL CO_2 EXTRACTION OF OIL FROM RENDERED MATERIALS: FISH MEAL AND OIL CHARACTERIZATION

S.L. Bucio[1,2], M. Teresa Sanz [2*], Sagrario Beltrán[2], R. Melgosa[2], A. G. Solaesa[2], María O. Ruiz[2]

[1]Biotechnology, Technological University of Morelia, 58200 Morelia, Mich. México.

[2] Department of Biotechnology and Food Science. University of Burgos. Plaza Misael Bañuelos s/n. 09001 Burgos. Spain. Tel.: +34 947 258810. Fax: + 34 947 258831.

E-mail: tersanz@ubu.es

Abstract

Liquid and supercritical CO_2 has been used to extract the remaining fat content from rendered fish meal. The effect of pressure (10 – 40 MPa) and temperature (25 – 80 ºC) on the extraction kinetics and extraction yield has been investigated as well as the effect on the rendered fish meal. The extraction curves are initially linear with a slope close to the oil solubility value in pressurized CO_2. Based on previous fish oil solubility data reported in the literature, a general equation has been proposed to correlate fish oil solubility data as a function of temperature and density of CO_2. Fish meal has been

* Corresponding author

characterized before and after extraction by determining the fat and protein content and its colour. Toxic trace elements have been also determined by ICP-MS in the fish meal showing that most of the toxic elements remained in the fish meal after extraction. Characterization of extracted oil was also performed by determining the fatty acid group composition and some physical parameters such as colour.

Keywords

Fish meal, liquid and supercritical CO_2, fish oil solubility

1. Introduction

Fish meal is one of the primary products resulting from the rendering process of fish discards, being Peru and Chili the two major producers [1]. The other main product is the oil fraction. Fish meal is the clean, dried, ground tissue of undecomposed whole fish or fish cutting, with or without the extraction of part of the oil [2].

Total protein in fish meal can be higher than 70 % with good digestibility of its amino acids which makes it an excellent source of nutritive protein. It is used to supplement other proteins in diets for farmed animals. Due to its high nutritional quality, fish meal could be used to obtain fish protein concentrates (FPC) for human consumption. The specification for some types of FPC demands very low fat content, to eliminate fishy taste and odor and rancid during storage since most of the flavor is in the oil fraction [3]. Additionally, low-fat protein hydrolyzate from fish is a promising product for the future.

The method of solvent extraction has been frequently employed when producing fish protein concentrated with a fat content less than 1 % (FPC, type A). The solvent most commonly used is isopropanol, although other solvents such as ethanol [4] and

isohexane [5] have been also successfully used. In the conventional solvent production method, temperature reached values up to 75 ºC [6]. Removal of the oil by solvent extraction is expensive and traces of solvent in the final product made solvent extracted FPC commercially unsuccessful [4].

In this work, high pressure CO_2 has been employed to reduce the fat content of rendered fish meal. Liquid and supercritical CO_2 can selectively extract the fat without affecting the protein [7]. CO_2 is a gas under ambient conditions that can be easily separated from processed products, leaving no residual solvent in the feed matrix. It is considered a "green solvent", being non-toxic, non-flammable and relative non-expensive. SC-CO_2 extraction has been successfully used in the literature to extract oil from different by-products of the fish industry [8] and also from the muscle [9].

The quality of the oil extracted by liquid or supercritical CO_2 can be of better quality than the oil extracted by solvent extraction, with lower total oxidation values than other extraction methods [8, 9]. Although solvent extraction of rendered material with organic solvents reached a high yield extraction (> 99%), the quality of the oil extracted is quite low [10]. The oil obtained after conventional solvent extraction from fish meal is frequently dark and polymerized and it is not suitable for refining for human consumption [11]. SFE has been also investigated in the literature as a promising technique to reduce some types of pollutants in fish oil during extraction, such as dioxins and dioxins-like PCBs (polychlorinated biphenyls) and toxic elements such as lead, cadmium, arsenic and mercury [12].

The aim of this work is the study of the influence of some extraction parameters such as pressure (10 – 40 MPa) and temperature (25 – 80 ºC) on the extraction of the residual oil from rendered fish meal coming from the last step of the rendering process. A

general equation correlating previous fish oil solubility data similar to the one obtained by Del Valle et al [13] for vegetable oils in high-pressure CO_2 has been also obtained. This way, the slope of the first part of the extraction curves is compared with the expected oil solubility data at the operating conditions. The Sovova's mathematical model [14] was used to describe the extraction kinetics. The remained fish meal has been characterized by determining the total protein content and toxic elements as well as its colour. Characterization of fishmeal extracts obtained by pressurized-CO_2 has been also performed in terms of their fatty acid group composition and other parameters such as colour.

2. Experimental section

2.1. Raw material

The raw material used in this work was fish meal kindly donated by SARVAL Bio-Industries Noroeste, S.A.U. The composition of the fish meal was 7.2 ± 0.2 % fat content determined by Sohxlet extraction with n-hexane [15]. The moisture content, 5.2 ± 0.3 %, was determined by drying in an oven at 105 ºC for 16 h up to constant weight. Crude protein content was determined with the Kjeldahl method and multiplying the nitrogen content by 6.25 [16], being 65.4 ± 0.7 %. Total ash content, 22.2 ± 0.3 %, was determined according to AOCS (ignition at 600 ºC for 2 h) [17]. The particle size distribution of fish meal was determined by using a vibratory sieve shaker (CISA, model RP.09) resulting that nearly 85 ± 5 % of the fish meal particles have a particle size between 0.15 and 1 mm (Table 1). Fish meal was used for pressurized CO_2 extraction without sieving.

2.2. Supercritical fluid extraction equipment and procedure

The extraction experiments were carried out in a semi-batch laboratory SFE-equipment whose P&I diagram has been previously describe [18]. In a SFE experiment, around 14 grams of fish meal were loaded in the extractor (40 mL capacity). Two syringe pumps (ISCO 260 DM), that work alternatively, provide an uninterrupted flow of CO_2 (Carburos metálicos, liquid $CO_2 \geq 99.9$ %) compressed up to the desired operating pressure. The pressurized solvent was pre-heated up to the desired extraction temperature before entering the extractor. The extractor was located in an oven whose temperature is controlled within an accuracy of ± 0.5 ºC. The carbon dioxide flow was set to 9.5 ± 0.5 g/min. Depressurized CO_2 was quantified with a totalizer flow meter. Extraction yield was determined gravimetrically by measuring the extract weight at different time intervals.

A total of ten experiments were carried out under different extraction conditions (Table 2). Runs 1 to 7 were performed to evaluate the influence of extraction pressure and temperature on the extraction yield under supercritical conditions and runs 8 to 10 were carried out with liquid CO_2 at 25 ºC and different operating pressures.

2.3. Analytical methods

2.3.1. Determination of fatty acid group composition

The fatty acid profile was determined by the AOAC method [19]. The fatty acid methyl esters were firstly prepared and then analyzed by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization detector (FID). The separation was carried out with helium (1.8 mL/min) as carrier gas. A fused silica capillary column

(OmegawaxTM-320, 30m×0.32mm i.d.) was used. The column temperature was programmed starting at a constant temperature of 180 ºC during 20 min, heated to 200 ºC at 1 ºC/min, held at 200 ºC during 1 min, heated again to 220 ºC at 5 ºC/min and finally held at 220 ºC for 20 min. A split injector (50:1) at 250 ºC was used. The FID was also heated to 250 ºC. Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co.). Their quantification was made by relating the peaks area to the area of an internal standard (methyl tricosanoate) as indicated by the AOAC method [19]. Calibration curves were made for several pairs formed by the internal standard + several representative chromatographic standards in order to find the corresponding response factors.

2.3.2. Colour determination

Colour (CIELab parameters) was evaluated for fish meal before and after extraction, as well as for some of the oils extracted. CIELab parameters were calculated automatically by a suitable programme installed in a Konica Minolta CM-2600d/2500d spectrophotometer using the illuminant D65 (daylight source) and a 10º standard observer (perception of a human observer) following the CIE recommendations. L*, a* and b* values describe lightness, redness-greenness, and yellowness-blueness. Changes in colour of the fish meal before and after extraction were expressed as [20]:

$$\Delta E = \sqrt{\left(L^*_{original} - L^*_{after\ treatment}\right)^2 + \left(a^*_{original} - a^*_{after\ treatment}\right)^2 + \left(b^*_{original} - b^*_{after\ treatment}\right)^2} \quad (1)$$

2.3.2 Toxic trace element determination

Toxic trace elements, arsenic, cadmium, mercury and lead were analyzed using inductively coupled plasma mass spectrometry (ICP-MS-Agilent 7500cx). All samples

were added to a HNO_3 solution (2 wt%) and digested by using a microwave system, along with internal standards in the temperature range from room temperature to 170 ºC for 43 min. The digested samples were cooled and dilute with deionized water. Concentrations of the toxic elements were determined using standard solutions prepared in the same acid matrix from 0 to 40 ppb for Pb, Cd and As and from 0 to 20 ppb for Hg.

3. Results and discussion

3.1. Influence of pressure and temperature on the extraction yield

The effect of extraction pressure on the extraction yield was evaluated under supercritical conditions from 20.0 MPa to 39.5 MPa at a constant temperature of 40 ºC (runs 1-3). Runs 8 to 10 have been carried out with LCO_2 at 25ºC and pressure has been varied from 10.0 to 30.0 MPa. The results are shown in Figures 1a and 1b respectively. All the extraction curves consist of one straight section forming the equilibrium part, followed by a curved line whose shape is controlled by internal diffusion. It can be also observed that most of the extraction is performed in the first part of the extraction. The extraction curves indicate that, at a constant temperature, the higher the pressure the higher the extraction rate, what may be attributed to the higher density of CO_2 which leads to higher solvent power. An increase of oil solubility when extraction pressure is increased has been also reported for similar extractions of fat from other rendered materials such as poultry meal [10]. As it will be explained in section 3.2 the first part of the extraction is controlled by this thermodynamic parameter and it can be fitted to a straight line. From Figures 1a and 1b it can be also observed that extraction yields show a significant dependence on extraction pressure (ranging from 64 to 91 % at 20.0 and

39.5 MPa respectively under SC conditions and from 63 to 83 % at 10.0 and 30.0 MPa with LCO_2), due probably to the lower solvation power of the CO_2 the lower its density. However in the extraction of fat from poultry meal, Orellana et al. [10] observed no significant dependence on extraction yield with pressure and temperature (6.9 – 34.5 MPa).

The temperature has been changed in the range from 40 ºC to 80 ºC at constant operating pressure, 30.0 MPa. The results are shown in Figure 2. Under constant operating pressure, an increase in temperature leads to a decrease in CO_2 density while the solute volatility increases. For many supercritical fluids extractions a retrograde solubility phenomenon below a "cross-over point" has been described. That means that below the "cross-over" point the lower density of CO_2 at higher operating temperature is not compensate by an increase in the solute volatility. From Figure 2 it can be observed that the slope of the initial part of the extraction curves at the five temperatures studied, corresponding to the fish oil solubility (see section 3.2), are close to each other. That means that the decrease of solvating power of CO_2 due to lower density by increasing operating temperature is of the same order as the increases in the vapor pressure of the solute with temperature. In the literature it has been reported a "cross over" pressure of approximately 35.0 MPa for the solubility of oils and fats [21]. Based on extraction curves of fat from poultry meal, Orellana et al. [10] expected a "cross-over" around 40.0 MPa. This value is also of the same order as the crossover pressure usually observed in vegetable oils [22,23]. This fact can be also observed in Figures 3a and Figure 3b where extraction curves under LCO_2 and SC-CO_2 are compared at operating pressures of 20.0 and 30.0 MPa, respectively. Figure 3a shows that LCO_2 can be advantageous when working at low operating pressure, since high

solubility of fish oil is obtained at low temperature, due to the mentioned retrograde solubility phenomenon, which is beneficial for capital and operating costs [10]. This fact could be also interesting, since in the literature it has been reported that fish protein concentrates produced at low temperatures (20ºC) have better emulsifying properties than at high temperatures (50ºC) [4]. At higher operating pressure, 30 MPa (Figure 3b), the slope of the first part of the extraction curves becomes closer at the two operating temperatures (40 and 25 ºC).

3.2. Modelling of the supercritical fluid extraction

In this work, the model proposed by Sovová [14] was used to describe the experimental extraction curves. This type of model assumes that the solute is regarded as a single pseudo compound which can lead to some errors since several components are generally involved in the extraction of the fish oil. In the model of Sovová the extraction yield is expressed as:

$$e = \frac{E}{N_m} \tag{2}$$

where E is the amount of extract (kg) and N_m the charge of insoluble solid (kg) in the extractor. The dimensionless amount of solvent consumed is obtained by:

$$q = \frac{Q\,t}{N_m} \tag{3}$$

where Q is the solvent flow rate (kg/h) and t the extraction time (h). Based on this model, the extraction curves consist of two parts, a straight section followed by a curved line. During the first one, the easily accessible solute from broken cells is transferred directly to the fluid phase, while in the second one the solute from intact cells diffuses first to broken cells and then to the fluid phase.

Orellana et al. [10] established that in the fat extraction of poultry meal the first part of the extraction is governed by the solubility equilibrium, corresponding to the slope of the extraction curve. For vegetable oil extraction, Sovová [14] found that extraction curves are initially linear with a slope close to the value of oil solubility in CO_2. However, Rubio Rodriguez et al. [8] observed different initial slopes in the extraction curves for different fish by-products which were attributed to the different internal structures, that affects the internal mass transfer and to the different solubility of the fish oil in SC-CO_2 due to the different lipid composition. In this work, the initial slope from the extraction curves was evaluated and compared with data of fish oil solubility in carbon dioxide. All the solubility data of fish oil in high pressure CO_2 found in the literature (Table 3) have been correlated with two equations: Chrastil equation [24] and a General Model proposed by del Valle et al. [13] to predict the solubility of vegetable oils in high-pressure CO_2. The Chrastil equation [24] is a log-log relationship between c_{sat} and the density of the pure SCF, rewritten by del Valle et al. [13] to express the solubility in weight-by-weight ($g \cdot kg^{-1}$, oil/SCF):

$$\log c_{sat} = \log c_{sat}^{o} + (k-1)\log\left(\frac{\rho}{\rho^{0}}\right) - \frac{\Delta H}{2.303R}\left(\frac{1}{T} - \frac{1}{T^{0}}\right) \quad (4)$$

where c_{sat}^{o} is the solubility of the oil at reference conditions of absolute temperature T_o and SCF density (ρ_o); k provides the amount of solvent molecules to form the so-called solvat-complex; ΔH is the total heat required to synthesize the solvato complex and R the universal gas constant. Recently, del Valle et al. [13] proposed a General Model to correlate vegetable solubility data obtained by introducing two empirical modifications to improve the fitting capabilities of Chrastil equation:

$$\log c_{sat} = \log c_{sat}^{o} + \left[\left(k^{o} - 1\right) + \alpha\left(\frac{\rho - \rho^{o}}{\rho^{o}} \right) + \beta\left(\frac{\rho - \rho^{o}}{\rho^{o}} \right)^{2} \right] \log\left(\frac{\rho}{\rho^{0}} \right) - \frac{\Delta H^{o}}{2.303R}\left[1 + \gamma\left(\frac{1}{T} - \frac{1}{T^{o}} \right) \right]\left(\frac{1}{T} - \frac{1}{T^{0}} \right) \quad (5)$$

where k^o is the association number at ρ^o, ΔH^o is the total heat required to synthesize the solvato complex at T^o, and α, β and γ are empirical parameters. The same reference condition (40ºC and 30 MPa) as the one adopted by del Valle et al. [13] has been considered in this work. The parameters for both models (equation 4 and 5) has been obtained for solubility data presented in Table 3 by means of the Levenberg-Marquardt method for nonlinear least squares curve-fitting [25] and are listed in Table 4. In the fitting procedure some solubility data were not included when they were identified as outliers when plotting the corresponding isotherm. Table 4 also summarizes some statistical parameters of the fitting such as the correlation coefficient (r^2) and the mean relative deviation for all experimental fish oil solubility data employed in the correlation procedure:

$$MRD = \frac{\sum_{all\ \exp.\text{solubility}\ data} abs\left(c_{sat}^{\exp} - c_{sat}^{calc}\right) / c_{sat}^{\exp}}{n_{\text{solubility}\ data}} \cdot 100 \quad (6)$$

where $c_{sat}^{\exp}$ is the experimental solubility data and c_{sat}^{calc} the solubility data calculated by the two models used in this work. From Table 4 it can be observed that the lowest relative deviation was found for the General Model proposed by del Valle et al. [13] (10.4 % vs 12 % for the Chrastil model), although it has double of model parameters. For the General Model the fish oil solubility data at the reference condition (40ºC and 30 MPa) has been found to be 8.18 $g \cdot kg^{-1}$. This value is very close to the value found by del Valle et al. [13] at the same reference condition when correlating vegetable oil

solubility data (8.07 $g \cdot kg^{-1}$). Lopes et al. [26] also showed that solubility of fish oils are of the same order of magnitude as the solubility of vegetable oils. Solubility data obtained at temperatures different from 40ºC were corrected by dividing the reported solubility data by the temperature-correction term (TCT) of the General Model in a similar way as del Valle et al. [13] in the correlation of vegetable oil solubility data:

$$TCT = \exp\left\{-4035\left[1-307\left(\frac{1}{T}-\frac{1}{313}\right)\right]\left(\frac{1}{T}-\frac{1}{313}\right)\right\} \quad (7)$$

The corrected (at 40 ºC) initial slope values obtained from the first part of the extraction curves have been plotted in Figure 4 as a function of pure CO_2 density together with the solubility data calculated by the General Model and all the experimental fish oil solubility data used in the fitting procedure. As it can be observed, the values of the slope of the first part of the extraction are of the same order of the solubility of fish oil in CO_2. Rubio-Rodríguez et al. [8] established that when the oil is strongly bounded to the protein matrix of the fish by-products, the internal mass transfer resistance is important and the initial slopes can be lower than the oil solubility values. Taking into account the values of the initial slope obtained in this work, fat content of the fish meal might be considered as extracellular oil or weakly bound to the protein matrix.

Based on these findings, equation (8) and equation (9) proposed by Sovová [14] were used to evaluate the first and second part of the extraction curve respectively:

$$e = q\, y_s \text{ , for } 0 \le q \le q_c \quad (8)$$

$$e = x_u\left[1 - C_1 \exp(C_2 q)\right] \text{ , for } q > q_c \quad (9)$$

C_1 and C_2 are adjusting constants, y_s is the experimental solubility datum, q_c the crossing point and x_u is the solute concentration in the untreated solid (kg solute/kg insoluble solid). The constants C_1 and C_2 of the model and the concentration in the

untreated solid, x_u, were estimated by nonlinear regression through Marquardt's algorithm in Statgraphics X64. The values of experimental and calculated extraction yields, e, were compared though the mean relative deviation:

$$MRD = \frac{1}{n}\left(\sum_n \left|\frac{e_{\exp} - e_{calc}}{e_{\exp}}\right|\right)\cdot 100 \quad (10)$$

The calculated extraction curves are plotted in Figures 1-3. From these Figures a good agreement can be observed between experimental data and model correlation. According to Sovová [14], the volumetric fraction of broken cells in the particles, called grinding efficiency, r, and the solid-phase mass transfer coefficient, $k_s a_s$, can be estimated from constants C_1, C_2 and co-ordinate q_c at the crossing point:

$$r = 1 - C_1 \exp\left(-C_2 q_c / 2\right) \quad (11)$$

$$k_s a_s = (1 - r)(1 - \varepsilon)\dot{Q} C_2 / N_m \quad (12)$$

In equation (11) solvent flow rate is expressed in $kg \cdot s^{-1}$. Fitting parameters along with the estimated values of the grinding efficiency and solid-phase mass transfer coefficients are presented in Table 5. The grinding efficiency estimated was quite high in all extractions and it can be concluded that the volumetric fraction of broken cells in the fish meal can be as high as 0.77. The crossing point, q_c, was found to increase with a decrease in the solubility value. The solid-phase mass transfer coefficient, $k_s a_s$, increases with operating pressure (R1-R3 and R8-R10) and decreases at high operating temperatures (R7)

3.3 Characterization of the extracts and fish meal

The lowest oil content remaining in the fish meal after CO_2 extraction was around 0.7 % reached at 39.5 MPa and 40 ºC. On the other hand, the highest oil content in the treated

fish meal was around 2.5 %, obtaining the same value both under supercritical conditions 20.0 MPa/40ºC and with LCO_2 (10.0 MPa/25ºC).

3.2.1 Fatty acid group composition of the extracts

Table 6 shows the fatty acid group composition of the lipids of the different extracts obtained from fish meal. The extracts obtained with SC-CO_2 and LCO_2 showed similar fatty acid profiles containing approximately 31.8-33.3 % of saturated fatty acids, 38.0-40.3 % of monounsaturated and 27.0-28.9 % of polyunsaturated. Table 6 also presents the fatty acid group composition of the lipid fraction obtained with n-hexane in a Soxhlet's extractor. Comparing the different fatty acid group composition, it can be observed that slightly higher percentage of PUFA (31.2 %) and slower percentage of SFA (31.6 %) are obtained with n-hexane than with CO_2. In the SC-CO_2 extraction of lipids from Brazilian redspotted shrimp waste [21] a similar trend in the fatty acid group was observed when comparing with petroleum ether Soxhlet's extract. These authors explained the difference in terms of the selectivity of SC-CO_2 to fractionate the oil.

3.2.2 Colour

L*, a* and b* values were measured for the oil extracted with SC-CO_2 and with LCO_2 (Table 7). SC-CO_2 and LCO_2 oil had values of the same order for yellowness (b*). However oil extracted by SC-CO_2 present lower lightness value (L*) and much higher redness values (a*) that could be also visually observed. The higher a* value of the lipid extract could be due to higher levels of pigments such as astaxanthin as it has been described in the literature [20]. Oil extracted by Soxhlet with n-hexane presented a darker colour probably due to partial degradation that causes a change in the colour [11]. According to this, CIELab presented much lower values for the yellowness (b*) and lower value for the lightness (L*) and the redeness (a*) than the oil extracted with

pressurized CO_2 (Table 7). Table 7 also shows CIELab parameters determined before and after extraction for the fish meal to show any effect of treatment on colour. Both, SC-CO_2 and LCO_2 treated fish meal had higher L* values, lower a* values and higher b* values compared to the untreated fish meal. Lighter colour of SC-CO_2 extracted samples has been attributed in the literature to the extraction of pigments with SC-CO_2 [27], according to the colour of SC-CO_2 extracted oil. Therefore, the colour change, ΔE, was slightly higher in the fish meal after SC-CO_2 extraction. This change in colour after extraction would be positive since a product with lighter colour could be desirable as a protein source in certain prepared foods [28].

3.2.3. Trace toxic elements

Fish is one of the food commodities where higher levels of arsenic, cadmium and mercury can be found. In this work, toxic elements, arsenic, cadmium, mercury and lead were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) in the fish meal before treatment and after SC-CO_2 and LCO_2 extraction (Table 8). Table 8 also lists the maximum level for these elements according to the directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed [29]. In any case, lower maximum levels in foodstuffs for human consumption can be found (for instance the maximum levels in fish meat for Pb, Cd and Hg are 0.3, 0.05-0.1, 0.5-1 ppm respectively). Concentration values for the elements are below the maximum levels determined by the directive 2002/32/EC for products intended for animal feed. Cadmium and mercury were not detected in the oil extracted and therefore they remained in the treated fishmeal. Most of the Cd found in fish is believed to be bound to protein, and therefore it is not easily co-extracted with the oil. Mercury content in fish meal is important since one possible source of human intake might arise from the

consumption of products from animals fed with fish meal containing methylmercury, being its most toxic form. Hajeb et al. [12] stated that mercury, cadmium, and lead are most likely attached to polar compounds, which are not extracted by SFE. However, the oil extracted contained 3.4-3.6 ppm of As. Among As derivatives, the water soluble form, arsenobetaine, is the the major form in fish and is widely assumed to be of no toxicological concern. Although considerable amounts of non-polar bound As compounds or arsenolipids have been also found [8]. Therefore, extraction of As in oil will strongly depend on the type of As species. Similar trends were found by Rubio-Rodríguez et al. [8] in the heavy metals analysis in marine oils obtained by SFE from different marine by-products at 25 MPa and 40 ºC. In any case, it must be highlighted that the amount of toxic elements co-extracted with the oil depends on the type of elemental species in the fish tissue [12].

Conclusions

High pressure carbon dioxide extraction has been studied as a procedure to reduce the fat content from rendered fish meal to obtain fish protein concentrates. Extraction experiments have been performed at different extraction pressure (10.0 – 39.5 MPa) and temperature (25 – 80 ºC). The initial oil content of fish meal was 7.2 ± 0.2 % and the lowest fat content in the fish meal was 0.7 % at 39.5 MPa and 40ºC.

The extraction curves obtained indicate that the extraction process may be controlled by the solubility of the oil in SC-CO_2 in the first stage of the extraction since initial slopes are close to values of fish oil solubility data when comparing with fish oil solubility data previously published.

Fish meal after SC-CO_2 extraction presents lighter colour than the original fish meal and the LCO_2 extracted meal. This fact agrees with the redness colour of the SC-CO_2 lipid extract as a consequence of extraction of pigments such as astaxanthin. Most of the toxic elements remained in the fish meal after SC-CO_2 or LCO_2 extraction, although important amounts of As have been found in the extracted oil.

Nomenclature

a_s = specific area between the regions of intact and broken cells (m^{-1})

C_1, C_2 = fitting parameters

e = extraction yield, (kg extract·kg insoluble $solid^{-1}$)

E = extract (kg)

k_s = solid-phase mass transfer coefficient (s^{-1})

n = number of experimental data

N_m= charge of insoluble solid (kg)

O.F. = objective function

Q = solvent flow rate ($kg \cdot h^{-1}$)

q = relative amount of the passed solvent (kg solvent·kg insoluble $solid^{-1}$)

q_c = relative amount of the passed solvent when all the solute in broken cells has been extracted (kg solvent·kg insoluble $solid^{-1}$)

r = grinding efficiency (fraction of broken cells)

t = extraction time (h)

x_u = concentration in the untreated solid (kg solute · kg solid $insoluble^{-1}$)

y_s = solubility (kg solute·kg $solvent^{-1}$)

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Table 1. Size distribution of fish meal.

Diameter range (mm)	wt%
< 0.15	5 ± 1
0.15 - 0.25	20 ± 2
0.25 – 0.5	38 ± 1
0.5 - 1	27 ± 2
1 - 2	7.7 ± 0.3
> 2	3.0 ± 0.3

Table 2. Experimental conditions in the extraction with LCO_2 and $SC\text{-}CO_2$ of fish meal

Run	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
p, MPa	20.0	30.0	39.5	30.0	30.0	30.0	30.0	10.0	20.0	30.0
T, ºC	40	40	40	50	60	70	80	25	25	25

Table 3. Oil solublity values considered in the correlation to equation (3) and (4).

Fish oil	T, ºC	p, MPa	c^{sat} ($g \cdot kg^{-1}$oil /CO_2)	Reference
Fish oil	40 - 80	20 - 35	0.6 – 12.7[a]	Ikawa et al. [30]
Sand eel	20 - 120	10 – 65	0.4 – 92.5	Staby et al. [31]
Cod liver	40 - 60	20 - 30	1.60 – 7.08	Catchpole et al. [32]
Ropufa 30 w-3 Food oil	28 - 50	7.8 – 29.4	0.52 – 7.1	Correa et al. [33]
Speckled	40-60	10 - 40	0.13 – 14.4	Lopes et al. [26]

(a) Graphical lecture.

Table 4. Parameters of Equation 3 and 4 in the correlation of fish oil solubility data

Model	c_{sat}^{o}	k^{o}	α	β	ΔH^{o} ($kJ \cdot mol^{-1}$)	γ	r^2	MRD (%)
Chrastil	7.86	11.194	--	--	88.69	--	0.9787	12.0
General Model	8.18	10.400	-8.863	-18.32	77.25	-306.8	0.9839	10.4

Table 5. Values of the C_1, C_2 parameters, x_u, q_c, estimated grinding efficiency r, solid-phase mass transfer coefficient, $k_s a_s$ and mean relative deviation.

Experiment	y_s	x_u	C_1	C_2	q_c	r	$k_s a_s$	MRD
R1	0.0017	0.0612	0.3835	0.0071	25.3	0.65	$1.2 \cdot 10^{-5}$	3.3
R2	0.0044	0.0560	0.3899	0.0562	8.3	0.69	$8.0 \cdot 10^{-5}$	1.1
R3	0.0070	0.0688	0.3254	0.0838	8.2	0.77	$8.8 \cdot 10^{-5}$	1.1
R4	0.0045	0.0571	0.4303	0.0625	8.6	0.67	$9.8 \cdot 10^{-5}$	1.7
R5	0.0048	0.0582	0.3937	0.0619	8.3	0.70	$8.7 \cdot 10^{-5}$	2.2
R6	0.0050	0.0603	0.3944	0.0631	8.3	0.70	$8.8 \cdot 10^{-5}$	3.6
R7	0.0054	0.0642	0.2626	0.0669	8.2	0.80	$6.1 \cdot 10^{-5}$	2.5
R8	0.0010	0.0508	0.7349	0.0477	48.4	0.77	$5.0 \cdot 10^{-5}$	9.8
R9	0.0026	0.0580	0.5475	0.0550	16.7	0.65	$8.9 \cdot 10^{-5}$	1.8
R10	0.0045	0.0637	0.8304	0.8716	8.8	0.44	$2.2 \cdot 10^{-4}$	3.0

Table 6. Fatty acid composition of n-hexane Soxhlet extract and SC-CO_2 and LCO_2 extracts from fish meal.

Fatty acid	n-hexane	40ºC 30 MPa	50ºC 30 MPa	60ºC 30 MPa	70ºC 30 MPa	80ºC 30 MPa	40ºC 20 MPa	40ºC 40 MPa	25ºC 10 MPa	25ºC 20 MPa	25ºC 30 MPa
SFA	31.6 ± 0.7	33.2 ± 0.6	33.1 ± 0.6	33.2 ± 0.7	33.3 ± 0.6	31.8 ± 0.7	33.1 ± 0.6	33.0 ± 0.5	32.9 ± 0.7	32.4 ± 0.4	32.3 ± 0.6
MUFA	37.2 ± 0.8	38.0 ± 0.8	39.8 ± 0.5	39.2 ± 0.4	39.4 ± 0.5	40.3 ± 0.5	39.9 ± 0.7	38.8 ± 0.7	38.9 ± 0.5	38.9 ± 0.6	38.8 ± 0.5
PUFA	31.2 ± 0.6	28.8 ± 0.8	27.1 ± 0.4	28.0 ± 0.5	27.2 ± 0.4	27.9 ± 0.3	27.0 ± 0.7	28.2 ± 0.4	28.2 ± 0.8	28.7 ± 0.8	28.9 ± 0.9

Table 7. Effect of extraction on the fish oil and residual meal colour ($SC\text{-}CO_2$ conditions: 30 MPa and 40 ºC, L-CO2: 20 MPa and 25 ºC)

Sample	Colour parameters			ΔE
	Lightness (L*)	Redness (a*)	Yellowness (b*)	
Fish oil				
$SC\text{-}CO_2$	19 ± 1	15 ± 1	13.1 ± 0.7	--
LCO_2	30 ± 1	2.1 ± 0.5	16 ± 1	--
Soxhlet (hexane)	16 ± 2	0.7 ± 0.4	1.0 ± 0.3	--
Fish meal				
Untreated	34.5 ± 0.5	5.2 ± 0.2	14.2 ± 0.6	
After $SC\text{-}CO_2$	42.7 ± 0.7	3.7 ± 0.1	16.8 ± 0.5	9 ± 2
After LCO_2	42 ± 1	4.01 ± 0.04	16.4 ± 0.7	8 ± 2

Table 8. Effect of SC-CO_2 and LCO_2 extraction on the content of toxic element in fish meal (SC-CO_2 conditions: 30 MPa and 40 ºC, L-CO2: 20 MPa and 25 ºC).

Toxic element		As	Cd	Hg	Pb
Concentration in fish meal, ppm		7.8 ± 0.2	1.6 ± 0.1	0.29 ± 0.01	0.34 ± 0.06
Limit by 2002/32/EC (ppm)*		10	2	0.5	5-10
Concentration in fish meal after extraction and in the oil extracted					
After SC-CO_2	Fish meal	8.2 ± 0.4	1.7 ± 0.1	0.31 ± 0.03	0.32 ± 0.02
	Oil	3.4 ± 0.2	0.004 ± 0.001	n.d.	0.10 ± 0.03
After L-CO_2	Fish meal	8.1 ± 0.2	1.7 ± 0.1	0.30 ± 0.01	0.31 ± 0.03
	Oil	3.6 ± 0.3	n.d.	n.d.	0.12 ± 0.03

(*) Relative to a feedingstuff with a moisture content of 12 %

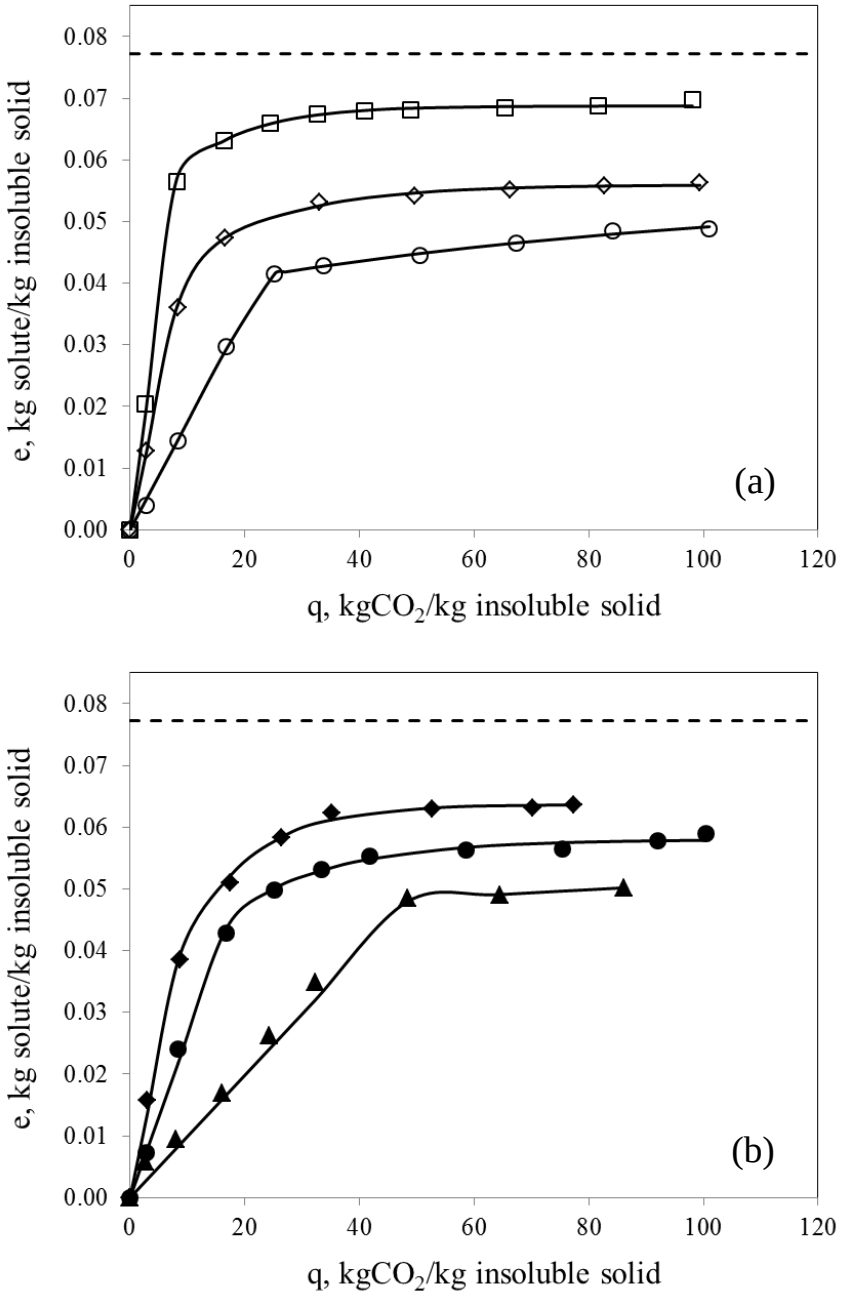

Figure 1. Influence of extraction pressure on fish oil yield from fish meal (a) SC conditions at 40ºC (○ 20.0 MPa; ◇ 30.0 MPa; □ 39.5 MPa) (b) LCO_2 at 25ºC ▲ 10.0 MPa; ● 20.0 MPa; ◆ 30.0 MPa. The solid lines correspond to the model of Sovová [14]. The discontinuous line represents the amount of oil in fish meal as obtained by Soxhlet hexane extraction.

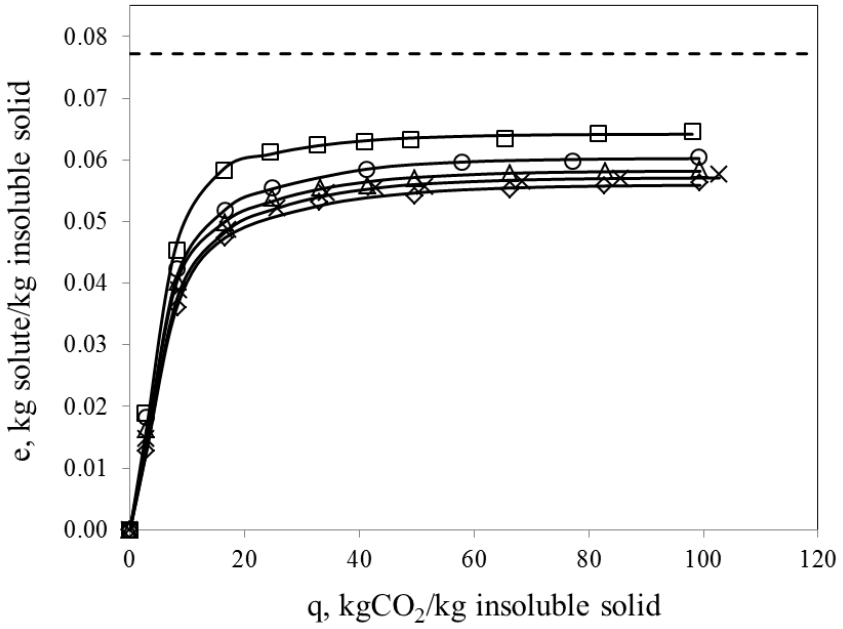

Figure 2. Influence of extraction temperature on fish oil yield from fish meal at constant extraction pressure of 30.0 MPa (□ 80ºC; ○ 70ºC; △ 60ºC; × 50ºC; ◇ 40ºC). The solid lines correspond to the model of Sovová [14]. The discontinuous line represents the amount of oil in fish meal as obtained by Soxhlet hexane extraction.

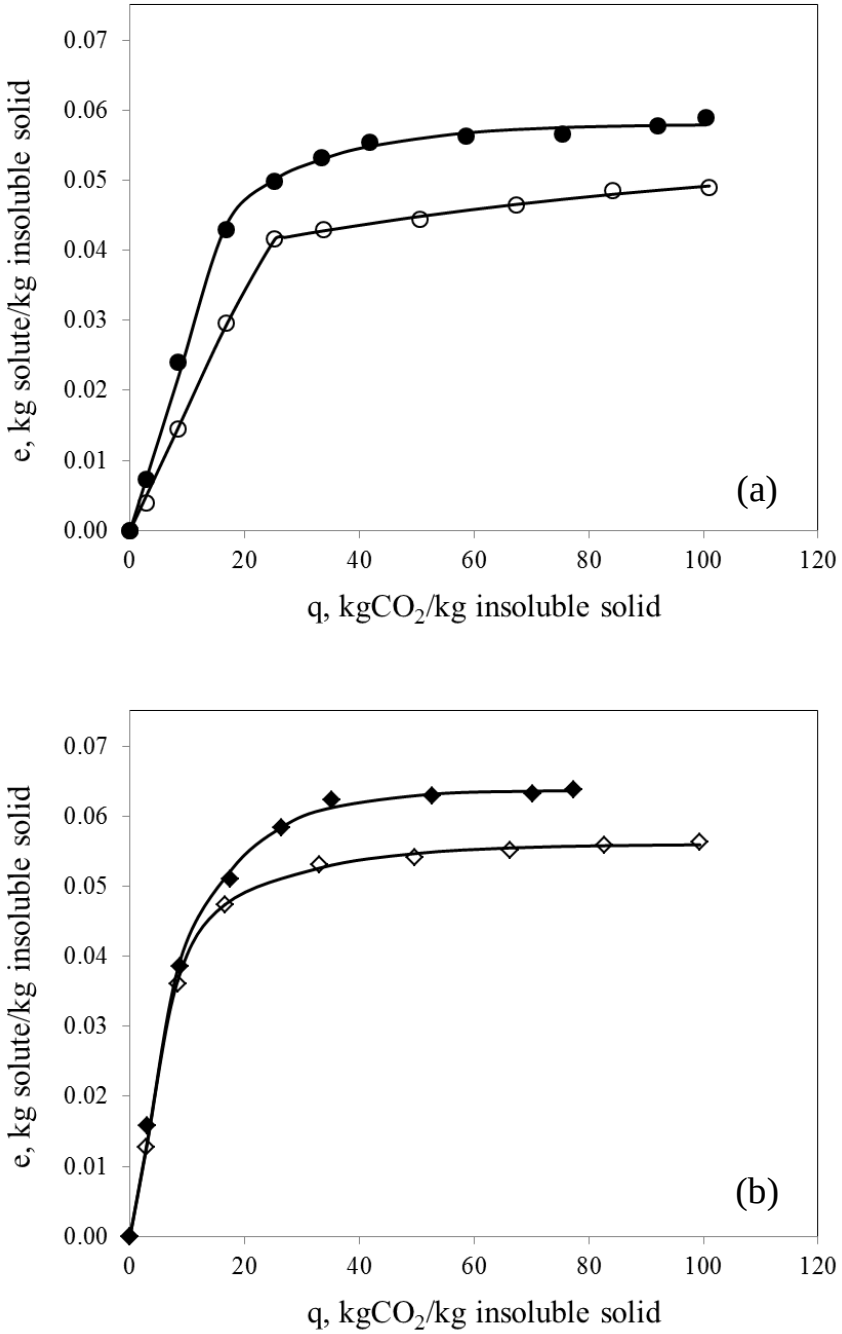

Figure 3. Influence of pressurized CO_2 state on fish oil extraction yield from fish meal (a) 20.0 MPa (○ 40ºC, ● 25ºC) (b) 30.0 MPa (◇ 40ºC, ◆ 25ºC). The solid lines correspond to the model of Sovová [14].

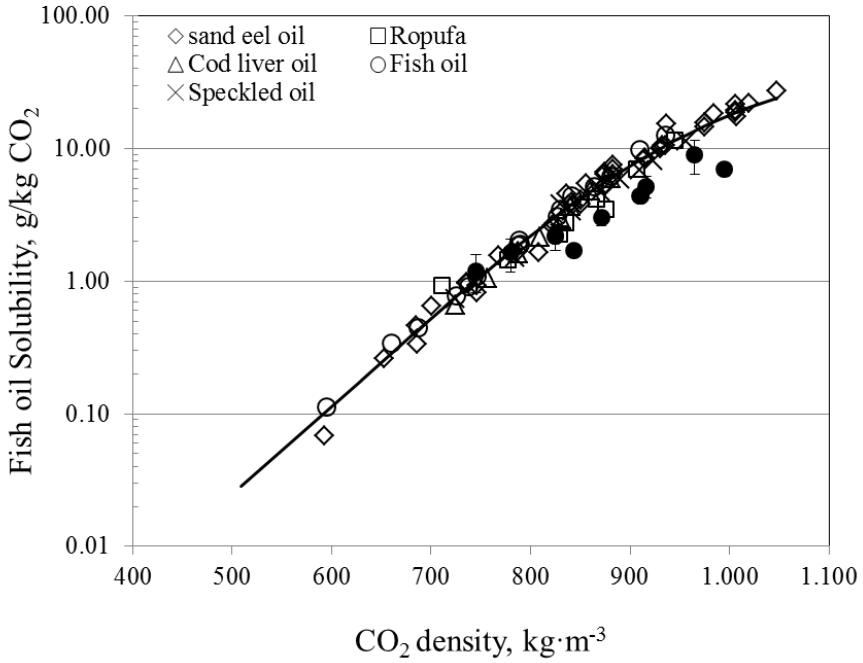

Figure 4. Corrected (at 40ºC) experimental solubility values of fish oil as function of pure CO_2 density. (● experimental data points of oil extraction from fish meal); (—) prediction of del Valle et al. [13] General Model.