Supercritical Fluid Extraction of High Value Lipids from Canned Sardine Waste Streams

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ABSTRACT

In this work supercritical carbon dioxide (SC-CO_2) extraction was explored to recover high added value lipids from canned sardine waste streams, namely heads and offal. Aiming at optimizing the recovery of target lipids, in particular ω-3 polyunsaturated fatty acids (ω-3 PUFA), different extraction conditions were applied, including operating pressure (300, 425 and 550bar) and temperature (35, 55 and 75ºC). All extractions were carried out for 135min at a fixed CO_2 flow rate of 25g/min. A Bligh and Dyer (B&D) extraction was performed on canned sardine residues for total lipids quantification and to evaluate the performance of SC-CO_2 extractions when compared with a conventional solvent extraction. The resulting samples were characterized in terms of global yield and analysed by gas chromatography coupled with a flame ionization detector (GC-FID), after transesterification, in order to identify and quantify the different fatty acids present in each extract. To evaluate their potential application as bioactive ingredients, extracts were screened for their antimicrobial activity on *Staphylococcus aureus* and *Escherichia coli*, selected as representative of Gram-positive and Gram-negative species, respectively; and anti-inflammatory activity using a yeast cell model system, employing the yeast Ca2+/calcineurin/Crz1 reporter assay.

INTRODUCTION

It is estimated that in EU-28, 88 million tons of food are wasted per year, representing 20% of all food produced, with around 143 billion euros of associated costs [1]. For this reason, reducing food loss is now an integral part of the European Commission's Circular Economy Package, ensuring a sustainable growth and development [2]. Among all wasted food, fish is the main source of surpluses when processed for human consumption, generating residues equivalent to 20 to 75% (w/w) of starting raw materials. In particular, the canning process is the second biggest source of fish-processing residues, generating more than 1.5 million tonnes per year of waste in the EU alone, being only preceded by curing [3, 4].

In the last years, several studies pointed marine by-products and waste streams as a remarkable source of important bioactive molecules, which hold a much higher market value than the starting materials. These residues represent an opportunity to convert low status wastes in valuable resources that can be used in a broad range of economically interesting applications [4]. In particular, over the past decades, extensive studies have addressed the therapeutic effects of marine ω-3 PUFA against different diseases such as cardiovascular, neurodegenerative, cancer (being even able to improve efficacy and tolerability of chemotherapy), amongst others [5]. Furthermore, ω-3 PUFA have also attracted attention for their ability to kill or inhibit the growth of different bacterial strains [6]. Sardine is one of the main species caught in Portuguese waters and has been recognized as one of the most prevalent sources of long-chain marine ω-3 PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [7].

Nowadays, with the challenges arisen by the competitiveness of the globalized market and by an increasing awareness of the need to protect the environment, there has been a growing interest in developing green processes and technologies that enable a selective and effective recovery of high added value compounds from natural and renewable raw materials [8]. SC-CO_2 is cheap, environmentally friendly and generally recognized as safe by FDA and EFSA. Furthermore, its high diffusivity combined with its easily tuneable solvent power, make this alternative solvent highly attractive for the extraction of bioactive compounds, at both laboratorial and industrial scales [9, 10]. In fact, SC-CO_2 extraction is considered to be an important process in pharmaceutical, nutraceutical, food and cosmetic industries, as it does not involve the use of toxic solvents, increasing the selectivity towards valuable bioactive compounds, while preserving their bioactivity [10]. In particular, SC-CO_2 extraction has already proven to be a good technology to isolate fatty acids from different natural sources, including fish [9].
Within this context, in this study, SC-CO_2 technology was explored to extract valuable lipids from canned sardine heads and offal, namely ω-3 PUFA. The resulting extracts were characterized in terms of global extraction yield, analysed by GC-FID and further tested for their potential antimicrobial and anti-inflammatory activities.

MATERIALS AND METHODS

Raw material. Canned sardine (*Sardina pilchardus*) heads and offal (hereinafter referred to as steamed sardine residues, SSR) were kindly provided by Fábrica de Conservas A Poveira, Portugal, in January 2017, and stored at -20ºC in the absence of light. Part of the raw material was dehydrated using a Coolsafe Superior Touch 55-80 freeze dryer (Scanvac) at -55ºC. After approximately 72h, the residues were milled using a cutter-emulsifier CKE-8 (Sammic). The frezze-dried residues (hereinafter referred to as freeze-dried steamed sardine residues, FDSSR) were protected from light and stored at room temperature, in a low moisture environment, until the day of experiments.
Chemicals. Carbon dioxide ALPHAGAZ™ 1 (99.99%, Air Liquide) was used for SC-CO_2 extraction experiments and acetone (99.8%) from Fisher Chemical was used for the recovery of extracts from the separators. Chemicals used for conventional solvent extractions included chloroform (95%, Carlo Erba Reagents), methanol (99.9%, Carlo Erba Reagents) and bi-distilled water. For chemical characterization of the raw material and resulting extracts, xylene (mix of isomers, Carlo Erba Reagents), isooctane (99.8%, Merck) and potassium hydroxide in methanol (Sigma-Aldrich) were used. The standard mixture of fatty acid methyl esters (52 FAMES) used for fatty acid identification and quantification was purchased from Nu-Chek-Prep, Inc.
Moisture content determination. The moisture content in sardine residues was determined by conventional Dean–Stark distillation, according to the procedure described by Jacobs (1939) [11]. Briefly, 75mL of xylene were added to 2g of SSR or 10g of FDSSR. At the beginning of the experiment, the electrical heating was maximized until the collection of the first droplets in the Dean–Stark receiver. Afterwards, the heating was reduced to a slower distillation rate. Distillation was conducted for 2h, after which the volume of water contained in the Dean-Stark receiver was measured and the moisture content calculated.
Extraction procedures
Conventional solvent extraction. Total lipid content in sardine residues was determined using the methodology described by Bligh and Dyer (1959) [12]. Briefly, 2g of SSR or FDSSR were homogenized, using a vortex, with a mixture of 2mL of chloroform and 2mL of methanol. After 2min of homogenization, 2mL of chloroform were added to the mixture. The mixture was further homogenized for 30s, after which 2mL of bidistilled water were added and homogenization continued for another 30s. The resulting mixture was filtrated under vacuum and transferred to a tube where it was left until complete phase separation and clarification. The aqueous layer was discarded and the chloroform layer was evaporated under a nitrogen stream. The global extraction yield of the resulting extracts was determined and samples were stored at -20ºC, in the absence of light, until further analyses.

Supercritical carbon dioxide extraction. SC-CO_2 extractions were carried out in a supercritical fluid extraction system (SFE-500F-2-C50, Thar Technology) described elsewhere [13]. Briefly, 10g of FDSSR were placed on the extraction vessel packed with laboratory glass beads to ensure a uniform distribution of solvent flow. Taking into account, the work developed by Létisse and co-workers (2006) concerning the extraction of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from sardine [14], herein SC-CO_2 extractions were performed by varying pressure (300, 425 and 550bar) and temperature (35, 55 and 75ºC). All experiments were conducted under a continuous fixed CO_2 flow rate of 25g/min. CO_2 was expanded into the first fraction collector, set at 60bar and 25ºC, in which the extracts were recovered during 135min. After extraction, the extract remaining in both separators was recovered using acetone. The solvent was evaporated under a nitrogen stream and the global extraction yield of the resulting samples determined. Samples were stored at -20ºC, in the absence of light, until further analyses.

Extracts characterization

Transesterification of extracts. Glycerides contained in the extracts were converted in methyl esters following the transesterification method described in the NP EN ISO 5509 2003 [15]. Briefly, after solubilisation of the dry extracts in isooctane, a solution of potassium hydroxide in methanol (2N) was added to the mixture. The resulting sample was then vigorously mixed for 30s. After complete phase separation and clarification, 2µL of the top layer were collected and injected in the gas chromatograph.

Gas Chromatography analyses of extracts. GC-FID analyses were carried out using a ThermoQuest Trace GC 2000 (CE Instruments, Ltd.) gas chromatograph coupled with a flame ionization detector. The separation of sample components was achieved using a J&W DB-23 capillary column (Agilent Technologies, Inc.), 60m × 0.25mm I.D. and 0.25µm phase thickness. The oven temperature program started at 70ºC up to 195ºC, at a rate of 5ºC/min. Temperature was kept at 195ºC for 30min. Then continued increasing at a rate of 5ºC/min until 220ºC, and kept at 220ºC for 65min. Helium was used as carrier gas. The injector temperature was 220ºC and the detector temperature was 280ºC. Fatty acid methyl esters (FAMES) were identified by comparison of relative retention times in the samples with those obtained for a standard mixture of 52 FAMES.

Antimicrobial activity

Bacterial test strains. *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 were the strains selected as representative of Gram-positive and Gram-negative species, respectively, which are relevant as foodborne pathogens.

Preparation of extract stock solutions. Extract stock solutions were prepared in cation-adjusted Mueller Hinton broth (CAMHB, BD Difco) immediately before the antimicrobial susceptibility testing assay. Pipetting up and down 20 times was performed to ensure a homogeneous extract stock solution.

Evaluation of antimicrobial susceptibility. Antimicrobial susceptibility testing assay was performed according to the broth microdilution method of CLSI M07-A10 guidelines [16]. Briefly, extract stock solutions were dispensed in a 96-well round bottom microtiter plate and 2-fold serially diluted in CAMHB to obtain a concentration range of solutions. The inoculum was prepared using the direct colony suspension method and adjusted in saline solution to achieve a turbidity equivalent to a 0.5 McFarland standard. The adjusted inoculum was further diluted in CAMHB to ensure that, after inoculation, each well contained approximately 5×10^4CFU. Each inoculated microtiter plate was incubated under aerobic conditions at 37ºC for 24h. The lowest concentration of an antimicrobial agent that completely inhibited visible growth as detected by the unaided eye was recorded as the minimal inhibitory concentration (MIC). MICs were determined by examining the growth of each strain for three replicates. If two of the three results were the same, that MIC was used as the composite result. If the three results differed, the middle result was used. For each stock solution assayed, a positive control (CAMHB and diluted inoculum), a medium sterility control (CAMHB), and an extract sterility control (CAMHB and extract stock solution) were also tested.

Anti-inflammatory activity

Yeast test strains. *Saccharomyces cerevisiae* yeasts, strain BY4742_CDRE-*lacZ*, were acquired from Euroscarf. Yeast cells were cultured using Synthetic Complete Supplement Mixture liquid medium, with 0.79g/L Complete Supplement Mixture (QBiogene), 6.7g/L Yeast Nitrogen Base (Difco), and 2% (w/v) glucose (Sigma-Aldrich).

Evaluation of anti-inflammatory potential. Anti-inflammatory potential of extracts was evaluated as described in the literature [17]. Briefly, cell suspensions were treated with the extracts for 90min at 30ºC under orbital shaking at 200rpm. After this incubation period, CaN/Crz1 inducer $MnCl_2$ was added to one aliquot of each condition to achieve a final concentration of 3mM. Induced yeasts were further incubated for 90min under the same conditions. After thorough homogenization, cells were submitted to a 20min incubation with Yeast Protein Extraction Reagent (Thermo Fisher Scientific, Inc.) at 37ºC. Following cell lysis, ortho-nitrophenyl-β-galactoside, a β-galactosidase substrate, was added to the suspension. After an 80min incubation at 30ºC, OD_{420} was measured. Results were expressed in Miller units, considering several correction factors, and calculated as follows [17, 18]:

$$Miller\ unit = \frac{1000*(OD420-1.75*OD550)}{80*0.01*OD600} \quad \text{(Equation 1)}$$

RESULTS

The method proposed by Bligh and Dyer (1959) is by far the most commonly used method for the extraction of total lipids. For this reason, in order to determine the total lipid content of canned sardine residues and to evaluate the performance of SC-CO_2 extractions, a B&D extraction was applied to both SSR and FDSSR. Furthermore, the moisture content of the residues was also evaluated. The results obtained are summarized in Table 1.

Table 1. Moisture content and global extraction yield obtained after B&D extraction for SSR and FDSSR.

Residue	Moisture content (%)	Global extraction yield ($g_{extract}/100g_{dry\ residue}$)
Steamed sardine residues	67.5	38.39
Freeze-dried steamed sardine residues	2.9	24.99

When applying the conventional B&D extraction to SSR, the results obtained for total lipid content ($38.39g_{lipids}/100g_{dry\ residue}$) were similar to those obtained by Batista et al. (2009) for raw sardine by-products [19]. In that work, after a 7h Soxhlet extraction with ethyl ether, the authors found that the residues were composed by 14.5% of lipids, which is equivalent to $44.34g_{lipids}/100g_{dry\ residue}$. However, there was a decrease in the extraction yield when the B&D extraction was performed on FDSSR, in which only 65.10% of the total lipids were extracted, taking the yield obtained by B&D extraction of SSR as reference. This decrease may be due to a loss of lipids during the freeze-drying process, or due to a promotion of the extraction of more polar lipids by the water present in SSR (67.5% vs. 2.9% of water in FDSSR). In fact, the method proposed by B&D enables the extraction of all lipids present in the tissues subjected to extraction (originally performed on frozen cod muscle), which may include polar lipids, phospholipids and possibly lipids bound with other components from cellular membranes [14]. Regarding the moisture content, the results obtained for SSR (67.5%) are also in accordance with the value reported by Batista et al. (2009) (67.3%), after drying the sardine samples in an oven at 105ºC for 24h [19].

Once the total lipid content was known, in order to obtain natural extracts rich in bioactive lipids with potential health-promoting effects, SC-CO_2 extraction was explored and applied to FDSSR. The impact of pressure and temperature on the global yield and on the composition of each extract was studied and compared with the results obtained after B&D extraction. Table 2 shows the experimental conditions at which the SC-CO_2 experiments were carried out, as well as the global extraction yield obtained for each experiment. Extraction yields ranged from $19.95g_{extract}/100g_{dry\ residue}$ (at 55ºC and 300bar) to $27.41g_{extract}/100g_{dry\ residue}$ (at 75ºC and 550bar). Considering the yield obtained by B&D extraction of FDSSR as reference ($24.99g_{lipids}/100g_{dry\ residue}$), at higher pressures

(425 and 550 bar) SC-CO_2 was able to produce extracts with similar or even higher yields than those obtained by a conventional B&D extraction. In particular, the yield obtained at 300bar and 75ºC was 52.48% higher than the one achieved by other authors for freeze-dried sardine heads, at optimal conditions of 300bar and 75ºC [14]. However, in the referred work, extractions where conducted on 2.5g of residue for 45min at a CO_2 flow rate of 1.9g/min. The reduced sardine/CO_2 ratio and short extraction time may have led to a lower yield [14].

Table 2. Extraction conditions applied to FDSSR and respective global extraction yields. (Global extraction yields take into account the extract that is recovered during extraction and the extract that is recovered from the separators after depressurization of the system).

Temperature (°C)	Pressure (bar)	CO_2 density (g/mL)	Global extraction yield ($g_{extract}/100g_{dry\ residue}$)
35	300	0.93	20.10
	425	0.98	25.81
	550	1.02	26.98
55	300	0.85	19.95
	425	0.92	24.63
	550	0.97	26.84
75	300	0.77	21.80
	425	0.85	23.97
	550	0.91	27.41

The extraction curves, which plot the cumulative extraction yield as a function of the extraction time, are shown in Figure 1 for all temperatures and pressures studied. The extraction yield increased with pressure for a fixed temperature, which follows the usual trend in supercritical fluid extraction of lipids from sardine and other natural sources [20, 21]. For instance, when working at 35ºC, by increasing pressure from 300bar to 550bar, a 41.66% increase in the extraction yield can be observed. This increase may be explained by the increase in CO_2 density with the pressure (for a constant temperature), thus enhancing the CO_2 capacity to solubilize the lipids. However, increasing temperature had no effect on extraction yields. For economic reasons, and to prevent the exposure of lipids to high temperatures, it is preferable to work at a lower temperature (35ºC). In Figure 1 is also possible to distinguish the two different mechanisms that are acting in the extraction. In the first minutes of extraction, the lipids that are readily available at the surface of sardine particles are extracted at a rapid and constant rate, in which the external mass transfer resistance is the controlling factor. A few minutes later, the lipids from deeper zones of the solid matrix start to be extracted by CO_2, being the internal mass transfer resistance the controlling factor [21].

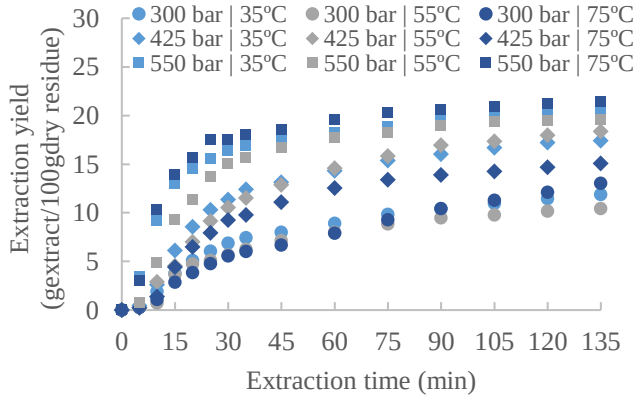

Figure 1. Extraction curves for SC-CO_2 extraction of FDSSR at different temperatures (35, 55 and 75ºC) and pressures (300, 425 and 550bar). (Extraction curves take into account the extract that is recovered during the extraction time, but not the extract that is recovered from the separators after depressurization).

Table 3 shows the main fatty acids (>5$g_{fatty\ acids}$/100$g_{fatty\ acids}$) identified in sardine extracts obtained by SC-CO_2 and B&D extractions. The major components of all extracts are palmitic acid (C16:0), DHA (C22:6 (n-3)), oleic acid (C18:1 (n-9)), EPA (C20:5 (n-3)), myristic acid (C14:0), cetoleic acid (C22:1 (n-11)), stearic acid (C18:0), palmitoleic acid (C16:1 (n-7)), gadoleic acid (C20:1 (n-11)) and cis-vaccenic acid (C18:1 (n-7)). These results are in accordance with those reported in the literature for sardine extracts [14, 20].

The antimicrobial potential of sardine extracts was evaluated on *S. aureus* and *E. coli*, which were selected as representative of Gram-positive and Gram-negative bacteria, respectively. MIC results have shown that all extracts inhibited both bacterial test strains and that samples were more efficient (at least 15-fold higher) in inhibiting the Gram-positive than the Gram-negative selected bacteria (data not shown). Among all extracts, the sample obtained from SSR after B&D extraction was the most effective antibacterial agent against both *S. aureus* (0.29$mg_{\omega3\text{-}PUFA}$/mL) and *E. coli* (9.24$mg_{\omega3\text{-}PUFA}$/mL). Nevertheless, SC-CO_2 extracts were as effective in inhibiting bacterial growth as the B&D extract resulting from FDSSR of both *S. aureus* (0.32$mg_{\omega3\text{-}PUFA}$/mL) and *E. coli* (10.14$mg_{\omega3\text{-}PUFA}$/mL).

In order to evaluate the anti-inflammatory effect of canned sardine residues extracts, tests were conducted in a yeast cell model system (yeast Ca2+/calcineurin/Crz1 reporter assay). Results showed that sardine extracts displayed a protective activity, showed by the inhibition of the induction of Crz1 (used to infer the anti-inflammatory potential of samples).

CONCLUSION

In the study reported herein, SC-CO_2 extraction was explored for the recovery of lipids from canned sardine residues, with special focus on PUFA. The influence of the main operating conditions on the extraction yield was studied, namely temperature and pressure. The best extraction yields were obtained when higher pressures were applied (up to 550bar), however, increasing temperature (up to 75ºC) did not enhance extraction yields or greatly modified the fatty acid profile of the resulting extracts. A minimum recovery of 19.95$g_{extract}$/100$g_{dry\ residue}$ and a maximum of 27.41$g_{extract}$/100$g_{dry\ residue}$ were obtained, which for higher pressures (425 and 550bar) represented an improvement (up to 8.83%) on global extraction yields, when compared with the results obtained for FDSSR after B&D extraction (24.99g_{lipids}/100$g_{dry\ residue}$). Extracts were analysed for their fatty acid profile and the main compounds present in the extracts were found to be palmitic acid, DHA, oleic acid and EPA, which account for about half of the fatty acid composition of sardine residues. Results showed that sardine extracts inhibited both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, in ranges of 0.29$mg_{\omega3\text{-}PUFA}$/mL to 1.01$mg_{\omega3\text{-}PUFA}$/mL and 9.24$mg_{\omega3\text{-}PUFA}$/mL to 16.08$mg_{\omega3\text{-}PUFA}$/mL, respectively. Sardine extracts have also shown an anti-inflammatory potential when tested in a yeast cell model system.

Since the solvent mixture used on the B&D extraction is not authorized for food applications, from the results obtained it can be concluded that SC-CO_2 extraction technology can be considered as a good alternative to this traditional solvent extraction method, thus enabling the valorisation of canned sardine heads and offal through their use in nutraceutical or food applications.

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Table 3. Fatty acid composition (major compounds) identified in canned sardine extracts by GC-FID.

Sample	Fatty acid	C14:0	C16:0	C16:1 (n-7)	C18:0	C18:1 (n-9)	C18:1 (n-7)	C20:1 (n-11)	C20:5 (n-3)	C22:1 (n-11)	C22:6 (n-3)	ω-3 PUFA	ω-6 PUFA	SFA	MUFA	Total FA
Steamed sardine residues	$mg_{FA}/g_{dry\ residue}$	26,05	73,75	15,72	16,82	30,33	8,08	14,92	28,27	18,27	50,73	94,61	10,72	129,67	101,53	336,53
	$mg_{FA}/g_{extract}$	67,86	192,13	40,96	43,82	79,01	21,06	38,87	73,66	47,61	132,16	246,47	27,92	337,81	264,49	876,69
Freeze-dried steamed sardine residues	$mg_{FA}/g_{dry\ residue}$	15,82	49,01	10,13	9,64	23,21	3,85	11,33	22,35	9,27	32,28	67,57	8,59	83,08	63,91	223,14
	$mg_{FA}/g_{extract}$	63,31	196,08	40,53	38,58	92,88	15,39	45,32	89,42	37,09	129,14	270,35	34,35	332,39	255,69	892,78
300 bar | 35ºC	$mg_{FA}/g_{dry\ residue}$	7,95	27,45	5,14	5,53	11,74	2,80	5,01	6,46	4,87	8,71	21,62	3,59	46,00	32,58	103,79
	$mg_{FA}/g_{extract}$	66,88	230,93	43,28	46,49	98,76	23,58	42,15	54,32	40,94	73,32	181,88	30,19	387,03	274,09	873,20
300 bar | 55ºC	$mg_{FA}/g_{dry\ residue}$	9,28	24,46	4,77	4,50	8,88	2,37	4,41	5,18	4,37	7,25	17,60	2,65	42,50	28,87	91,62
	$mg_{FA}/g_{extract}$	88,86	234,27	45,73	43,12	85,04	22,68	42,24	49,64	41,88	69,42	168,61	25,38	407,02	276,52	877,53
300 bar | 75ºC	$mg_{FA}/g_{dry\ residue}$	10,62	30,88	5,82	5,83	12,34	2,79	4,99	6,61	4,63	9,07	22,29	3,52	52,94	35,45	114,19
	$mg_{FA}/g_{extract}$	81,45	236,81	44,65	44,69	94,66	21,42	38,25	50,66	35,52	69,55	170,92	26,97	406,03	271,86	875,78
425 bar | 35ºC	$mg_{FA}/g_{dry\ residue}$	14,17	40,01	7,59	7,54	15,19	3,73	6,97	9,78	6,83	15,24	34,13	4,65	69,28	46,29	154,36
	$mg_{FA}/g_{extract}$	81,35	229,76	43,58	43,30	87,24	21,42	40,02	56,15	39,23	87,53	196,00	26,71	397,82	265,83	886,37
425 bar | 55ºC	$mg_{FA}/g_{dry\ residue}$	16,82	45,45	8,64	7,80	15,82	3,99	6,96	7,93	7,64	12,40	29,23	4,42	77,48	49,84	160,97
	$mg_{FA}/g_{extract}$	91,50	247,25	46,98	42,42	86,03	21,69	37,87	43,13	41,57	67,46	159,00	24,03	421,46	271,11	875,60
425 bar | 75ºC	$mg_{FA}/g_{dry\ residue}$	11,47	33,21	6,25	6,51	13,03	3,28	6,11	8,00	8,43	12,92	28,67	3,78	57,27	43,37	133,09
	$mg_{FA}/g_{extract}$	76,07	220,29	41,45	43,20	86,44	21,78	40,55	53,08	55,91	85,71	190,16	25,07	379,92	287,72	882,87
550 bar | 35ºC	$mg_{FA}/g_{dry\ residue}$	14,23	42,51	8,39	9,03	18,63	4,47	7,62	12,76	10,25	19,64	43,68	6,03	74,09	57,06	180,86
	$mg_{FA}/g_{extract}$	69,85	208,63	41,19	44,31	91,46	21,96	37,42	62,61	50,31	96,38	214,39	29,60	363,63	280,05	887,68
550 bar | 55ºC	$mg_{FA}/g_{dry\ residue}$	16,89	49,16	9,53	8,77	17,48	4,61	7,66	8,06	8,09	10,99	28,69	4,68	82,47	54,90	170,74
	$mg_{FA}/g_{extract}$	86,11	250,70	48,61	44,73	89,13	23,49	39,08	41,11	41,24	56,06	146,30	23,87	420,53	279,98	870,68
550 bar | 75ºC	$mg_{FA}/g_{dry\ residue}$	18,32	52,55	9,94	9,66	19,74	4,70	1,53	9,74	8,58	13,17	33,32	5,77	89,49	53,09	181,67
	$mg_{FA}/g_{extract}$	85,51	245,33	46,39	45,08	92,14	21,96	7,16	45,47	40,04	61,51	155,55	26,95	417,78	247,85	848,12

ω-3 PUFA: omega-3 polyunsaturated fatty acids
ω-6 PUFA: omega-6 polyunsaturated fatty acids
SFA: saturated fatty acids
MUFA: monounsaturated fatty acids
Total FA: total fatty acids

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