FOOD BY-PRODUCTS AS SOURCES OF VALUABLE LIPIDS AND THEIR EXTRACTION BY CO_2-ETHANOL

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ABSTRACT

Supercritical extraction of lipids was carried out on two oil press-cakes, flaxseed and sesame, and one marine source, the scallop leftovers. The objective was to recover various extracts containing different lipids and polyunsaturated fatty acids (PUFA) thanks to the by-products specific composition. Since the recovery of phospholipids (PL) was foreseen, the extraction was performed by using CO_2+EtOH mixtures. The materials were first characterized by regards of their total lipid and phospholipid contents and their fatty acids composition. Extractions were then performed by collecting regularly fractions. Phospholipids were detected in several fractions indicating that conditions of 27% of EtOH, 45°C and 25 MPa were suitable for their extraction from the matrices. The use of two EtOH contents during the extraction allowed for separating other lipids from phospholipids although the purification was not total. The rich PL fractions contained as well non lipidic compounds due to the use of 27%EtOH. However, with PL purity of 25wt% for scallops to 35wt% for flaxseed, extracts compared well with phospholipid extracts on market.

INTRODUCTION

Industrially, edible oils from plants are produced in two steps. The first step consists in pressing seeds in screw or expeller press to remove around 70-80% of the oil. The residual oil is further chemically extracted by solvent usually hexane. Like free fatty acids, color bodies, oxidation products or trace metals, phospholipids (PL) are contaminants that are removed during a complex refining process of pressed and solvent-extracted oils. Phospholipids are natural emulsifiers that find widespread utilization in food, cosmetic and pharmaceutical products. So far, the main industrial sources of PL are soybean oil and egg yolk, from which PL are recovered by multi-steps procedures that consume and therefore produce wastes of acidified water and organic solvents. As alternative sources, marine species are scrutinized

owing to the potential presence of n-3 polyunsaturated fatty acids (PUFA). So far the leading marine product on market is a krill extract that contains around 35% of PL.

In the context of giving value to leftovers, we investigate the supercritical CO_2 route for recovering phospholipids (1) from first-pressed cakes based on the assumption that they might still contain appreciable amounts of phospholipids (2) from a marine by-product never investigated to author's best knowledge, scallop non edible parts, i.e. all parts except muscle and roes. Not only extraction but separation between phospholipids and other lipids is foreseen. A two-step method based on increasing amount of ethanol as co-solvent was thus selected according to literature [1-3].

MATERIALS AND METHODS

Raw materials and chemicals

Sesame and flaxseed cakes, provided by Bioplanete (Bram, France) came from the cold-pressing of sesame seeds (*Sesamum indicum*) and flaxseed (*Linum usitatissimum L.*). Fresh scallops (*Pecten maximus*) wastes were collected at a local store. Wastes comprised all parts except the shellfish muscle and roe. The collected material was ground under vacuum at 4°C and dried by zeodration before being stored at -18°C.

Solvent for supercritical fluid extraction (SFE) were CO_2 (99.95%, Air Liquide, France) and ethanol 96% (Atlantic Labo, France). Solvents for extraction of total lipids were of 99% purity (Atlantic Labo); reagents and standards to prepare and identify fatty acid were from Sigma-Aldrich.

Supercritical fluid extraction

Extractions were carried out in a home-made set-up comprising mostly a 0.49L cylindrical vessel (TOP Industrie, France), two Gilson pumps (Model 305) for the CO_2 and co-solvent delivery, and various stop valves, pressure and temperature sensors. The CO_2 flow was measured by a gas flowmeter after its expansion whereas the ethanol co-solvent flow was measured by monitoring the consumption from a graduated in-take flask. The extractor was loaded, from bottom to top, by 10cm of glass beads followed with alternative layers of 2cm of samples and 1cm of glass beads to avoid the material caking. The matrix amount in the vessel was of 30-35g.The vessel temperature was regulated by an electrical mantle (Watlow). Leaving the extractor, the stream flew through a metering valve into a home-made cyclonic separator, whose bottom was immersed in ice. The collector was emptied regularly to collect fractions. Set temperature and pressure of extractions were kept constant whatever the procedure and matrix, at 45°C and 25 MPa, respectively.

Characterization of raw materials and extracts

The total lipids of dried by-products (TL) were extracted with 20 volumes of chloroform/methanol (2:1 vol:vol) according to Folch method [4]. The total phospholipids content (TPL) was determined by spectrophotometry according to Ames method [5]. Fatty acids of the total lipid extract were identified by gas chromatography (GC) after methyl ester derivatization [6]. GC (GC 2010 plus, Shimadzu, Japon) was performed on a BPX 70 capillary column (60 m long, 0.25µm film, 0.25mm i.d. SGE, Australia) a FID detector, hydrogen as carrier gas and a split ratio of 1:50 to 1:60. Phospholipids were detected and quantified by ^{31}P NMR performed on a Bruker Avance 400 spectrometer (Bruker Biospin, Wissembourg, France) with a 5 mm QNP probe $^{1}H/^{13}C$-^{31}P-^{19}F.

Since SFE was carried out with CO_2+ethanol mixture, all extracts were recovered as ethanolic fractions. The total extracted amount was measured by gravimetry after evaporating aliquot under nitrogen. Total lipids and phospholipids in fractions were quantified by Folch extraction and ^{31}P NMR, respectively. Matrices after CO_2 treatment were extracted as well by Folch reagent to evaluate the amount of residual lipids and residual phospholipids.

RESULTS

By-products characteristics

Main characteristics of the food by-products are summarized in Table1.

Table 1. Food by-products characterization. ω3 and ω6 PUFA: polyunsaturated fatty acids; FA: fatty acids, TL: total lipids.

By-product	Water wt%	Total Lipids wt%	ω3 and ω6 PUFA wt% of FA	Ratio w3/w6	Phospholipid wt% of TL	Phospholipid wt% of matrix
Scallop	9.3 ± 0.8	12.7 ± 0.6	41.5 ± 0.4	9.05	11.5 ± 1.2	1.5 ± 0.2
Sesame	7.6	22.6 ± 2.4	45.8 ± 0.1	< 0.01	3 ± 0.1	0.7 ± 0.0
Flaxseed	6.6	10.9 ± 1.4	69.1 ± 0.1	2.08	7.6 ± 0.7	0.8 ± 0.1

The three matrices contain various total lipids content, sesame being the richest in lipids with 22% of dry weight. Among the plant cakes, flaxseed exhibits the highest level of ω3 and ω6 PUFA that represent almost 70% wt of the total lipids. Fatty acids of flaxseed are mostly C18:3 ω3 (46.7%), C18:2 ω6 (22.0%) and the monounsaturated C18:1 ω9 (18.7%). Sesame does not contain notably C18:3 ω3 (0.35%), hence, its high content in PUFA comes mostly from abundance of C18:2 ω6 (44.5%) and C18:1 ω9 (38.6%). Regarding phospholipids, this class of compounds represents 3 to 8% of total lipids, which means around 0.75 ± 0.1g of PL/ 100g of matter. The new marine by-product (scallops leftovers) combines appreciable

abundance of PUFA (41%) and phospholipids (11% of lipids). Moreover, it is a unique source of C20:5 ω3 (17.4%) and C22:6 ω3 (10.3%).

The by-product characteristics allowed for selecting matrices depending on extraction aims. By exhibiting high level of total lipids but the smallest level of phospholipids, sesame cake is a good candidate for recovering appreciable volume of residual oil. By exhibiting the highest content in phospholipids and a low lipid content, scallop leftovers are better candidate to recover phospholipids.

Supercritical extractions

The kinetic curve of the total mass extracted from the three by-products is given Figure 1. Supercritical extractions were performed with CO_2 + 7% EtOH for the two first fractions and CO_2 + 27% EtOH above 2 kg of extracting fluid.

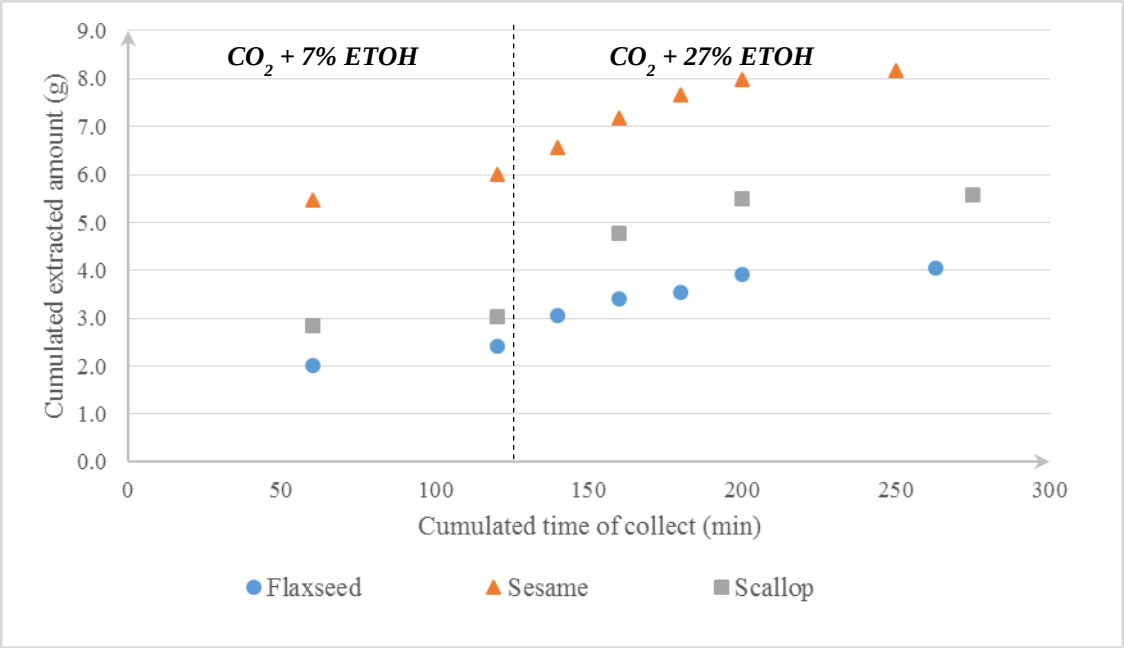

Figure 1. Cumulated extracted amounts obtained from the three by-products at 25 MPa, 45°C and 2-steps of CO2+%EtOH.

Appreciable part of total extracted amount was recovered in the first fraction, i.e. during the first hour of extraction. For sesame, this represented 67% ± 1 of the total extracted amount whereas it was only 50% for flaxseed. The mass recovered during the second period of 60 min was significantly lower but the 27% of EtOH provided a second steady rate of extraction up to the passing of 4kg of fluid (250min). Pre-pressed cakes behave differently in terms of extracted mass: for flaxseed cake, only 4g of extract was obtained whereas sesame produced 8 g (Table 2). Coincidently, the trend of extracted mass fit the increasing lipid content of cakes.

The first fractions of sesame and flaxseed were bi-phasic, with an oily phase of 9 and 3 ml at the bottom, respectively. Oily phases were analyzed by gas-chromatography to identify and quantify fatty acids. Composition of the extracted oil was similar to the initial composition of the cake, indicating that conditions of supercritical treatment did not yield any major selectivity.

Generally, colors of extracts faded as extraction proceeded, from yellow to pale for both cakes. Cakes after supercritical processing were grey and colorless. Their content in residual lipids was measured allowing for estimating the yield of lipid extraction (Table 2): 87-92% of the total lipids present in by-products were removed by the supercritical processing. In case of scallop matrix, the first fraction did not contain any oily phase, probably because of the longer fatty acids of that material, and was brown. Global and total lipid yields were in the same range than for the other matrices.

Table 2. Yields of supercritical extraction carried out by a two-steps path using first 7% of EtOH then 27% as cosolvent with CO_2. Mass loaded = 35g for sesame and flaxseed, 30g for scallop. TL: total lipids; TPL: total phospholipid.

	Flaxseed	Sesame	Scallop
Global yield (g extract / 100g matter)	11%	23.4%	18.7%
Residual lipids matrix (g TL/ 100g matter)	0.8g/100g	3.5g/100g	2.0g/100g
Lipid extraction yield, extracted/initial TL (%)	94%	86%	87%
Phospholipid yield, extracted/initial TPL (%)	82%	70%	80%

Distribution of lipids and phospholipids over fractions is shown in Figure 2A in case of scallop by-product, together with the total extracted amount per fraction (amounts are expressed in g for lipid and total amounts, and in mg for phospholipids). In the first fraction obtained by using CO_2+7%EtOH, lipids were in majority and represented about 90% of the overall amount extracted. Continuing further the extraction for 60 minutes at same conditions yielded only to few hundreds of mg of extract, indicating that the kinetics of extraction had considerably slowed down. As mentioned before, the increase of EtOH content to 27mol% yielded a second step of extraction, in which lipids were again extracted. However, non lipidic compounds were now significantly co-extracted, and lipids represented only 0.7g of the 1.7g of the fraction (i.e. 41%). The higher %EtOH yielded as well the apparition of phospholipids in the fraction that were present in the appreciable amount of 400mg. Although their extraction could have been delayed for kinetic reasons, e.g. slower mass transfer, the higher dissolving ability of the CO_2+27%EtOH mixture is likely to be responsible of their extraction. As the process continued, the extraction of phospholipids slowed down. After 4kg

of extracting fluid, their extraction was not completed since 20% of the PL initial amount remained in the matrix.

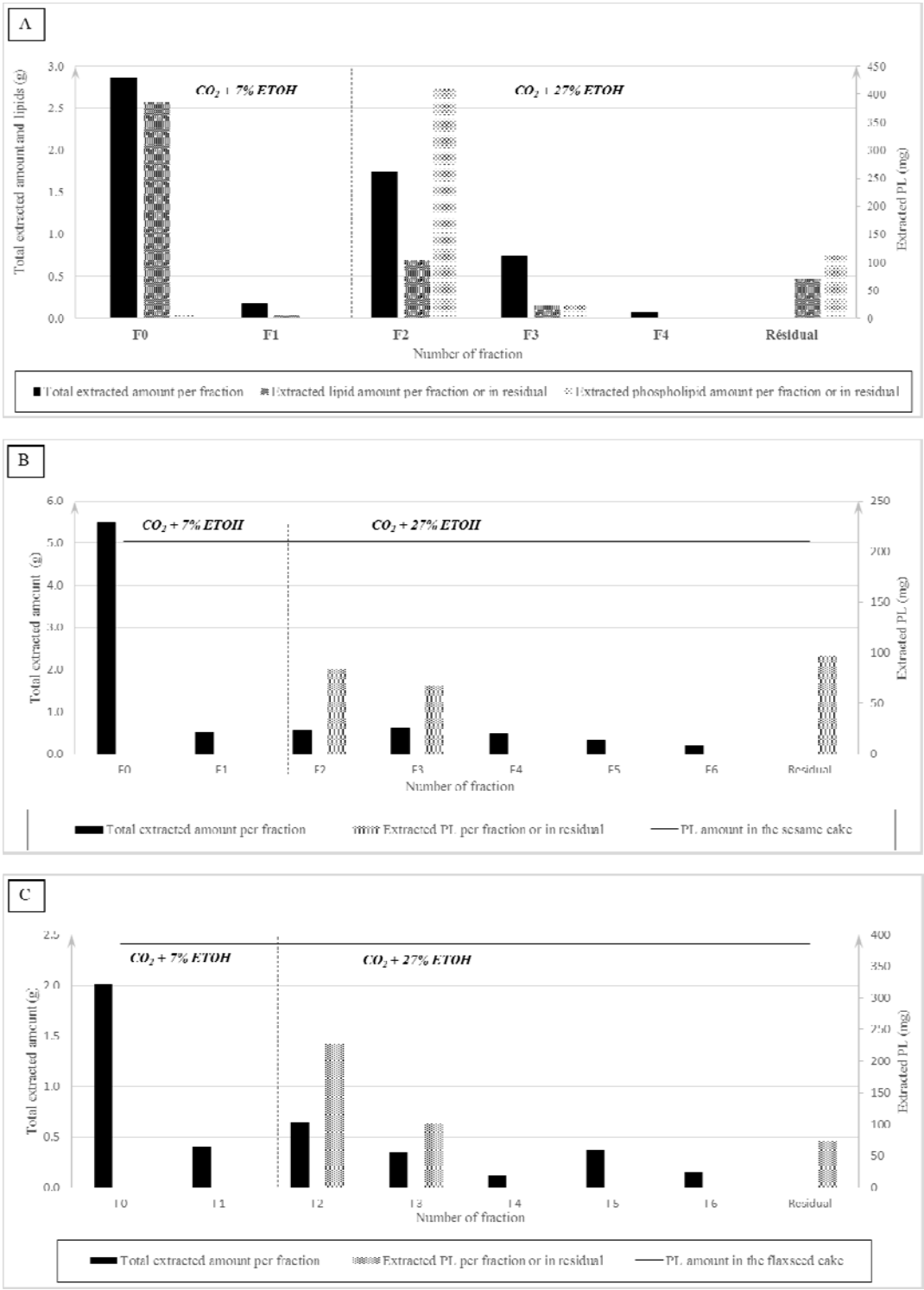

Figure 2. Composition of fractions obtained during SFE of (A) scallop leftovers, (B) sesame pressed cake, (C) flaxseed pressed cake, SFE performed at 25 MPa, 45°C with 7%EtOH then 27% of EtOH added to CO_2.

For sesame and flaxseed, phospholipids were extracted and concentrated as well in the first fraction obtained by 27%EtOH. However, their extraction kinetic was slower than in scallop wastes since fractions F3 and residue were found to contain significant amounts as well. Slower mass-transfer due to probably different matrix structures or sizes is likely to occur.

CONCLUSIONS

Looking for new sources of valuable lipids issued from food by-products, three matrices were characterized for their content in total lipids, type of fatty acids and content in phospholipids. Flaxseed and sesame pressed cakes and scallop leftovers exhibit different specificities that make them exploitable in different ways. By exhibiting high level of total lipids, sesame cake provides appreciable volume of residual oil, whereas the 1.5g of phospholipids per 100g dry matter in scallops make them appreciable sources of marine phospholipids; flaxseed cake contains less oil than sesame but it contains the valuable w3 – w6 PUFA. Extraction by supercritical fluids allowed for recovering the various lipids, and specially phospholipids providing the use of CO_2 plus ethanol as cosolvent. For those compounds, extraction yield between 70 and 82% were obtained with 4kg of fluid, i.e. for a fluid/matrix ratio of 130. Besides quantity, purity is another criteria of importance when dealing with extraction process. The high level of ethanol percentage used in this work (27%vol) was detrimental to the extract purity. However, the procedure developed allowed for recovering phospholipids mostly in one fraction, with a purity ranging from 25% (scallop) to 35% (flaxseed). It is interesting to note that the lowest source of PL, flaxseed, provided the highest extract purity.

ACKNOWLEDGMENTS

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