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Purification of structured lipids using $SCCO_2$ and membrane process

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Abstract

The aim of this study was to evaluate the combination of supercritical carbon dioxide ($SCCO_2$) and membrane separation technologies in the purification of structured lipids (SLs) previously obtained. The SLs (modified triacylglycerols) were obtained from enzymatic interesterification between medium-chain triacylglycerols (MCT) and ethyl esters of long-chain fatty acids (EtELCFA). This process generates by-products that must be removed from the reaction medium to obtain pure SLs. The purification process was carried out by extraction of the by-products from the reaction medium with supercritical CO_2 and further selective retention of SLs using membranes integrated within the extractor. Two nanofiltration membranes (Osmonics DL4040-C and HL4040-F) and one reverse osmosis membrane (DOW Chemical BW30-4040) were evaluated. Pressures of 9, 11 and 13 MPa were used in the retentate side, and transmembrane pressures (TMP) in the range 0.7–4 MPa were applied. All experiments were conducted at 40 °C. The effect of these pressures on selectivity during the reaction's by-product extraction and retention of SLs by the membrane were analyzed. Results showed that pressures on the retentate side and the TMP are very important parameters in the solubilization and permeation of triacylglycerols, fatty acids and ethyl esters of fatty acids. Retention of 100% of SLs and maximum permeation of reaction by-products were reached at pressure of 9 MPa and TMP of 0.7 MPa, using the BW30-4040 reverse osmosis membrane.

Keywords: Structured lipids; Extraction; $SCCO_2$; Polymeric membrane

1. Introduction

Structured lipids (SLs) are defined as chemically or enzymatically modified triacylglycerols. This modification results in the alteration of their fatty acid composition and/or positional distribution in the glycerol molecule [1]. The benefits of the ingestion of specific SLs are related to their composition and an adequate balance between medium-chain (MCFA) and long-chain (LCFA) fatty acids. Among them are omega-6, omega-3 and omega-9 fatty acids, which may be used to reduce seric levels of low density lipoprotein (LDL) and triacylglycerols, to prevent thrombosis and to improve immunological functions [2].

Acidolysis of triacylglycerols or transesterification between triacylglycerol and esters of fatty acids to obtain SLs produces a reaction mixture composed of triacylglycerols, containing the desired SLs, fatty acids, and/or esters of fatty acids. Structured lipids should be processed under mild conditions of temperature, not only due to the presence of essential fatty acids that may otherwise oxidize, but also to avoid acyl migration [3].

The use of supercritical fluids (SCF) to process oils and fats has been proposed as an alternative to the extraction process and purification of lipid matrices involving organic solvents. The extraction and purification of lipid compounds, such as triacylglycerols, free fatty acids, tocopherols, carotenoids and sterols using $SCCO_2$ has been investigated by several authors [4–10]. Through these studies, the advantages of using SCF for the extraction of non polar compounds of low molecular weight are well known, as compared to the conventional solvent extraction processes, have become know. The main advantages are: the low viscosity and high diffusivity of supercritical fluids; the low process temperature, which assures protection against oxidation of thermolabile compounds; the fact that their solvation properties may be modified by adjusting pressure and

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temperature; that they may be easily removed from the extracted product by decompression and, in the case of $SCCO_2$, that it is considered environmentally safe, not generating chemical residues [5]. Carbon dioxide is the SCF most-used as a solvent for food applications. This is not only due to its low cost and availability at high purity, but also because of its ease of use and safety regarding its manipulation and removal by depressurization [11].

In the last few years there has been an increasing interest in the application of membranes to process lipids. The use of membranes is promising when one considers the reduction in energy consumption and residual water, absence of chemical products, and easy scale-up procedures [12]. Most of the recent research that uses membrane technologies to process lipids has focused on solvent-recovery of micelle, degumming, bleaching, neutralization, hydrolysis of oils and fats, synthesis of structured lipids in membrane reactors, and deacidification [12–13].

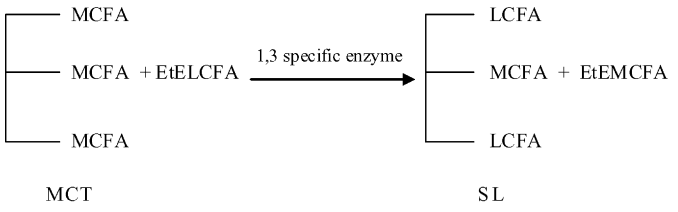
The combination of supercritical fluids extraction and separation by membranes allows the integration of the extraction reaction by the $SCCO_2$ (improved by the high solvent power of $SCCO_2$) and the selective separation by the membrane (through the $SCCO_2$ and extracted solutes filtration–reaction mixture). The combination of extraction with $SCCO_2$ and membrane separation allows the obtention of extracted fractions with a narrow range of molecular weights [14]. This combination has been investigated for the fractionation of triacylglycerols, for the extraction and purification of carotenoids, and for the separation of essential oils [14–17]. After extraction with SCF, costs for recompression of gas to the liquid phase or to the supercritical phase are high, and the combination with the membrane separation process allows the retention of the solutes extracted, and permeation of the SCF with a low pressure drop, leading to a reduction in energy and costs during recompression [14,16].

The objective of this study was to evaluate a low temperature method of purifying structured lipids by combining extraction using $SCCO_2$ with the membrane separation process. Two processes were evaluated simultaneously: (a) the extraction by $SCCO_2$ of undesirable by-products (free fatty acids and/or esters from fatty acids) resulting from the interesterification reaction and (b) the retention of SLs by polymeric membranes which are integrated within the extractor. Different values of transmembrane pressure and CO_2 pressure were evaluated to find the most efficient removal of by-products and the best retention of SLs.

2. Material and methods

2.1. SL production

The SLs used with the selected membrane were obtained by enzymatic interesterification between medium-chain triacylglycerol (MCT) (Trigliceril CM–Support) and ethyl esters from long-chain fatty acids (EtELCFA) of fish oil, with 36% of omega-3 fatty acids, following conditions described by Moura et al. [18]. Eq. (1) describes the interesterification reaction between MCT and EtELCFA in the presence of sn-1,3 specific enzyme:



(1)

where MCFA-medium chain fatty acids, LCFA-long chain fatty acids, EtEMCFA—ethyl esters medium chain fatty acids, EtELCFA–ethyl esters long chain fatty acids. The amount and kind of by-products released from the interesterification reaction depends on the level of substitution attained and on the substrate molar ratio used. For the SLs' purification, the by-products must be removed from the reaction medium. Experiments were carried out in an interesterification jacketed reactor (2 L capacity), under vacuum, with temperature of 60 °C, agitation of 1.66 Hz (100 rpm), 5% (weight/substrate) of the enzyme Lipozyme IM TL (Novozymes) and substrate molar ratio of 1:4 (MCT/ EtELCFA). In each run, 500 g of substrates were used. The MCT and EtELCFA showed a molecular weight averages of 494 and 313 g/mol, respectively.

2.2. Separation and composition of fatty acids from the SLs

Separation of SLs was carried out by thin layer chromatography, using preparative silica plates (0.05 mm) and a mobile phase of hexane/ethyl ether/acetic acid (80:20:1, v/v/v) [19]. After elution of the reaction mixture components, triacylglycerols were removed from the chromatographic plate and evaluated as to their fatty acid composition, following the AOCS Ce 1–62 method [20]. Analytical conditions: chromatograph: CGC Agilent 6850 GC System; column: DB-23, Agilent (50% Cyanopropyl–methylpolysiloxane), 60 m × 0.25 mm × 0.20 μm; oven temperature: 195 °C for 20 min; 195–215 °C at 5 °C min^{-1}; 215 °C for 16 min; carrier gas: He; column gas flow: 1.0 mL min^{-1}; injector temperature: 250 °C; detector temperature: 280 °C; split ratio: 1:50. Nu-chek GLC-87 Standard was used to identify fatty acid methyl esters (FAMEs).

2.3. Membrane properties

One reverse osmosis and two nanofiltration membranes were evaluated in terms of their capacity for triacylglycerol retention and permeation of free fatty acids and/or ethyl esters in supercritical media. Membrane properties are given in Table 1.

2.3.1. Conditioning of the membranes

The membrane pretreatment or conditioning consists of its immersion in an organic solvent in order to wash out the additives and humectants used in the manufacturing process from the surface and the pores [21,22]. The method of membrane conditioning also plays an important role in the solvent flux,

Table 1
Characteristic properties of membranes used in the experiments

Membrane	Type	Composition	Manufacturer	Rejection[a]
DL4040-C	Nanofiltration	Polyamide	Osmonics	$MgSO_4$-96%
HL4040-F	Nanofiltration	Polyamide	Osmonics	$MgSO_4$-98%
BW30-4040	Reverse osmosis	Polyamide	Filmtec (Dow)	NaCl-99.5%

[a] Information provided by the manufacturer.

the membrane integrity and the pressure rating of polymeric membranes [23].

The HL4040-F and DL4040-C nanofiltration membranes were tested without any kind of conditioning and showed a good permeation of $SCCO_2$ and lipidic fraction. However, the BW30-4040 reverse osmosis membrane did not permeate the $SCCO_2$ and lipidic fraction. Thus, the BW30-4040 reverse osmosis membrane was conditioned by immersion in ethanol for 4 h, and further immersion in hexane for the same amount of time. The relative polarities of these solvents are 0.65 and 0.01, respectively [24]. This treatment was carried out in order to gradually adapt the membrane to the hydrophobic conditions of the $SCCO_2$ and lipidic fraction.

2.4. Pilot plant unit for membrane-integrated supercritical extraction

SL purification and concentration experiments were carried out in a pilot plant unit with an integrated membrane, as schematized in Fig. 1.

Two filtration cells, #6 and #7, are shown in the scheme. The cells are jacketed and made of stainless steel, each one with a capacity of 30 cm^3, 2.3 cm internal diameter, placed in series. For the experiments reported here, only cell #7 was used. Filtration area of the membranes was 3.14 cm^2, and a dead-end flow regime was used (flow through a membrane module in which the only outlet for upstream fluid is through the membrane), therefore, there is no recirculation of the retentate. In this specific case, the "stage cut" (permeate flow/feed flow) was considered to be equal to 1. However, in no experiment was the final ratio of permeate mass/retentate mass greater than 0.29. Cell temperature was kept at 40 °C by a thermostatic bath (#11). TMP was monitored by a pressure transducer (#5) (Model RTP12/BE53R, AEP, Italy), and controlled by a pneumatic valve (#4) (Model 807, Badger Meter, USA). Operating pressure in the buffer tank (#3) and cells was maintained by the booster (#2) (Model DLE 15-1, Maxpro, Germany). Buffer tank and filtration cell temperatures were set to be equal.

2.5. Experimental procedures

To start the experiments, valves #14 and #17 were initially closed (to isolate cell #6) and #15 and #16 were open. This procedure was necessary to equalize the pressure on the two

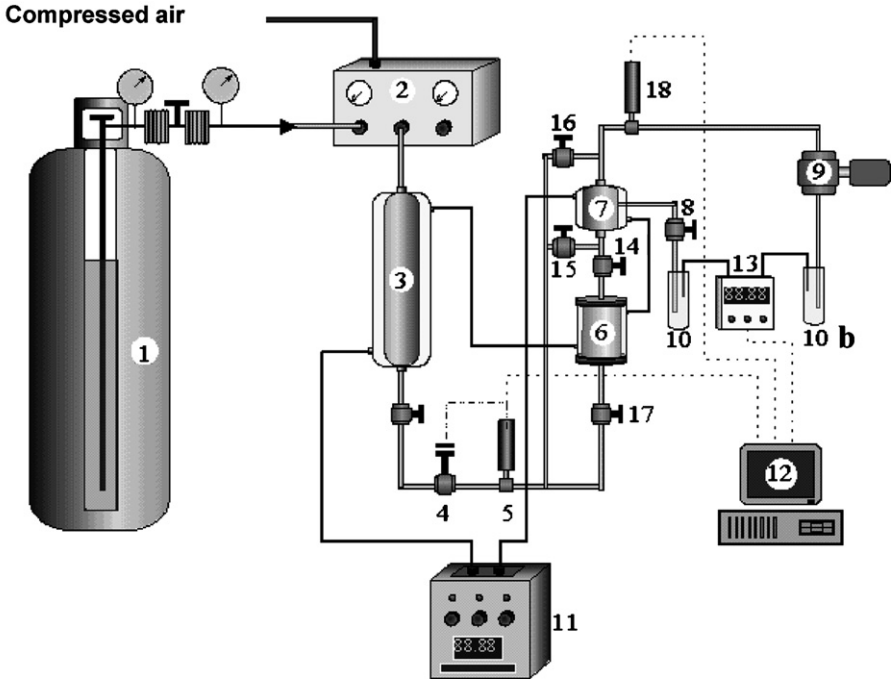


Fig. 1. Scheme of the pilot plant SCF extraction unit with integrated membrane. (1) CO_2 cylinder, (2) booster, (3) buffer tank, (4) pneumatic valve, (5) and (18) pressure transducers, (6) and (7) filtration cells, (8) micrometric valve, (9) back-pressure valve, (10 and 10b) samplers, (11) thermostatic bath, (12) microcomputer, (13) flowmeter, (14–17) manual valves.

sides of the membrane. Then, valve #16 was closed and the TMP controlled by the back-pressure valve #9. TMP was monitored by the pressure transducer #18. All experiments were carried out with pressures above atmospheric on the permeate side. $SCCO_2$ flow was measured by a bubble flowmeter (#13). In all runs, 10 g of the lipidic fraction (triacylglycerols, free fatty acids and/or ethyl esters) were used.

Initially, trial runs were carried out in order to select the membrane with the best performance for triacylglycerol (TG) retention and permeability to free fatty acids (FFA). A lipidic fraction with a substrate molar ratio of 1:4 (TG/FFA) was used. The TMP applied was 1 MPa for the DL4040-C membrane, and 1.5 MPa for the BW30-4040 and HL4040-F membranes. The permeate flux was sampled after 1 h of filtration. Operating pressure was kept at 13 MPa. These preliminary essays were carried out only to select the membrane with regard to their capacity for triacylglycerol retention. Evaluations of $SCCO_2$ and lipid fluxes were not carried out.

The membrane with the greatest retention of triacylglycerols was used in the experiments, with the lipidic fraction obtained from the enzymatic SLs' obtention, using MCT and EtELCFA. The selected membrane was then evaluated for $SCCO_2$ and lipid fluxes, in order to verify its retention of triacylglycerols and permeation of ethyl esters. Runs were carried out with pressures ranging from 9 to 13 MPa on the retentate side and TMP ranging from 0.7 to 4.0 MPa. TMP was adjusted by keeping the retentate-side pressure constant, and reducing the pressure on the permeate side. Lipids permeate fluxes were determined by accumulated sampling in separator #10b, as a function of time. These measurements were carried out until a constant flux was achieved for each TMP applied. All runs were carried out at 40 °C. Under these conditions, even when setting TMP to 4 MPa, the CO_2 was within its critical temperature and pressure range (critical pressure = 7.38 MPa; critical temperature = 31.06 °C) on both sides of the membrane.

2.6. Retention of triacylglycerols

The percentage of triacylglycerols retention by the membrane (*%R*) was calculated according to Eq. (2):

$$\%R = \left[1 - \left(\frac{C_{tp}}{C_{tr}}\right)\right] \times 100 \quad (2)$$

where C_{tp} is the concentration of triacylglycerols in the permeate and C_{tr} is the concentration of triacylglycerols in the retentate. Since the initial mass of the cell lipid fraction was known, along with its concentration of triacylglycerols, the value of C_{tr} was calculated through the mass balance, subtracting the mass of triacylglycerols present in the permeate at each time interval. This procedure was necessary because the determination of the triacylglycerol concentration in the retentate only, would not take into account a possible fraction of these compounds adsorbed and/or accumulated on the membrane surface, because was used dead-end filtration, without agitation.

2.7. Chemical analysis of permeates

The chemical composition of the permeate was determined by High Performance Size Exclusion Chromatography (HPSEC) using the following conditions: two columns connected in series, Hewlett Packard–DVB (Polydivinylbenzene), with pore diameter of 100 and 500 Å; refraction index detector (Differential Refractometer LCD 201–SICON, ANALYTIC); isocratic pump (Perkin-Elmer-Liquid Chromatograph); 20 μL injector, and mobile phase (Tetrahydrofuran), flow of 1 mL min^{-1} [25].

3. Results and discussion

3.1. Preliminary tests to select the membrane

Table 2 shows the triacylglycerol retention for the three membranes tested, using $SCCO_2$ and a lipidic fraction composed of triacylglycerols and free fatty acids. The lipidic fraction was composed of 68% triacylglycerols, 15% medium chain triacylglycerols and 16.8% long chain fatty acids.

The nanofiltration membranes HL4040-F and DL4040-C were submitted to trial runs without any conditioning treatment, and showed good permeability to the reaction mixture composed of $SCCO_2$ and the lipidic fraction. However, the BW-30-4040 membrane, showed a total resistance to the permeation of the reaction mixture. This resistance could be caused by its dense layer, characteristic of reverse osmosis membranes, and/or manufacturing conditions. Although made of the same material as the other membranes (polyamide), the BW-30-4040 membrane came from a different manufacturer. The low affinity may be related to the possible presence of polar compounds on the membrane surface. For example, the glycerol used by manufacturers to maintain membrane hydration, would alter the membrane polarity. For this reasons for the reverse osmosis membrane a pretreatment needs to be applied before initiating system start-up procedures. Thus, in this study, a membrane pretreatment was carried out to gradually adapt the membrane to the hydrophobic conditions of the $SCCO_2$ and lipidic fraction. The reaction mixture did not permeate through the BW-30-4040 membrane without conditioning. Thus, only this membrane was subject to the conditioning treatment.

According to Table 2, the BW-30-4040 membrane showed the greatest retention of triacylglycerols and, therefore, was selected to proceed with the experiments.

In Table 3, it can be observed that the BW30-4040 membrane showed preferential permeation of the fatty acids present in the reaction medium in comparison to the triacylglycerols, and

Table 2
Triacylglycerol retention by the three tested membranes

Membrane	TMP (MPa)	Triacylglycerol retention (%)[a]
DL4040-C	1.0	75
HL4040-F	1.5	80
BW30-4040	1.5	91

Pressure on the retentate side was 13 MPa and temperature 40 °C.
[a] Sample taken after 1 h of filtration.

Table 3
Composition of lipidic fraction before and after filtration with a BW30-4040 membrane

Compounds	Composition (%)	
	Before filtration	After filtration (lipidic permeate)[a]
Triacylglycerols	68.0	4.00
MCFA	15.0	74.0
LCFA	16.8	21.2

[a] Sample taken after 1 h of filtration.

also a greater permeation of MCFA with respect to LCFA. This can be explained by the differences in the molecular weights of the compounds and the affinity between these compounds and the membrane. It was observed in this study that medium-chain fatty acids with a molecular weight average of 152 g/mol were permeated.

The work of Spricigo et al. [16] showed that a reverse osmosis membrane, in the presence of $SCCO_2$, may swell affecting its selective properties. In this way, the permeation of molecules with molecular weights greater than that of the molecular weight cut-off (MWCO) of membranes may be possible.

During filtration, fatty acids, especially those of lower molecular weight (MCFA) were lixiviated by $SCCO_2$ which explains their greater concentration in the lipidic permeates. Longer filtration time could help to extract a greater amount of fatty acids, thus increasing the purification in terms of triacylglycerols.

3.2. Composition of the lipidic fraction used in the purification experiments of SL

For the SL purification runs, a lipidic fraction obtained from the enzymatic interesterification between MCT and esters of long-chain fatty acids was used, and the following centesimal composition was found: 42.0% of TG (including the structured lipids), 53.2% of ethyl esters of long-chain fatty acids, and 4.8% of ethyl esters of medium-chain fatty acids.

Table 4 presents the fatty acid composition of MCT used in the obtention of the SLs (before interesterification) and Table 5 shows the fatty acid composition of the SLs obtained from the interesterification between MCT and ethyl esters from long-chain fatty acids, after 25 h of interesterification. The SLs obtained are a source of rapid energy absorption and essential fatty acids due to the presence of medium chain fatty acids (MCFA) and essential fatty acids omega-3 (Linolenic, Eicosapentaenoic and Docosahexaenoic), respectively.

Table 4
Fatty acid composition of the MCT

Fatty acid	%Molar
Caproic (C6:0)	1.30
Caprylic (C8:0)	43.97
Capric (C10:0)	15.20
Lauric (C14:0)	0.20

Table 5
Fatty acid composition of the SLs

Fatty acid	%Molar
Caproic (C6:0)	0.40
Caprylic (C8:0)	13.26
Capric (C10:0)	5.04
Lauric (C12:0)	0.13
Miristic (C14:0)	2.38
Isopentadecanoic (C15:0 iso)	0.09
Pentadecanoic (C15:0)	0.18
Palmitic (C16:0)	5.03
Palmitoleic (C16:1)	2.14
Isomargaric (C17:0 iso)	0.06
Isomargaric (C17:0 anteiso)	0.02
Hexadienoic (C16:2)	0.18
Margaric (C17:0)	0.41
Margaroleic (C17:1)	0.27
Isostearic (C18:0 iso)	0.24
Stearic (C18:0)	1.02
Oleic (C18:1n9cis)	3.30
Linoleic (C18:2n6)	0.50
γ-Linolenic (C18:3n6)	0.03
Linolenic (C18:3n3)	0.29
Stearidonic (C18:4)	0.61
Arachidic (C20:0)	0.10
Gadoleic (C20:1)	0.33
(C20:2n6)	0.06
Arachidonic (C20:4)	0.34
Eicosapentaenoic (C20:5n3)	3.56
Behenic (C22:0)	0.13
Cetoleic (C22:1)	0.05
Docosapentaenoic (22:4n6)	0.07
Docosapentaenoic (C22:5n3)	0.54
Docosahexaenoic (C22:6n3)	1.68

3.3. Performance of the BW30-4040 membrane under different operating conditions

3.3.1. Effect of TMP on the $SCCO_2$ flux

Fig. 2 shows the $SCCO_2$ flux through the BW30-4040 membrane under different TMP conditions and a constant retentate-side pressure of 13 MPa. The permeability with regard to $SCCO_2$ was 27.8 $kg\,h^{-1}\,m^{-2}\,MPa^{-1}$, showing a linear relationship with the TMP applied, whether for a progressive increase or reduction in TMP. Therefore, hysteresis seems not to be a relevant phenomenon, at least in the applied TMP range. Similar results were found by Spricigo et al. [16] working with a reverse osmosis membrane prepared from cellulose acetate, for the same TMP range.

3.3.2. Effect of TMP on the $SCCO_2$ flux and triacylglycerol retention

Fig. 3 shows that an increase in the TMP improved the $SCCO_2$ and lipids fluxes. However, a greater effect of the increased TMP on the $SCCO_2$ permeation can be noted, probably due to its lower molecular weight. In addition, a drop in both permeate fluxes with filtration time, for all TMP ranges, was observed. This flux reduction could be related to the concentration polarization and fouling. We must also consider the effect of membrane com-

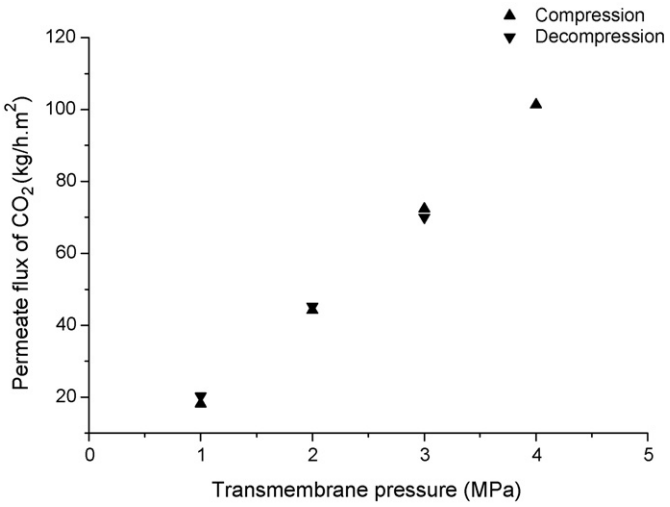


Fig. 2. $SCCO_2$ flux for the BW30-4040 membrane during compression and decompression under different transmembrane pressures (TMP). Constant retentate-side pressure of 13 MPa and $T = 40\,^{\circ}C$. $y = 27.796x - 10.505$, $R^2 = 0.9994$.

paction, which might have the effect of reducing the membrane pore diameter, mainly when using higher TMP values.

The effect of TMP on the lipid permeate flux and triacylglycerol retention, at constant pressure on the retentate side, is shown in Fig. 4. An increase in the triacylglycerol retention as a function of TMP and process time is observed. We notice that in the initial period there was a significant increase in the triacylglycerol retention. This may be attributed to a membrane pore blockage, thus increasing its selectivity. A drop in lipids flux was also observed as a function of time, at a TMP of 4 MPa, possibly due to membrane compaction at high differential pressures.

3.3.3. *Effect of retentate-side pressure on the $SCCO_2$ and lipid fluxes at constant TMP*

In this experiment, pressure was varied on the retentate side and TMP was kept constant at 1 MPa. It can be observed in Fig. 5 that an increase in pressure on the retentate side caused a reduction in the $SCCO_2$ flux and an increase in lipid permeation.

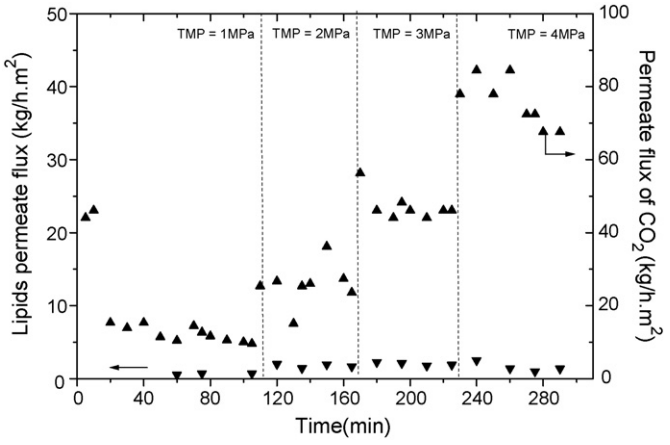


Fig. 3. Effect of transmembrane pressure (TMP) for the BW30-4040 membrane on the $SCCO_2$ and lipids permeate fluxes for a retentate-side pressure kept at 13 MPa and temperature of 40 °C.

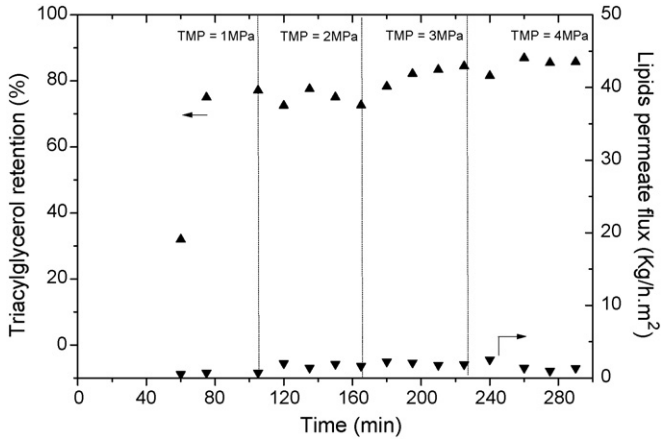


Fig. 4. Effect of transmembrane pressure (TMP) for the BW30-4040 membrane on the lipids permeate flux and triacylglycerol retention for a retentate-side pressure kept at 13 MPa and temperature of 40 °C.

It is known that CO_2 density in the supercritical region increases considerably with pressure at constant temperature, which might result in its flux reduction at high pressures. At 40 °C, the CO_2 density increase from 0.278 g/cm^3 at 8 MPa to 0.744 g/cm^3 at 13 MPa. Under these conditions, intermolecular distances are reduced, favoring an increase in specific interactions between solute and solvents, thus improving the solvent power of the SCF [26]. Therefore, as a consequence of the higher pressure on the retentate side, there was an improved $SCCO_2$ solvent effect, characterized by a greater extraction of the lipidic fraction.

Fig. 6 shows the retention of triacylglycerols as a function of different pressures on the retentate side. At 9 MPa, stabilization of the membrane when exposed to a mixture of $SCCO_2$+lipidic fraction was observed and a greater retention of triacylglycerols was obtained. At the other pressures a lower extraction of triacylglycerols was recorded. An increase in the pressure of the retentate side from 9 to 13 MPa led to a reduction in the retention of triacylglycerols, attributed to a greater extraction of lipids and a loss of extraction selectivity.

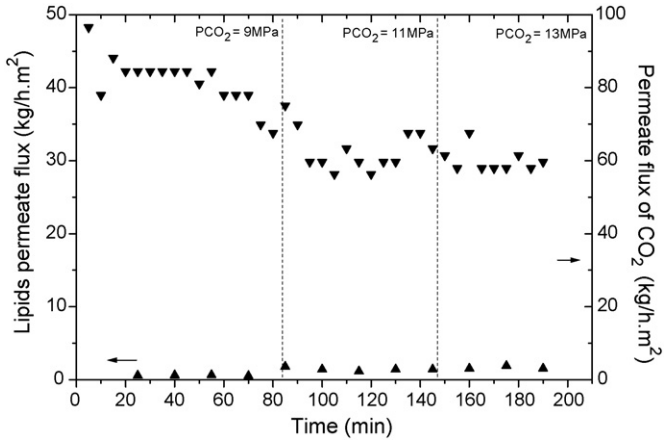


Fig. 5. Effect of pressure on the retentate-side for the BW30-4040 membrane on the $SCCO_2$ and lipids permeate fluxes for a constant transmembrane pressure of 1 MPa and temperature of 40 °C.

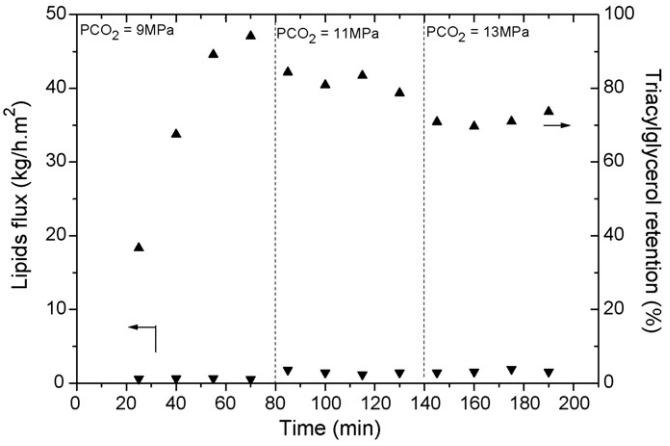


Fig. 6. Effect of pressure on the retentate-side for the BW30-4040 membrane on the triacylglycerol retention and lipids permeate flux for a constant transmembrane pressure of 1 MPa and temperature of 40 °C.

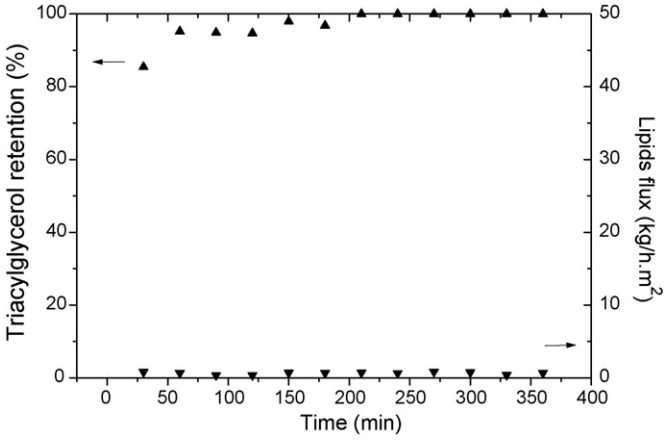


Fig. 8. Lipids flux and triacylglycerol retention for the BW30-4040 membrane at pressure on the retentate side of 9 MPa and transmembrane pressure of 0.7 MPa.

After a more detailed investigation into the lipidic permeate composition, as shown in Fig. 7, a change in the selectivity of lipidic component extraction as a function of different pressures applied to the retentate side was evident.

It is known that solubility of organic compound in a SCF is greatly affected by their polarity, which in turn may directly affect extraction selectivity. The solubility of $SCCO_2$ is favored by the presence of insaturations, ramifications, etherification and estherification. On the other hand, solubility is unfavored by increasing carbon atom numbers, and the presence of aromatic substituents, and polar groups such as hydroxylic, carboxylic and amine groups. At a given temperature, the solvent power of a gas may be improved by increasing its pressure, with this effect being more pronounced in the region immediately above the critical pressure [26]. Brunetti et al. [4] investigated the deacidification of olive oil with high acidity by means of $SCCO_2$ extraction under pressures between 20 and 30 MPa and temperatures between 40 and 60 °C. They reported a greater solubility of fatty acids in $SCCO_2$ in regard to their respective triacylglycerols, under particular conditions of temperature and pressure (60 °C and 20 MPa).

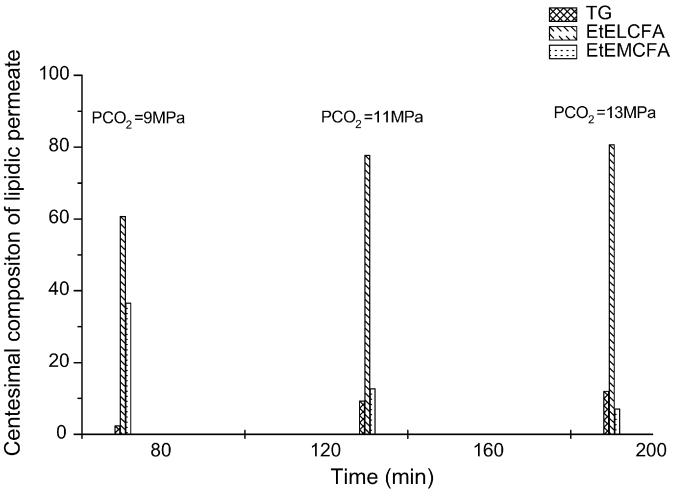


Fig. 7. Centesimal composition of lipidic permeates extracted under different $SCCO_2$ pressures and constant transmembrane pressure of 1 MPa and 40 °C.

We can see in Fig. 7 that for lower pressures on the retentate side there was a less efficient extraction of triacylglycerols and a greater extraction of ethyl esters of long- and medium-chain fatty acids. The increase in pressure on the retentate side from 9 to 13 MPa resulted in an improved solubility of the lipidic fraction, characterized by a greater extraction of triacylglycerols, thus altering the extraction selectivity.

3.3.4. Effect of processing time on the lipid flux and triacylglycerol retention

In order to verify membrane behavior during a longer filtration period, experiments using constant pressure on both retentate and permeate sides were carried out (constant TMP). Working pressure on the retentate side was 9 MPa, resulting in the least effective triacylglycerol extraction (Fig. 7). A TMP of 0.7 MPa was used in order to have the minimum loss in the triacylglycerol retention in the first hours of filtration. Higher triacylglycerol permeations were found when using TMP values higher than 0.7 MPa.

As shown in Fig. 8, during the first few hours the membrane showed an average retention of triacylglycerols of 95%; 100% was reached after 3 h of filtration, without a significant reduction in the lipid flux.

4. Conclusions

The results presented show the technical feasibility of the use of the BW30-4040 commercial reverse osmosis membrane for the retention of SLs (modified triacylglycerols) and a significant permeation of fatty acid esters and/or free fatty acids present in the reaction medium ($SCCO_2$ + lipidic fraction). The membrane demonstrated a good resistance for the pressures used on the retentate side and for the transmembrane pressures (TMP) applied. The triacylglycerol retention was found to stabilize after a period of approximately 2 h. Higher pressures on the retentate side reduced the triacylglycerol retention due to an increase in the solvent power of $SCCO_2$, thus reducing the extraction selec-

tivity. The use of a low TMP (0.7 MPa) and a low pressure on the retentate side (9 MPa) resulted in a retention of 100% of SLs. In addition, a temperature of 40 °C for the extraction and purification of SLs confers good protection of polyunsaturated fatty acids against oxidation and acyl migration.

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