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Isoflavone-rich extracts from okara using supercritical fluid extraction: Kinetic modeling and characterization

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ABSTRACT

The lipid fraction and bioactive compounds from okara, a by-product of soybean processing, have been extracted by supercritical fluid extraction. The effect of operating parameters, pressure (20–40 MPa), temperature (40–80 °C), and cosolvent usage (0–10 %wt. ethanol), on the extraction kinetics has been investigated, satisfactorily correlating the experimental data to the Sovová's model. Extraction yield by SFE ranged from 0.1014 g extract/g insoluble solid (IS) at 20 MPa and 80 °C to 0.1081 g extract/g IS at 30 MPa and 60 °C (from 9.2 to 9.8 g extract/100 g okara, respectively), similar to Soxhlet extraction with n-hexane (9.2 ± 0.2 g extract/100 g). Pressure positively affected the initial extraction rate, which also increased with temperature except for the lowest pressure of 20 MPa, exhibiting a typical cross-over behavior. The use of ethanol as cosolvent promoted the extraction of phenolic compounds and isoflavones, and was less important in the extraction of tocopherols. Extraction yield using ethanol increased up to 0.1100 g extract/g IS at 40 MPa and 40 °C. As expected, the higher content of bioactive compounds in the sc-CO_2 + ethanol extracts positively affected the antioxidant capacity, with the maximum found at 20 MPa, 40 °C and 10 %wt. ethanol.

1. Introduction

Raw okara, or soy pulp, is a by-product obtained from ground soybeans after extraction of the water-extractable fraction to produce soymilk and tofu. It has been estimated that around 1.1 kg of okara is produced per kg of processed soybeans (Khare et al., 1995). As a result, large quantities of okara are produced in the soymilk and tofu industries, which are generally used for animal feed or discarded due to its tendency to rapid microbial spoilage. However, okara still has an interesting nutritional profile, with proteins (15.2–33.4 % dry wt.), lipids (8.3–10.9 % dry wt.), and dietary fiber (42.4–58.1 % dry wt.) (Vong and Liu, 2016). Additionally, okara contains bioactive phenolics, such as a variety of isoflavones and tocopherols (Vong and Liu, 2016).

Peptide and protein derived from okara have demonstrated antioxidant properties (Fang et al., 2021) and inhibitory activity against angiotensin-converting enzyme (ACE) (Nishibori et al., 2017). Okara constitutes an important source of dietary fiber and polysaccharides that have also shown prebiotic potential (Vong and Liu, 2016). The primary constituents of okara fiber include galacturonic acid, galactose, arabinose, glucose, xylose, fucose and a low amount of rhamnose and mannose (Mateos-Aparicio et al., 2010). Furthermore, phenolic compounds and isoflavones within okara are recognized as bioactive agents, demonstrating antioxidant and estrogenic activity (Faraj and Vasanthan, 2004). Traditionally, these valuable compounds have been extracted using organic solvents, which raise concerns regarding their toxicity and potential health risks when employed in food processing applications (Wiboonsirikul et al., 2013).

Over the past years, alternative extraction methodologies have been investigated as potential substitutes for conventional extraction procedures. Supercritical fluid extraction (SFE) stands out due to numerous advantages over traditional extraction methods, including selectivity, swiftness and automation, environmental friendliness and significant reduction in organic solvent consumption (Sethupathy et al., 2021). Carbon dioxide (CO_2) is the most commonly employed solvent in SFE since it is chemically inert, non-toxic, non-flammable, and recognized as a food-grade solvent (Brunner, 2005). These attributes have attracted the attention of industry and researchers, particularly in the food, pharmacy and cosmetics industries.

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Nevertheless, the low solubility of polar organic compounds in supercritical CO_2 (sc-CO_2) limits the application of SFE to the extraction of interesting okara compounds such as isoflavones (Rostagno et al., 2002). Isoflavones are heterocyclic phenols found in soybeans, considered as phytoestrogens due to their similar structure to estrogens and because they can influence sex hormone metabolism and their biological functions when ingested (Faraj and Vasanthan, 2004). Additionally, isoflavones are associated with the prevention of several age-related conditions, including human cancers, cardiovascular diseases, osteoporosis and post-menopausal symptoms (Křížová et al., 2019). The 12 main isoflavones are daidzin, genistin, glycitin, and their respective aglycone, acetyl, and malonyl forms (Zuo et al., 2008), all of them with a strong polar character and low solubility in neat sc-CO_2. The incorporation of a polar modifier (e.g., ethanol or methanol) into sc-CO_2 represents the simplest and most effective method to achieve the desired solvent polarity and increase the solubility of isoflavones. Modifiers can also mitigate interactions between the analyte and matrix, thereby enhancing the extraction efficiency of polar organic compounds (Rostagno et al., 2002).

The utilization of modified sc-CO_2 for the extraction of bioactive compounds from soybean and derivatives has been previously suggested in the past years. In the simplest configurations, the cosolvent can be added directly to the sample (Rostagno et al., 2002). However, using a second high-pressure pump to incorporate the cosolvent into the sc-CO_2 stream yields more reproducible results and facilitates operation, maintaining the polarity and other solvent characteristics unchanged throughout the extraction process (Quitain et al., 2006; Zuo et al., 2008). According to literature, optimal conditions for isoflavone extraction range from 30 to 60 MPa and 40–60 ºC, and the most used cosolvents are methanol, ethanol, and its aqueous mixtures, in concentrations up to 10 % mol in sc-CO_2. (Jokić, Nagy, et al., 2012; Quitain et al., 2006; Rostagno et al., 2002; Zuo et al., 2008). Technical viability of isoflavone extraction by sc-CO_2 has been previously investigated (Lummaetee et al., 2017), obtaining a maximum profitability of 46.18 $ per batch of 100 g of soybean meal using methanol-modified sc-CO_2 at 50 ºC and 60 MPa. Extraction temperature and solvent consumption stand out as the most relevant parameters affecting profitability (Lummaetee et al., 2017), although extractor capacity and system scale was not considered. Besides, downstream processing of isoflavone extracts should not be overlooked since the presence of water and methanol calls for costly and time-consuming solvent removal steps.

Several authors have studied the SFE of oil from unprocessed soybean meal and reported extraction kinetic data and the effect of extraction parameters on extract composition (Jokić et al., 2013; Jokić, Nagy, et al., 2012). However, SFE kinetics of okara is scarcely reported. Recently, Aussanasuwannakul et al. (2023) studied the valorization of okara by SFE and distinguished three stages in the extraction curve: the constant extraction rate (CER), the falling extraction rate (FER), and the diffusion-controlled (DC) periods. However, SFE was carried at 30 MPa and 50 ºC, not considering the effect of extraction parameters. This work attempts to present a more comprehensive and systematic approach, not only reporting the extraction kinetics but also employing modeling techniques to understand the impact of pressure, temperature and solvent polarity on the extraction yield, the extract composition and its bioactive properties. This information will constitute an important advancement for the development of alternative methods for okara valorization, and will improve the circularity of soybean processing.

2. Experimental

2.1. Materials

The okara used as raw material in this work was kindly supplied by Frías Nutrición S.A.U. (Burgos, Spain) with an average moisture content of 82.5 ± 0.1 %wt. As soon as obtained, the raw material was frozen and stored at −18 ºC. Since wet okara is very prone to microbial spoilage, small amounts were thawed and dried in short time, using an air convection oven at 45 ºC. Then, the batches of dried okara were collected and homogenized, constituting the sample for characterization and SFE experiments. Soxhlet extraction with n-hexane was performed in a Büchi B-811 automatic Soxhlet extractor, using the standard procedure with 2–3 g of sample and 100 mL n-hexane during 25 cycles, 10 min cleaning and 10 min drying, giving an oil content of 9.2 ± 0.2 %wt. Final moisture of dried and homogenized okara resulted in 3.6 ± 0.1 %wt., as determined by oven-drying at 105 ºC during 24 h.

2.2. Methods

2.2.1. Supercritical carbon dioxide extraction (SFE)

The sc-CO_2 extraction experiments were carried out in a laboratory SFE-plant. Further description and P&I diagram can be found elsewhere (Benito-Román et al., 2018). In a typical SFE experiment, 8.5 g of dry okara were loaded in the extractor (26.5 mL capacity, with ½" internal diameter). Two syringe pumps (ISCO 260D), that work alternately, provided an uninterrupted flow of CO_2 (Air Liquide, liquid CO_2 ≥ 99.9 %). The liquid CO_2 was first cooled down to −5 ºC by means of a cryostat and then compressed up to the desired operating working pressure. Then, the pressurized solvent was pre-heated up to the desired extraction temperature before entering the extractor, which was held in an oven to control the temperature within an accuracy of ± 0.5 °C. Pressure inside the extractor was controlled by means of a back-pressure regulator (BPR) valve (Dutch Technologies GRT8S). The outlet flow was collected in a cold trap and extraction yield was determined gravimetrically by measuring the extract weight at different time intervals.

Based on previous experiments and considering the maximum working pressure and temperature of the equipment, 9 experimental runs were carried out, varying pressure from 20 to 40 MPa, and temperature from 40 to 80 ºC. CO_2 mass flow was maintained at 10.5 ± 0.5 g/min, and extraction time was extended for 240 min in order to ensure the exhaustion of the okara samples.

In order to investigate the effect of solvent polarity on the SFE of bioactive compounds from okara, an additional set of experiments was performed incorporating ethanol (10 %wt.) as cosolvent by means of an HPLC pump (Gilson 5SC). Based on the results obtained in the previous runs, the cosolvent experiments were carried out at a fixed temperature of 40 ºC and pressures of 20 and 40 MPa. For these experiments, the extracts obtained at each time interval were collected separately and the extraction yield was determined gravimetrically after evaporating the remaining ethanol in a vacuum oven at 25 ºC.

2.2.2. Fatty acid profile

Analysis of the fatty acid profile of Soxhlet and SFE extracts was performed following the AOAC method 996.06–1996 (AOAC International, 2010) in a gas chromatography system (Hewlett Packard 6890 N) equipped with an auto-sampler (7683B series) and a flame ionization detector (FID). The chromatographic method has been previously described in detail (Rebolleda et al., 2013; Solaesa et al., 2014). Fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co.) and results were expressed as area percentage.

2.2.3. Total phenolic compounds

The total phenolic content of okara extracts was determined by the Folin-Ciocalteu method (Singleton et al., 1999). The okara extract was diluted in ethanol (5 mg/mL). Briefly, 500 μL of the diluted extract were mixed with 5.0 mL of water and then, 500 μL of the Folin-Ciocalteu reagent. After that, 1 mL of sodium carbonate 7.5 % (w/v) was added to the mixture. Samples were filtered and centrifuged, and the absorbance of optically clear supernatant was measured at 725 nm after 60 min of reaction in darkness (spectrophotometer V-750, Jasco, Japan). A calibration curve was prepared with standard solutions of gallic acid and results were expressed as mg of gallic acid equivalent (GAE)/g

extract.

2.2.4. Analysis of isoflavones

Identification and quantification of the isoflavones present in the okara extracts was carried out in an Agilent 1100 HPLC-DAD system. The equipment consisted of a degasser (G1322A), a quaternary pump (G1311A), an autosampler (G1329A), a column oven (G1316A) and a diode array detector (G1315A). The column used was a Kinetex® 5 μm Biphenyl 100 Å, 250 ×4.6 mm, with a guard column. The mobile phase was ammonium acetate 5 mM with acetic acid (1 % v/v) in water (solvent A) and ammonium acetate 5 mM with acetic acid (1 % v/v) in acetonitrile (solvent B). The composition of the mobile phase varied during the run according to a nonlinear gradient as follows: from 0 to 7 min 2 % of solvent B (isocratic), from 7 to 20 min from 2 % to 8 % solvent B, from 20 to 35 min from 8 % to 10 % solvent B, from 35 to 55 min 10–18 % solvent B, from 55 to 65 min 18–38 % solvent B, from 65 to 75 min 38–65 % solvent B, from 75 to 80 min 65–80 % solvent B, and post time of 10 min. Solvent flow rate was maintained at 0.8 mL/min during the whole run. Detection and quantification were performed at 254, 280, 330, and 370 nm. Samples were injected after extraction of isoflavones with methanol. In brief, 25 μL of okara extract were mixed with 975 μL of methanol and vortexed during 1 min. Subsequently, the mixture was centrifuged at 13,300 rpm for 5 min. Immediately afterwards, the methanolic phase was withdrawn and injected in the HPLC system. The Agilent OpenLab CDS software was employed to collect and analyze the chromatographic data delivered by the diode array detector, which was used to identify the different isoflavones by comparing retention times and spectral data with those of authentic standards: daidzin (TargetMol), glycitin (ChemCruz), genistin (TargetMol), daidzein (Thermo Scienctific), glycitein (Chengdu Biopurify Phytochemicals), genistein (Fluorochem), malonyldaidzin (ChemFaces) and malonylgenistin (ChemCruz). Individual stock solutions of these standards and their mixtures were prepared to plot the calibration curves, expressing the results as μg isoflavone/g extract.

2.2.5. Tocopherol profile

The identification and quantification of tocopherols present in the okara extracts were performed after isolation by solid-phase extraction (SPE) in the HPLC-DAD system described in Section 2.2.4. The SPE and chromatographic methods are described elsewhere (Rebolleda et al., 2012). The individual compounds of α-, β-, γ- and δ-tocopherols were monitored at 296 nm and identified by comparison of their retention times with those of the corresponding standards. Calibration curves relating peak area and concentration of the respective standard were also built for quantification. OpenLab CDS software was employed to collect and analyze the chromatographic data and results were expressed as μg of tocopherols/g extract.

2.2.6. Determination of antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed to evaluate the antioxidant activity of okara oil extracts. The free-radical scavenging activity was measured according to the methodology described by Benito-Román et al. (2018) with slight modifications. Briefly, the okara extracts were diluted in isooctane (20 mg/mL) and 0.5 mL were mixed with 2 mL of the DPPH solution (100 μM in isooctane). The quenching kinetics were measured at 517 nm and 25 °C using a Jasco V-750 spectrophotometer for 60 min. The decolouration percentages versus time (60 min) were compared with a calibration curve obtained using different dilutions of Trolox reagent, expressing the antioxidant activity as mmol of Trolox equivalents (meq Trolox)/g extract.

2.3. Modeling

The model proposed by Sovová (2005) was used to fit the experimental extraction curves. This model successfully describes the extractive curves of different seed oils (Alonso-Riaño et al., 2022; Benito-Román et al., 2018; Rebolleda et al., 2012). In the model of Sovová, the extraction yield is expressed as:

$$e = \frac{E}{N_m} \tag{1}$$

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

$$q = \frac{Q \cdot t}{N_m} \tag{2}$$

where Q is the solvent flow rate (kg/h) and t the extraction time (h).

The model of Sovová (2005) considers that extraction takes place in two stages. First, the easily accessible solute from broken cells is transferred directly to the fluid phase and extracted. In the second part of the extraction curve, the solute from intact cells diffuses first to broken cells and then to the fluid phase. This way, extraction curves are initially linear with a slope close to the value of oil solubility in CO_2 (Sovová, 2005). Assuming that the first part of the extraction curve is solubility-controlled, and the second part is controlled by diffusion, Eqs. 3 and 4 were proposed to evaluate the first and second part of the extraction curve, respectively:

$$e = q \cdot y_s; \text{ for } 0 \le q \le q_c \tag{3}$$

$$e = x_u[1 - C_1 \exp(C_2 \cdot q)]; \text{ for } q > q_c \tag{4}$$

where y_s is the initial solute concentration in the fluid phase, or the experimental oil solubility when no solvent-matrix interactions occur; q_c is the crossing point, x_u is the initial solute concentration in the insoluble solid (g oil/g IS), and C_1 and C_2 are adjusting constants. For experiments 1–9, x_u was estimated based on preliminary tests using Soxhlet extraction to determine the total extractable material, which was further adjusted based on the experimental yield achieved in the SFE process for experiments 10–11.

In this work, the adjustable parameters of the model were calculated by using the software Statgraphics Centurion 19 ×64 (version 19.4.01) using the Marquardt's algorithm. The mean relative deviations (MRD) were calculated using the Eq. 5 for each extraction curve:

$$\text{MRD}(\%) = \frac{1}{n}\sum_{i=1}^{n} \text{abs}\left(\frac{e_{exp} - e_{calc}}{e_{exp}}\right) \cdot 100 \tag{5}$$

where n is the number of experimental and calculated data points in each extraction curve.

2.4. Statistical analysis

SFE and Soxhlet experiments were duplicated to account for experimental uncertainties. Analysis of the extracts was performed at least in triplicate and statistically significant differences were evaluated by ANOVA (one-way) and Fisher's Least Significant Difference (LSD) at a $p < 0.05$ using the software Statgraphics Centurion 19 ×64 (version 19.4.01).

3. Results and discussion

3.1. Supercritical fluid extraction and modeling

The effect of extraction parameters on the SFE of okara was studied at three different temperatures (40, 60 and 80 ºC), and pressures (20, 30 and 40 MPa), giving 9 p-T combinations. From Fig. 1, it can be observed that an increase in pressure at a fixed temperature of 60 ºC promoted an increase in the initial extraction rate. The same was true for the other isotherms (40 and 80 ºC) and can be attributed to an increase of solvating power associated to the variation in CO_2 density with pressure

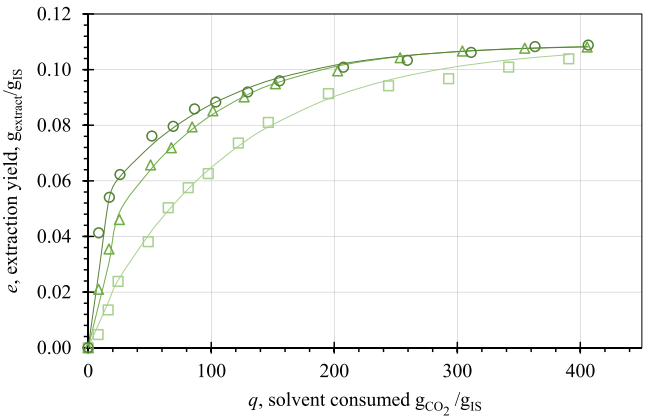

Fig. 1. Effect of pressure on the okara extraction kinetics by supercritical fluid extraction (SFE) with sc-CO_2 at 60 ºC. □: 20 MPa, △: 30 MPa, ○: 40 MPa. Lines are from the fitting of the Sovová's model.

and temperature.

Fig. 2 shows the effect of a) solvent density and b) pressure at constant temperature on the initial extraction rate of okara by SFE. At 40 ºC, CO_2 density slightly varies with pressure from 839.81 kg/m^3 at 20 MPa up to 956.07 kg/m^3 at 40 MPa (Span and Wagner, 1996), which translates into smaller variations of solubility and similar extraction profiles at this lower temperature. On the other hand, density variations with pressure at 60 and 80 ºC are more pronounced, ranging from 593.89 kg/m^3 at 20 MPa to 823 kg/m^3 at 40 MPa (Span and Wagner, 1996), which causes dramatic variations in the initial extraction rate and a significant effect of pressure on the extraction kinetics at these higher temperatures. Fig. 2b shows that increasing pressure translates into higher extraction rate for each isotherm. However, different trends were observed at the different pressures studied in this work. Specifically, at 20 MPa, an increase in temperature led to a reduction in the initial extraction rate. Conversely, the initial extraction rate increased when increasing temperature from 40 to 60 ºC at 30 MPa and then decreased at 80 ºC. Finally, increasing temperature at the highest pressure of 40 MPa led to increased solubility in all the temperature range, and especially from 40 to 60 ºC. This phenomenon aligns with established findings in the literature regarding SFE of oil from different seeds (Alonso-Riaño et al., 2022; Benito-Román et al., 2018; Rebolleda et al., 2012), and it is ascribed to the dual influence of temperature on the solubility of solids in sc-CO_2. Notably, increasing temperature results in a decrease in solvent density and, consequently, a reduction in the solvating power of sc-CO_2. Simultaneously, the solute's vapor pressure rises with temperature, facilitating its transfer to the solvent phase and increasing the extraction rate. Based on the outcomes of this study, it can be inferred that, at the lowest pressure of 20 MPa, the impact of temperature on solvent density predominates, while the significance of this effect diminishes at 30 and especially at 40 MPa, where it is compensated by the parallel increase in the vapor pressure of the solvent. Similar findings have been corroborated in previous studies (Alonso-Riaño et al., 2022; Rebolleda et al., 2012), which observed increased oil solubility with temperature when SFE was conducted at pressures around 30–40 MPa, where this typical crossover behavior is often observed.

Fig. 3 shows the effect of temperature on the SFE kinetics at constant pressure. Additionally, the cosolvent effect was tested at 40 ºC and two different pressures: 20 and 40 MPa (Fig. 3a and c). Compared to the extraction kinetics at the same conditions with neat sc-CO_2, the addition of 10 %wt. ethanol increased the initial extraction rate, especially at the lowest pressure of 20 MPa, whereas a slight increase was observed at 40 MPa. Final extraction yield with neat sc-CO_2 ranged from 0.1014 g extract/g IS at 20 MPa and 80 ºC to 0.1081 g extract/g IS at 30 MPa and 60 ºC (from 9.2 to 9.8 g extract/100 g okara, respectively). When ethanol was used as a cosolvent, final extraction yield did not significantly increase at the lowest pressure of 20 MPa (0.1035 g/g IS; 9.4 % wt.), whereas a slight increase was observed at 40 MPa, obtaining up to 0.1100 g of extract per g of insoluble solid loaded into the extraction vessel (9.9 g extract/100 g okara). On the other hand, Soxhlet extraction with n-hexane rendered a similar yield compared to SFE, with 9.2 ± 0.2 g extract/100 g okara. The extraction yield obtained in this work by Soxhlet extraction is slightly lower than others previously reported in the literature for okara, e.g. Ma et al. (1997) reported an oil content of 12.3 %wt. (0.1402 g extract/g IS), and Quitain et al. (2006) also reported a similar oil content (12.4 %wt., or 0.1416 g extract/g IS). However, the SFE yield obtained in this work is up to 3-fold higher than the obtained by Quitain et al. (2006) at similar conditions. Oil recovery by SFE was also higher in this work, with similar results for SFE and Soxhlet extraction with n-hexane. However, Quitain et al. (2006) found a maximum recovery of 63.5 % after 5 h of extraction at 20 MPa and 40 ºC and incorporating 10 %mol ethanol. The extraction kinetics shown in this work indicate that the amount of CO_2 needed to reach the extraction plateau is around 200–300 g CO_2 per g of insoluble solid loaded into the extractor, depending on the experimental conditions. However, Quitain et al. (2006), only used 17 g CO_2/g IS so higher extraction yields could have been likely obtained if experiments were performed at a higher solvent:mass ratios. Other authors have reported higher extraction yields for SFE of okara oil, reaching up to 18.5 ± 0.8 % wt. at 30 MPa and 50 ºC (Aussanasuwannakul et al., 2023). This value is similar to the yields reported for unprocessed soybean meal since Dobarganes Nodar

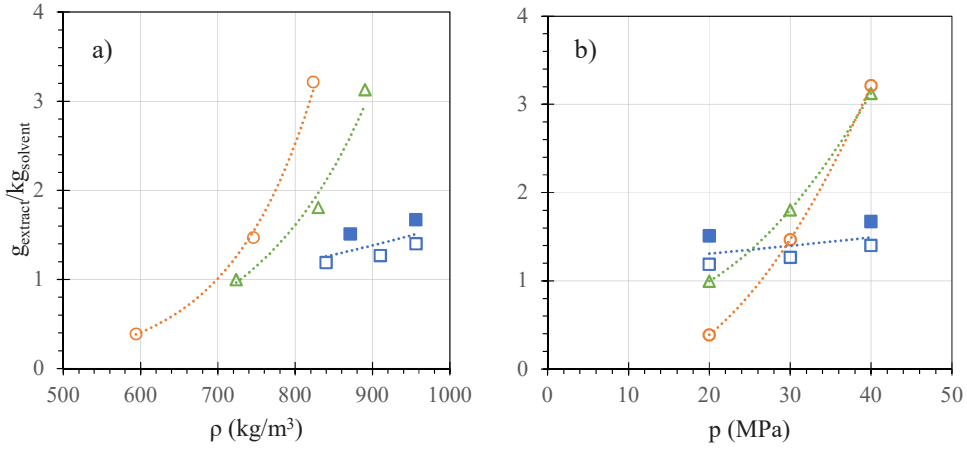

Fig. 2. Effect of a) solvent density and b) extraction pressure on the initial extraction rate of okara by supercritical fluid extraction (SFE). □■: 40 ºC, △: 60 ºC, ○: 80 ºC. Empty symbols: neat sc-CO_2; full symbols: ethanol 10 %wt. as cosolvent. Lines are drawn to guide the eye.

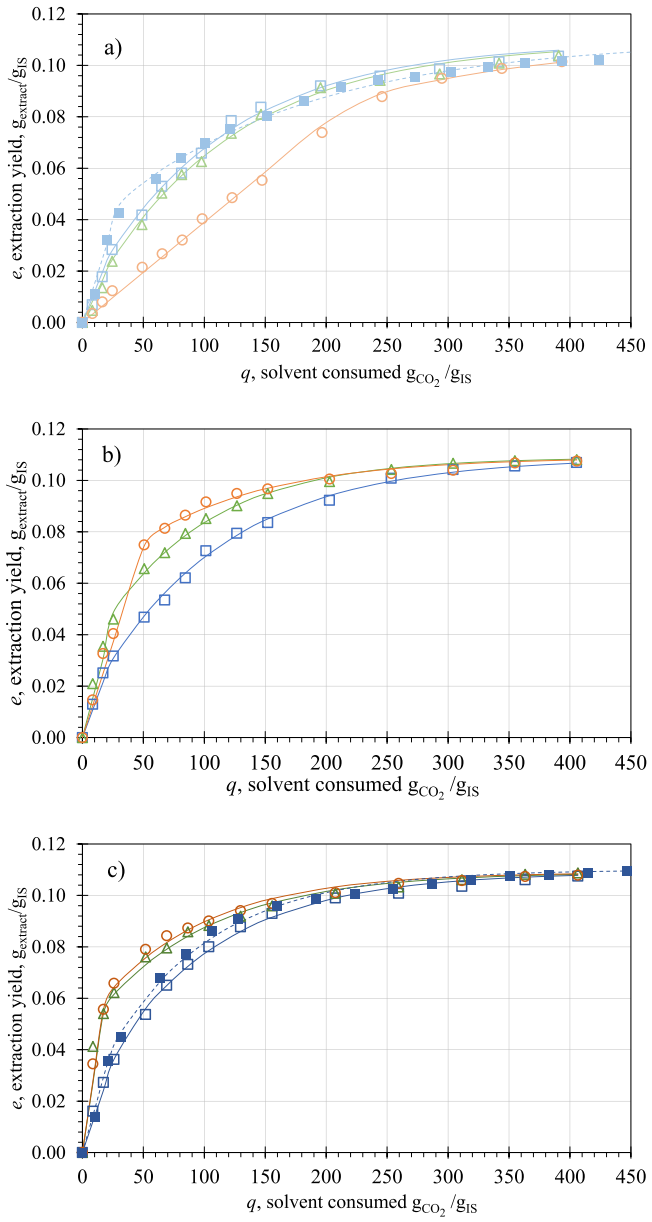

Fig. 3. Effect of temperature and cosolvent on the okara extraction kinetics by supercritical fluid extraction (SFE) at a) 20 MPa; b) 30 MPa; c) 40 MPa. □■: 40 ºC, △: 60 ºC, ○: 80 ºC. Empty symbols: neat sc-CO_2; full symbols: ethanol 10 % wt. as cosolvent. Lines are from the fitting of the Sovová's model.

et al. (2002) reported 19.5 % yield at 30 MPa and 40 ºC, using soybean seeds with 19.9 % oil according to Soxhlet extraction with n-hexane (Dobarganes Nodar et al. (2002)). Similar values were also reported for soybean cultivar "Ika" at higher pressures, with 19.3 ± 0.3 % at 40 MPa and 40 ºC, compared to the 20.1 ± 0.1 % obtained by Soxhlet extraction with n-pentane (Jokić, Nagy, et al., 2012). In general, the differences in extraction yield and recovery can be explained by extraction conditions and oil content of the starting material, which in turn depends on many factors, including soy variety, origin, and farming conditions. In the case of okara, some components of the lipid fraction could have been removed during soybean processing for soymilk production; therefore, direct comparison is difficult in many cases.

Model parameters for all the SFE experiments are shown in Table 1. Up to our knowledge, this is the first time that extraction kinetics are reported for SFE of okara with sc-CO_2. The Sovová's model parameters (y_S, C_1 and C_2) for okara are similar to previously published data for SFE

Table 1
Parameters of the Sovová's model for the supercritical fluid extraction (SFE) of okara oil.

Run	p (MPa)	T (ºC)	y_s ($g_{extract}/kg_{solvent}$)	q_c ($g_{solvent}/g_{IS}$)	C_1	C_2	MRD (%)
1		40	1.19	16.3	0.9243	0.0089	1.2
2	20	60	1.00	24.4	0.9589	0.0086	3.9
3		80	0.39	196.7	0.8791	0.0064	0.8
4		40	1.27	16.9	0.9090	0.0093	0.4
5	30	60	1.81	25.3	0.7433	0.0116	0.7
6		80	1.47	25.4	0.4796	0.0097	0.6
7		40	1.40	25.9	0.8474	0.0107	0.5
8	40	60	3.13	17.3	0.5743	0.0107	0.8
9		80	3.22	17.3	0.5501	0.0113	0.3
10*	20	40	1.51	20.2	0.6850	0.0061	1.3
11*	40		1.67	21.3	0.8311	0.0116	0.6

* Runs 10 and 11 were carried out with 10 %wt. ethanol as cosolvent.

of soybean oil and other seeds (Alonso-Riaño et al., 2022; Benito-Román et al., 2018; Jokić, Nagy, et al., 2012; Rebolleda et al., 2012), and satisfactorily describe the extraction curves. As previously mentioned, the fluid-phase solute concentration in the first stage of the extraction (y_S) varies with pressure and temperature following a cross-over behaviour. The amount of CO_2 that starts the depletion of the more accessible solute, q_c, ranges from 16.3 to 29.0 g CO_2/g IS in all SFE experiments except for run 3 that raises up to *ca.* 200 g CO_2/g IS due to the low density and hence low solubility at these conditions (20 MPa, 80 ºC), which extended the linear stage. For runs 1–9, the solute concentration in the insoluble solid, x_u, was fixed at 0.109 g/g IS according to Soxhlet and moisture analyses. However, for runs 10–11 x_u increased up to 0.110 g/g IS, taking into account the solubilization of more polar compounds due to the incorporation of ethanol.

3.2. Characterization of extracts

3.2.1. Fatty acid profile

Table 2 summarizes the results obtained in the analysis of the fatty acid profile of the okara extracts obtained in this work by Soxhlet extraction and by SFE at 30 MPa and 60 ºC. In general, no significant differences in the fatty acid profile were found between the extracts obtained at different SFE conditions (data not shown). Other authors have also reported that pressure and temperature do not significantly affect the fatty acid profile of SFE-extracted oil, although a moderate fractionation can be achieved by collecting the extracts obtained at different time intervals separately (Jokić et al., 2013). In this work, the analysis of the fatty acid profile indicated that the okara extracts are rich in polyunsaturated fatty acids (PUFAs) of the n-6 series, such as linoleic (C18:2) and γ-linolenic (C18:3). The most abundant fatty acid was linoleic acid, in amounts higher than 50 % no matter the extraction method or the SFE conditions used. Oleic acid, a monounsaturated fatty acid (MUFA), was the second most abundant fatty acid, in quantities around 20 %. Fatty acids from the linolenic series are important in okara since they constitute a source of omega-3 (Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, et al., 2010). Table 2 shows that sc-CO_2 was more efficient in the extraction of these compounds since the SFE extract contained 7 % and 0.3 % of γ- and α-linolenic isomers, respectively, while Soxhlet extraction with n-hexane provided slightly less γ-linolenic acid (ca. 6.5 %), and α-linolenic acid was not detected in the GC analysis.

In the literature, similar fatty acid profiles have been reported for soybean oil (Jokić et al., 2013) and other oils from other beans and soybean derivatives (Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, et al., 2010). In this work, the most distinctive feature of the okara extract obtained by SFE was the presence of *ca.* 5 % butyric acid as a representative of short-chain fatty acids (SCFA). The presence of this compound may be an indicative of fermentation by chiefly anaerobic

Table 2
Fatty acid profile of okara extracts obtained by Soxhlet extraction with n-hexane and supercritical fluid extraction (SFE) with sc-CO_2 at 30 MPa and 60 °C.

Trivial name	C:D	Fatty acid profile (Area %)	
		Soxhlet (n-hexane)	SFE (sc-CO_2 30 MPa, 60 °C)
Butyric acid	C4:0	0.4 ± 0.2	4.8 ± 0.8
Myristic acid	C14:0	0.4 ± 0.2	0.09 ± 0.01
Palmitic acid	C16:0	10.7 ± 0.5	9.3 ± 0.1
Stearic acid	C18:0	6.0 ± 0.5	5.2 ± 0.1
Oleic acid	C18:1 n−9	20.2 ± 0.1	19.8 ± 0.1
Vaccenic acid	C18:1 n−7	n.d.	1.15 ± 0.01
Linoleic acid	C18:2 n−6	52.3 ± 0.5	51.2 ± 0.4
α-linolenic acid	C18:3 n−3	n.d.	0.28 ± 0.09
γ-linolenic acid	C18:3 n−6	6.5 ± 0.5	7.16 ± 0.08
Stearidonic acid	C18:4 n−3	1.3 ± 0.5	0.05 ± 0.03
Arachidic acid	C20:0	0.18 ± 0.01	0.3 ± 0.1
Eicosadienoic acid	C20:2 n−6	2.0 ± 0.5	0.4 ± 0.2
Eicosatrienoic acid	C20:3 n−6	0.07 ± 0.03	0.3 ± 0.2
SCFA		0.4 ± 0.2	4.8 ± 0.8
SFA		18 ± 1	14.9 ± 0.3
MUFA		20.2 ± 0.1	21.1 ± 0.1
PUFA		60 ± 2	59 ± 1

SCFA: short-chain; SFA: saturated (except C4:0); MUFA: monounsaturated; PUFA: polyunsaturated fatty acids

bacteria (Mok et al., 2021) before the extraction, which is otherwise common in wet okara. Furthermore, butyric acid was less abundant in Soxhlet extracts, which is probably due to the high temperatures involved that promoted its partial loss by volatilization.

3.2.2. Total phenolic compounds and antioxidant activity

Total phenolic compounds determined in okara extracts are depicted in Fig. 4a, together with their antioxidant activity (Fig. 4b). From these results, it can be observed that the incorporation of 10 %wt. ethanol as cosolvent dramatically increased the extraction of total phenolic compounds, especially at 20 MPa and 40 °C with 26.8 ± 0.2 mg GAE/g extract, while less than 5 mg GAE/g extract were obtained without ethanol. Similar results have been reported in the literature (Quitain et al., 2006) and can be attributed to the increased polarity of the solvent mixture.

From Fig. 4b, it can be observed that increasing pressure at moderate temperatures led to increasing antioxidant activity. However, the highest temperature of 80 °C showed slightly lower antioxidant activity, which may be due to thermal degradation of some of the extracted bioactive compounds. As for TPC, the incorporation of 10 %wt. ethanol as cosolvent increased the antioxidant activity, especially in the extract obtained at 20 MPa and 40 °C. However, a direct correlation between TPC and antioxidant activity cannot be established in the samples with cosolvent, likely because not all the total phenolics determined in the extracts according to the Folin-Ciocalteu method presented antioxidant properties. Furthermore, the TPC analysis is subjected to several interferences, including the presence of coloured compounds that could have been extracted with the incorporation of the cosolvent, and its presence in the final extracts could have led to overestimations in the TPC of these samples. In any case, additional analysis of okara extracts is mandatory in order to obtain more information about the compounds responsible for their antioxidant properties. The following sections will present the results obtained in the determination of the isoflavone and tocopherol content and profile.

3.2.3. Isoflavone content

Table 3 summarizes the results obtained in the analysis of isoflavones contained in the okara extracts. As it can be seen and due to its low polarity, sc-CO_2 alone is not able to extract these polar compounds and only small amounts of isoflavones were detected. Results obtained with neat sc-CO_2 may indicate that isoflavone extraction is favored at lower temperatures, which is similar to previous findings (Quitain et al., 2006) and is consistent with the thermolabile character of isoflavones. For instance, Zuo et al. (2008) reported that increasing temperature from 40 to 70 °C drastically reduced the recovery of isoflavones at 50 MPa. The same authors found a positive correlation between extraction pressure and recovery of isoflavones in the range 30–60 MPa (Zuo et al., 2008), although the effect of pressure is not so clear in this work since isoflavone concentration in okara extracts was similar at 20 and 30 MPa and decreased at 40 MPa. On the other hand, we can clearly observe that the incorporation of ethanol as cosolvent and the increase in solvent polarity

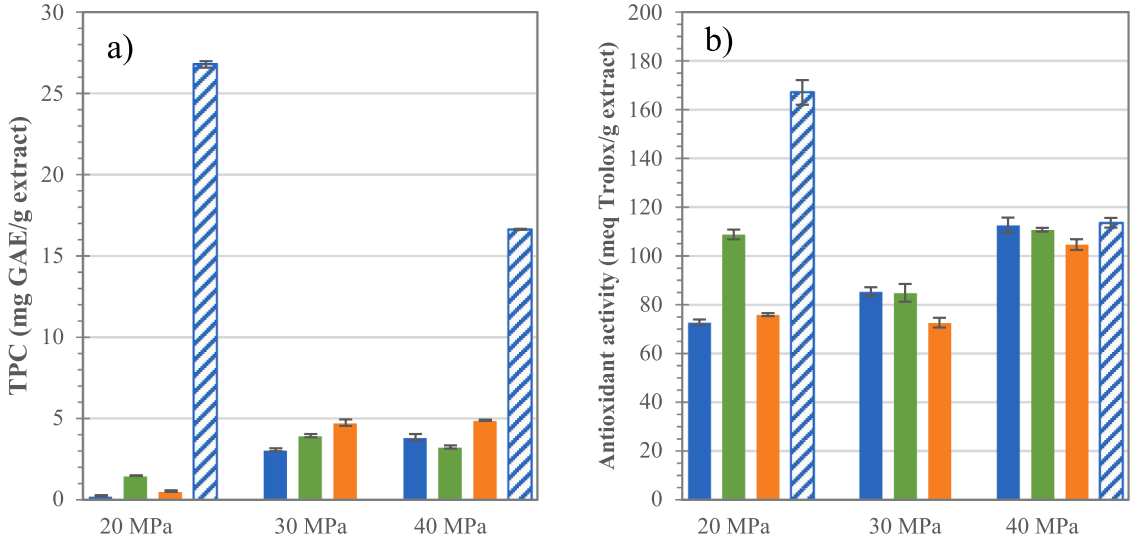

Fig. 4. a) Total phenolic compounds (TPC) and b) antioxidant activity of okara extracts obtained by supercritical fluid extraction (SFE) with sc-CO_2 at different extraction conditions. : 40 °C; : 60 °C; : 80 °C. : 40 °C and 10 %wt. ethanol.

Table 3
Isoflavones found in the okara extracts obtained by supercritical fluid extraction (SFE) with sc-CO_2 at different extraction conditions.

Compound name	Isoflavone concentration (μg/g extract)										
	20 MPa			30 MPa			40 MPa			40 °C, 10 %wt. ethanol	
	40 °C	60 °C	80 °C	40 °C	60 °C	80 °C	40 °C	60 °C	80 °C	20 MPa	40 MPa
Daidzin	2.6 ± 0.2^{c}	3.3 ± 0.2^{b}	3.9 ± 0.2^{b}	2.6 ± 0.2^{c}	1.21 ± 0.04^{f}	1.35 ± 0.07^{e}	1.43 ±0.09^{e}	1.15 ± 0.08^{f}	1.66 ± 0.09^{d}	5.5 ± 0.4^{a}	4.87 ± 0.3^{a}
Glycitin	0.14 ± 0.01^{c}	0.03 ± 0.01^{f}	0.05 ± 0.01^{e}	0.09 ± 0.01^{d}	0.02 ± 0.01^{f}	0.02 ± 0.01^{f}	0.03 ± 0.01^{f}	n.d.	0.29 ± 0.03^{b}	0.33 ± 0.01^{b}	0.37 ± 0.02^{a}
MalonylDaidzin	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}	0.02 ± 0.01^{c}	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}	0.05 ± 0.03^{c}	0.11 ± 0.01^{c}	0.07 ± 0.01^{c}	0.24 ± 0.01^{b}	8.1 ± 0.5^{a}	8.03 ± 0.3^{a}
Genistin	0.31 ± 0.01^{c}	0.27 ± 0.01^{c}	0.14 ± 0.01^{d}	0.49 ± 0.03^{b}	0.25 ± 0.01^{c}	0.15 ± 0.08^{d}	0.16 ± 0.06^{d}	0.44 ± 0.02^{b}	0.26 ± 0.01^{c}	0.59 ± 0.04^{a}	0.43 ± 0.02^{b}
MalonylGenistin	2.6 ± 0.1^{c}	1.14 ± 0.04^{g}	0.60 ± 0.02^{h}	3.7 ± 0.2^{a}	3.1 ± 0.1^{b}	2.1 ± 0.1^{de}	2.0 ± 0.1^{e}	2.4 ± 0.2^{cd}	2.0 ± 0.1^{e}	1.75 ± 0.08^{f}	2.45 ± 0.1^{c}
Daidzein	0.07 ± 0.01^{h}	0.12 ± 0.01^{g}	0.06 ± 0.01^{h}	0.12 ± 0.01^{g}	0.51 ± 0.02^{c}	0.42 ± 0.02^{d}	0.28 ± 0.02^{f}	0.35 ± 0.02^{e}	0.27 ± 0.02^{f}	23.1 ± 0.4^{a}	17.66 ± 0.2^{b}
Glycitein	2.9 ± 0.3^{b}	2.6 ± 0.2^{bc}	3.0 ± 0.2^{b}	3.0 ± 0.2^{b}	3.9 ± 0.1^{a}	2.51 ± 0.03^{c}	0.83 ± 0.03^{f}	1.22 ± 0.06^{e}	0.74 ± 0.03^{g}	3.2 ± 0.1^{b}	2.11 ± 0.1^{d}
Genistein	1.6 ± 0.3^{c}	0.61 ± 0.02^{d}	0.27 ± 0.02^{f}	1.16 ± 0.08^{c}	0.41 ± 0.02^{e}	0.52 ± 0.04^{d}	0.69 ± 0.04^{d}	0.40 ± 0.02^{e}	0.10 ± 0.01^{g}	13.8 ± 0.7^{a}	10.34 ± 0.4^{b}
Total	10.4 ± 0.9^{cd}	8.1 ± 0.5^{e}	8.0 ± 0.5^{e}	11.2 ± 0.7^{c}	9.4 ± 0.3^{d}	7.1 ± 0.3^{e}	5.5 ± 0.4^{f}	6.0 ± 0.4^{f}	5.6 ± 0.3^{f}	56 ± 2^{a}	46 ± 1^{b}
Total aglycones (Agly)	4.6 ± 0.6^{cd}	3.3 ± 0.2^{e}	3.3 ± 0.2^{e}	4.2 ± 0.3^{d}	4.8 ± 0.1^{c}	3.4 ± 0.1^{e}	1.8 ± 0.1^{f}	2.0 ± 0.1^{f}	1.1 ± 0.1^{g}	40 ± 1^{a}	30.1 ± 0.7^{b}
Total glucosides (Glu)	5.8 ± 0.3^{c}	4.8 ± 0.3^{d}	4.7 ± 0.3^{d}	7.0 ± 0.4^{b}	4.6 ± 0.2^{d}	3.7 ± 0.2^{f}	3.7 ± 0.3^{f}	4.0 ± 0.3^{ef}	4.5 ± 0.2^{de}	16 ± 1^{a}	16.2 ± 0.7^{a}
Ratio Glu/Agly	1.25	1.43	1.42	1.64	0.97	1.06	2.07	2.06	3.97	0.40	0.54

Different letters in each row denote statistically significant differences at $p < 0.05$.

enhanced isoflavone extraction. As Table 3 shows, up to 10 times more isoflavones were quantified in the extracts obtained by using 10 %wt. ethanol, compared to the same conditions without cosolvent. Nevertheless, these results are lower than others previously reported in the literature. Rostagno et al. (2002) obtained up 86.28 μg of isoflavones per g of soybean flour at 36 MPa, 50 °C and 10 %mol methanol:water (70:30 v/v) and Quitain et al. (2006) reported up to 170 μg isoflavones/g okara extract at 30 MPa, 40 °C and 5 %mol ethanol. As in the case of the differences found in the extraction yield, comparisons should be carefully made since the isoflavone content may vary depending on the soybean variety and processing conditions. Additionally, the polar isoflavones are even more to be removed during soymilk production. According to different authors, about 12–40 % of the isoflavones in soybeans are retained in okara during soymilk production (Jackson et al., 2002; Jankowiak et al., 2014).

Lastly, it is noticeable that aglycones, the less polar compounds among isoflavones, experienced a higher increase in their extraction yield and reversed the ratio glucosides/aglycones to less than 1. This phenomenon indicates that moderately polar compounds such as isoflavone aglycones can be more easily extracted by ethanol-modified sc-CO_2. However, the total solubilization of the more polar and complex isoflavone glycosides requires solvents with higher polarity. Some authors have obtained good isoflavone yields by using methanol, ethanol, or hydroalcoholic solutions (Zuo et al., 2008). However, the presence of water and the toxicity of methanol force the development of further downstream processing, which is often costly, time-consuming and can lead to bioactivity loss of the extract.

3.2.4. *Tocopherols*

Tocopherol profiles found in okara extracts obtained by SFE are reported in Table 4. Comparison with previously reported data of tocols content in soybean oil extracted by SFE (Jokić, Vidović, et al., 2012) reflects important similarities. Generally, the most abundant tocopherol isomer in soybean oil is γ-tocopherol, as it has been found for all the extracts obtained in this work. On the other hand, β-tocopherol is almost non-existent in soybean oil, being usually not detected or found in quantities below the limit of detection of the analytical method. Regarding the effect of extraction conditions on the tocopherols profile, Table 4 shows that extraction of tocopherols is favoured by increasing pressure at mild temperatures.

Table 4
Tocopherols found in the okara extracts obtained by supercritical fluid extraction (SFE) at different extraction conditions.

Run	p (MPa)	T (°C)	Tocopherols (μg/g extract)				
			α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total
1		40	19 ± 6^{bcde}	n.q.	113 ± 1^{e}	21 ± 1^{d}	153 ± 8^{c}
2	20	60	28 ± 6^{b}	n.q.	151 ± 2^{c}	56 ± 1^{c}	235 ± 9^{b}
3		80	16 ± 2^{e}	n.q.	50 ± 1^{g}	22 ± 1^{d}	92 ± 4^{d}
4		40	24 ± 1^{cd}	11 ± 1^{b}	129 ± 4^{d}	80 ± 7^{b}	244 ± 13^{b}
5	30	60	31 ± 2^{b}	14 ± 1^{a}	139 ± 7^{d}	73 ± 3^{b}	258 ± 12^{b}
6		80	17 ± 1^{e}	n.q.	45 ± 1^{h}	50 ± 6^{c}	112 ± 8^{d}
7		40	26 ± 1^{c}	n.q.	131 ± 2^{d}	78 ± 6^{b}	236 ± 9^{b}
8	40	60	25 ±1^{c}	11 ± 1^{b}	198 ± 3^{a}	72 ± 3^{b}	307 ± 8^{a}
9		80	22 ± 1^{d}	n.q.	65 ± 2^{f}	58 ± 3^{c}	145 ± 6^{c}
10*	20	40	43 ± 1^{a}	7 ± 1^{c}	133 ± 4^{d}	97 ± 6^{a}	280 ± 12^{a}
11*	40		41 ± 1^{a}	7 ± 1^{c}	175 ± 4^{b}	70 ± 9^{b}	292 ± 15^{a}

Different letters in each column denote statistically significant differences at $p < 0.05$.
n.q.: non-quantifiable (below limit of detection).
* Runs 10 and 11 were carried out with 10 %wt. ethanol as cosolvent.

The highest content of total tocopherols was found at 40 MPa and 60 ºC, with 0.307 ± 0.004 mg tocols/g extract. This trend is also found in the literature for SFE of soybean (Jokić et al., 2013) and other seeds (Benito-Román et al., 2018). High extraction temperatures likely promoted the degradation of tocopherols, since the lowest content at each pressure was found at the highest extraction temperature (80 ºC). The addition of cosolvent promoted an increase in the tocopherol extraction at 20 MPa and 40 ºC, compared to the runs carried out at the same conditions with neat sc-CO_2. However, the increased polarity of the solvent was not a determining factor for enhancing the extraction of this low-polarity compounds, as reflected in the non-significant differences found at 40 MPa and 40 ºC.

4. Conclusions

The findings of this study have significant implications for sustainable food processing and byproduct valorization. The results obtained in this work show that supercritical fluid extraction (SFE) with supercritical carbon dioxide can be used as an eco-friendly alternative to conventional solvent extraction methods for the recovery of the lipid fraction and bioactive compounds from okara. Extraction yield by SFE was similar to conventional Soxhlet extraction with n-hexane. However, the ability of SFE to preserve short-chain fatty acids and coextract phenolic compounds and isoflavones, particularly with ethanol as a cosolvent, suggests that SFE method can yield extracts with enhanced bioactive properties, such as improved antioxidant capacity, better stability and higher added-value. This enhances the value of okara, traditionally considered a waste byproduct, and opens new prospects for its application in functional foods, nutraceuticals, and cosmetics. Additionally, the successful correlation of experimental data to Sovová's model presents an effective tool for process optimization, facilitating more efficient extraction procedures and aiding in the scale-up of this green technology for future industrial applications.

Looking forward, several promising research directions emerge from this work. Prior to the scaling up of the process and the industrial application, the techno-economic analysis, and the analysis of the environmental impacts of SFE, compared to traditional extraction methods, will help solidify its position as a sustainable extraction technology. Future prospects also include exploring the extraction of other bioactive compounds in okara and investigating its bioavailability and health benefits through *in vivo* studies, which will pave the way for their inclusion in functional foods and nutraceutical products.

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CRediT authorship contribution statement

Sagrario Beltrán: Writing – review & editing, Funding acquisition. **María Teresa Sanz:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Pedro Barea:** Writing – review & editing, Methodology, Investigation. **Elixabet Díaz-de-Cerio:** Writing – review & editing, Methodology, Investigation. **Alba Ester Ester Illera:** Writing – review & editing, Methodology, Investigation. **Rodrigo Melgosa:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alonso-Riaño, P., Melgosa, R., Trigueros, E., Illera, A.E., Beltrán, S., Sanz, M.T., 2022. Valorization of brewer's spent grain by consecutive supercritical carbon dioxide extraction and enzymatic hydrolysis. Food Chem. *396* (November 2021). https://doi.org/10.1016/j.foodchem.2022.133493.

Aussanasuwannakul, A., Boonbumrung, S., Pantoa, T., 2023. Valorization of soybean residue (Okara) by supercritical carbon dioxide extraction: compositional, physicochemical, and functional properties of oil and defatted powder. Foods *12* (14). https://doi.org/10.3390/foods12142698.

Benito-Román, O., Rodríguez-Perrino, M., Sanz, M.T., Melgosa, R., Beltrán, S., 2018. Supercritical carbon dioxide extraction of quinoa oil: study of the influence of process parameters on the extraction yield and oil quality. J. Supercrit. Fluids *139*. https://doi.org/10.1016/j.supflu.2018.05.009.

Brunner, G., 2005. Supercritical fluids: technology and application to food processing. J. Food Eng. *67* (1–2), 21–33. https://doi.org/10.1016/j.jfoodeng.2004.05.060.

Dobarganes Nodar, M., Molero Gómez, A., Martínez de la Ossa, E., 2002. Characterisation and process development of supercritical fluid extraction of soybean oil. Food Sci. Technol. Int. *8* (6), 337–342. https://doi.org/10.1106/108201302031651.

Fang, J., Lu, J., Zhang, Y., Wang, J., Wang, S., Fan, H., Zhang, J., Dai, W., Gao, J., Yu, H., 2021. Structural properties, antioxidant and immune activities of low molecular weight peptides from soybean dregs (Okara). Food Chem.: X *12*, 100175. https://doi.org/10.1016/j.fochx.2021.100175.

Faraj, A., Vasanthan, T., 2004. Soybean isoflavones: effects of processing and health benefits. Food Rev. Int. *20* (1), 51–75. https://doi.org/10.1081/FRI-120028830.

International, A. (2010). *AOAC 996.06-1996, Fat (Total, Saturated, Unsaturated and Monounsaturated) in Foods. Hydrolytic extraction Gas Chromatographic Method.*

Jackson, C.J.C., Dini, J.P., Lavandier, C., Rupasinghe, H.P.V., Faulkner, H., Poysa, V., Buzzell, D., DeGrandis, S., 2002. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. Process Biochem. *37* (10), 1117–1123. https://doi.org/10.1016/S0032-9592(01)00323-5.

Jankowiak, L., Trifunovic, O., Boom, R.M., Van Der Goot, A.J., 2014. The potential of crude okara for isoflavone production. J. Food Eng. *124*, 166–172. https://doi.org/10.1016/j.jfoodeng.2013.10.011.

Jokić, S., Nagy, B., Zeković, Z., Vidović, S., Bilić, M., Velić, D., Simándi, B., 2012. Effects of supercritical CO 2 extraction parameters on soybean oil yield. Food Bioprod. Process. *90* (4), 693–699. https://doi.org/10.1016/j.fbp.2012.03.003.

Jokić, S., Sudar, R., Svilovic, S., Vidovic, S., Bilic, M., Velic, D., Jurkovic, V., 2013. Fatty acid composition of oil obtained from soybeans by extraction with supercritical carbon dioxide. Czech J. Food Sci. *31* (2), 116–125. https://doi.org/10.17221/8/2012-cjfs.

Jokić, S., Vidović, S., Zeković, Z., Kuzmanović, S.P., Jevrić, L., Marić, B., 2012. Chemometric analysis of tocopherols content in soybean oil obtained by supercritical CO2. J. Supercrit. Fluids *72*, 305–311. https://doi.org/10.1016/j.supflu.2012.10.008.

Khare, S.K., Jha, K., Gandhi, A.P., 1995. Citric acid production from Okara (soy-residue) by solid-state fermentation. Bioresour. Technol. *54* (3), 323–325. https://doi.org/10.1016/0960-8524(95)00155-7.

Křížová, L., Dadáková, K., Kašparovská, J., Kašparovský, T., 2019. Isoflavones. Molecules *24* (6). https://doi.org/10.3390/molecules24061076.

Lummaetee, K., Ku, H.M., Wongrat, W., Elkamel, A., 2017. Optimization of supercritical fluid extraction of isoflavone from soybean meal. Can. J. Chem. Eng. *95* (6), 1141–1149. https://doi.org/10.1002/cjce.22786.

Ma, C.-Y., Liu, W.-S., Kwokb, K.C., Kwokb, F., 1997. Isolation and characterization of proteins from soymilk residue (okara)*. Food Res. Int. *29* (8), 199–805.

Mateos-Aparicio, I., Redondo-Cuenca, A., Villanueva-Suárez, M.J., 2010. Isolation and characterisation of cell wall polysaccharides from legume by-products: Okara (soymilk residue), pea pod and broad bean pod. Food Chem. *122* (1), 339–345. https://doi.org/10.1016/j.foodchem.2010.02.042.

Mateos-Aparicio, I., Redondo-Cuenca, A., Villanueva-Suárez, M.J., Zapata-Revilla, M.A., Tenorio-Sanz, M.D., 2010. Pea pod, broad bean pod and okara, potential sources of functional compounds. Lwt *43* (9), 1467–1470. https://doi.org/10.1016/j.lwt.2010.05.008.

Mok, W.K., Tan, Y.X., Chen, W.N., 2021. Evaluating the potential of Bacillus subtilis fermented okara as a functional food ingredient through in vitro digestion and

fermentation. Food Biotechnol. *35* (2), 136–157. https://doi.org/10.1080/08905436.2021.1909615.

Nishibori, N., Kishibuchi, R., Morita, K., 2017. Soy pulp extract inhibits angiotensin I-converting enzyme (ACE) activity in vitro: evidence for its potential hypertension-improving action. J. Diet. Suppl. *14* (3), 241–251. https://doi.org/10.1080/19390211.2016.1207744.

Quitain, A.T., Oro, K., Katoh, S., Moriyoshi, T., 2006. Recovery of oil components of okara by ethanol-modified supercritical carbon dioxide extraction. Bioresour. Technol. *97* (13), 1509–1514. https://doi.org/10.1016/j.biortech.2005.06.010.

Rebolleda, S., Beltrán, S., Sanz, M.T., González-Sanjosé, M.L., Solaesa, Á.G., 2013. Extraction of alkylresorcinols from wheat bran with supercritical CO 2. J. Food Eng. *119* (4), 814–821. https://doi.org/10.1016/j.jfoodeng.2013.07.008.

Rebolleda, S., Rubio, N., Beltrán, S., Sanz, M.T., González-Sanjosé, M.L., 2012. Supercritical fluid extraction of corn germ oil: study of the influence of process parameters on the extraction yield and oil quality. J. Supercrit. Fluids *72*, 270–277. https://doi.org/10.1016/j.supflu.2012.10.001.

Rostagno, M.A., Araújo, J.M.A., Sandi, D., 2002. Supercritical fluid extraction of isoflavones from soybean flour. Food Chem. *78* (1), 111–117. https://doi.org/10.1016/S0308-8146(02)00106-1.

Sethupathy, P., Dutta, S., Moses, J.A., Anandharamakrishnan, C., 2021. Progress in supercritical extraction of nutraceuticals from herbs and spices. Innov. Food Process. Technol.: Compr. Rev. 567–583. https://doi.org/10.1016/B978-0-08-100596-5.22964-8.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. *299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

Solaesa, Á.G., Bucio, S.L., Sanz, M.T., Beltrán, S., Rebolleda, S., 2014. Characterization of triacylglycerol composition of fish oils by using chromatographic techniques. J. Oleo Sci. *63* (5), 449–460. https://doi.org/10.5650/jos.ess13202.

Sovová, H., 2005. Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation. J. Supercrit. Fluids *33* (1), 35–52. https://doi.org/10.1016/j.supflu.2004.03.005.

Span, R., Wagner, W., 1996. A new equation of state for carbon dioxide covering the fluid region from the triple-point temperature to 1100 K at pressures up to 800 MPa. J. Phys. Chem. Ref. Data *25* (6), 1509–1596. https://doi.org/10.1063/1.555991.

Vong, W.C., Liu, S.Q., 2016. Biovalorisation of okara (soybean residue) for food and nutrition. Trends Food Sci. Technol. *52*, 139–147. https://doi.org/10.1016/j.tifs.2016.04.011.

Wiboonsirikul, J., Mori, M., Khuwijitjaru, P., Adachi, S., 2013. Properties of extract from okara by its subcritical water treatment. Int. J. Food Prop. *16* (5), 974–982. https://doi.org/10.1080/10942912.2011.573119.

Zuo, Y.B., Zeng, A.W., Yuan, X.G., Yu, K.T., 2008. Extraction of soybean isoflavones from soybean meal with aqueous methanol modified supercritical carbon dioxide. J. Food Eng. *89* (4), 384–389. https://doi.org/10.1016/j.jfoodeng.2008.05.004.