SUPERCRITICAL FLUID EXTRACTION OF WHEAT BRAN OIL. STUDY OF EXTRACTION YIELD AND OIL QUALITY

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Running Title

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Abstract

The supercritical fluid extraction (SFE) of wheat bran oil with pure supercritical carbon dioxide at different extraction pressure (25, 40 and 55 MPa) and temperature (40, 70 and 95 ºC) has been studied. Since wheat bran is characterized by having an important content of bioactive compounds such as alkylresorcinols (AR) and other phenolic compounds, the content of such compounds in the extracted oil was evaluated in order to establish oil quality. The influence of extraction pressure and temperature and wheat bran moisture on the extraction yield and oil quality was studied. Oil quality was evaluated through parameters such as AR content and profile, total polyphenols index (TPI), antioxidant activity and fatty acids profile. SFE performed over fresh wheat bran at 55 MPa and 95 ºC was found to provide wheat bran oil with the highest AR and phenolic content and antioxidant capacity of all the runs performed in this work. Some SFE experiments focused on oil fractionation showed that this technique can provide oil fractions enriched with AR and phenolic compounds.

1. Introduction

Wheat bran is a by-product obtained in the processing of wheat flour and it is characterized by having an important content of biological active compounds like alkylresorcinols (AR) and other polyphenols [1-3].

Alkylresorcinols are phenolic lipids, 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring. They are found in the external layers of cereals, like wheat and rye, and in other plants like rice shoots, mango latex and peel, cashew nuts, etc. AR can be also produced by some microorganisms [4,5].

Alkylresorcinols have been reported to have antibacterial, antifungal, and antiparasitic activities [4,6,7]. Also their antioxidant activity, their effect on different enzymes as inhibitors and their anticancer and antimutagenic properties have been described [5,8,9]. AR could be also used to authenticity control of cereal products [10] and as biomarkers of the cereal intake due to its absorption by the human body [11]. Due to all these properties, extracts with high amounts of AR could be of interest in the food industry.

Cereal bran extracts containing AR have been obtained by using several solvent systems combined with different processes [12-15], although the disadvantages associated with the use of organic solvents make advisable to look for alternative extraction techniques. In recent years, supercritical fluid extraction (SFE) has been applied for the extraction of compounds from natural sources due to the several advantages reported for this type of extraction [16]: high selectivity, short extraction times, possibility of using GRAS (generally recognized as safe) and cheap solvents as carbon dioxide, etc. [17]. Most of the previous works on SFE applied to cereal bran have dealt with the use of supercritical CO_2 (SC-CO_2) as solvent together with some cosolvents that help to modify the polarity of the solvent system. Extraction of rye bran with cosolvents has been reported to be at least as effective as traditional organic extraction [18-20]. In some cases, AR have been concentrated using two stage SC-CO_2-extraction, a first SFE stage with pure SC-CO_2 [21] or modified with very low amount of ethanol (0.06 %) [22] for removing the non-polar lipids, and a second SFE stage using ethanol as cosolvent of the CO_2 for obtaining a polar fraction where AR were concentrated [21,22]. SFE of wheat bran has been less reported than rye bran extraction, but some previous results obtained by our research group have shown that rich AR wheat bran oil can be obtained by using pure SC-CO_2 [23]. Furthermore, some studies on wheat bran oil quality have shown the oil obtained by

SFE to have a slightly better quality than hexane extracted oil in terms of acidity, peroxide value and antioxidant activity [24].

The aim of this work was to study the quality of the wheat bran oil obtained by using pure SC-CO_2 under different conditions as extraction solvent. Parameters such as AR content and profile, total polyphenols index (TPI), antioxidant activity, and fatty acids profile were evaluated. Additionally, the influence of some process parameters, such as pressure and wheat bran moisture, not evaluated in our previous work [23] were addressed in this work. Also the range of extraction temperatures studied was increased in this work since previously [23] this parameter was shown to have a significant influence on the extraction yield and oil quality.

2. Materials and Methods

2.1. Raw material

The raw material used in this work was wheat bran (T*riticum aestivum L.*) kindly provided by HASENOSA (Spain). Fresh and dried bran were used. Fresh bran was dried in an oven at 70 ºC during 48 h for those experiments that required dried bran.

2.2. Solvents and reagents

Liquid CO_2 (≥ 99.9%) was provided by Carburos metálicos (Barcelona, Spain), methanol (≥ 99.9%) by LabScan (Gliwice, Poland), ethanol (≥ 99.9%) by Merck (Darmstadt, Alemania), Fast Blue RR salt, olivetol, AR standards, ABTS (2,29-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and fatty acid methyl esters by Sigma-Aldrich Co. (St. Louis, MO, USA), potassium carbonate (K_2CO_3) and potassium persulphate

($K_2O_8S_2$) by Panreac (Barcelona, Spain) and methyl tricosanoate by Larodan (Malmö, Sweeden).

2.3. Supercritical fluid extraction equipment and procedure

The extraction experiments were carried out in a semi-pilot SFE-plant whose P&I diagram has been presented elsewhere [25]. The usual elements of a SFE-plant with solvent recycling were installed, i.e.: pump, extractor, separator, heating and cooling systems and pressure dampers; rupture disks and safety valves were installed for safety and instruments for measurement and control of the process parameters.

In a SFE experiment, 300 g of wheat bran were placed in the extractor (2 L capacity) that was later pressurized with CO_2 up to the extraction pressure. Then, the solvent was circulated at the desired extraction temperature, *T*, with a solvent flow of 9 ± 1 kg CO_2/h and during an extraction time of 120 min. This extraction time was chosen based in previous work [23] to circulate enough amount of SC-CO_2 for completing extraction of the soluble compounds. The solvent was continuously recycled to the extractor after removing the solute in the separator that was kept at 4.9 ± 0.6 MPa and 24 ± 2 ºC.

Co-extracted water was removed from the extracts by vacuum evaporation at 45 ºC during 90 min using a Heidolph VV2000 rotary evaporator (Schwabach, Germany). Water-free oils were stored at -20 ºC until analysis.

Table 1 shows the experimental conditions used to evaluate the influence of wheat bran moisture (R1 and R6) and extraction pressure and temperature (R2 to R10) on the oil yield and quality. A two-stage extraction (R11) was carried out in order to obtain a rich AR oil fraction. The extraction time for each stage was 60 min. The rest of the

extraction conditions for each stage are shown in Table 1. All the runs were performed in triplicate and the results are given as average values ± standard absolute deviation.

2.4. Analytical methods

Determination of total AR

The total AR content in the extracted material was determined by a colorimetric method based on the use of Fast Blue RR salt [26]. A stock solution of 0.05% Fast Blue RR reagent was used to prepare a working solution by mixing 1 part of stock reagent with 4 parts of methanol. Aliquots (20 μL) of ethanol solution of wheat bran oil (5 mg/mL) were placed in assay tubes and made up to 200 μL with ethanol. Then, 2 mL of the working solution and 10 μL of a 10% K_2CO_3 solution were also added to each tube. Absorbance of the reaction mixture was measured at 480 nm after 20 min in a Hitachi U-2000 spectrophotometer (Tokyo, Japan). Olivetol (5-pentylresorcinol) was used as standard.

Determination of AR profile

Alkylresorcinols were determined according to a modification of Knölder et al. [27] method using an Agilent HPLC (series 1100) equipped with ChemStation software, a degasser (G1322A), a quaternary pump (G1311A), an autosampler (G1329A), a column oven (G1316A) a diode array detector (G1315A) and a mass spectrometry detector (G1916A) with an APcI source. The column used was Kromasil C18-5 250 x 4.6 mm and operated at 25 ºC. The mobile phase was methanol (A) and water (B) and the following gradient was used: 2% B to 0% B in 10 min. The total run time was 100 min. 100 μL of methanolic solutions of wheat bran oil (10 mg/mL) were injected**.** . All AR were monitored at 280 nm at a flow rate of 0.6 mL/min.

Positive-ion mass spectra of the column eluate compounds were recorded in the range m/z 100–500. Nitrogen was used both as the drying gas at a flow rate of 10 L/min and as the nebulizing gas at a pressure of 380 Pa. The nebulizer temperature was set at 350 ºC and a potential of 4000 V was used on the capillary.

Individual compounds were identified by their mass spectra [27] and quantified using a calibration curve of the corresponding standard compounds: $C_{21}H_{36}O_2$ (AR-C15), $C_{23}H_{40}O_2$ (AR-C17), $C_{25}H_{44}O_2$ (AR-C19) and $C_{31}H_{56}O_2$ (AR-C25). A linear relationship between the number of carbons of the alkyl chain and the response factor was found and it was used to calculate the response factor of the AR that were not available [23].

Determination of Total Polyphenol Index (TPI)

The TPI is based on the maximum absorbance that many phenolic compounds present at 280 nm. Ethanol solutions of wheat bran oil (5 mg/mL) were diluted with ethanol (1:10) and absorbance at 280 nm was measured in a Hitachi U-2000 spectrophotometer (Tokyo, Japan).

Determination of antioxidant capacity: ABTS assay

This assay is based on decolorization that occurs when the radical cation $ABTS^{\bullet+}$ is reduced to ABTS´ [28]. The radical was produced by reaction of 7 mM solution of ABTS in water with 2.45 mM $K_2O_8S_2$ (1:1). This mixture was kept in darkness at room temperature for 16 hours before use in order to obtain a stable radical $ABTS^{\bullet+}$ solution [29].

The assay was made with 980 µL of diluted $ABTS^{\bullet+}$ and 20 µL of ethanol solution of bran oil (5 mg/mL). The absorbance at 734 nm was measured after 20 min of reaction in

a Hitachi U-2000 spectrophotometer (Tokyo, Japan). Ethanolic solutions of known Trolox concentrations were used for calibration.

Determination of fatty acids profile

The fatty acids profile was determined by the AOAC method [30]. The fatty acid methyl esters were firstly prepared and then analyzed by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization detector (FID). The separation was carried out with helium (1.8 mL/min) as carrier gas. A fused silica capillary column (OmegawaxTM-320, 30 m×0.32 mm i.d.) was used. The column temperature was programmed starting at a constant temperature of 180 ºC for 20 min, heated to 200 ºC at 1 ºC/min, held at 200 ºC for 1 min, heated again to 220 ºC at 5 ºC/min and finally held at 220 ºC for 20 min. A split injector (50:1) at 250 ºC was used. The FID was also heated to 250 ºC. Fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards. Their quantification was made by relating the peaks area to the area of an internal standard (methyl tricosanoate) and using the corresponding chromatographic standards in order to find the response factors of each compound, as indicated by the AOAC method [30].

3. Results and discussion

3.1. Effect of wheat bran moisture on the extraction yield and oil quality

Table 2 shows the extraction yield and quality of the oil obtained by SFE from dry and fresh wheat bran under the same extraction conditions (40 MPa and 70 ºC). A reduction of 40 % can be observed for the total extraction yield when dry bran was used. This reduction might be explained by the role that water plays during the extraction process

of plant materials. The lipid pillars of an elementary membrane change with water content. If there is not enough water in the system, the pillars may close the membrane, making it impermeable, and therefore hindering solute extraction [17]. The lower yield obtained when extracting dry bran, also resulted in a lower extraction of AR and polyphenols, which corresponded with a minor antioxidant capacity of the extract. Since TPI is similar in both extracts, the lower antioxidant capacity of the extract obtained from dry bran can be attributed to the reduction in the AR content.

The AR profile of the extracts obtained from dry and fresh wheat bran (R1 and R6) was also determined by HPLC-DAD. The AR profile and one of the chromatograms are given in Table 3 and Figure 1 respectively. Similar profile was determined for both oils although it slightly differs from those reported in the literature where C15:0 is not always identified [31,32]. These differences might be explained by the different calibration methods used. The different compound used for calibration can also explain the differences obtained in this work between the colorimetric method with Fast Blue RR, calibrated with olivetol, and the HPLC method calibrated using several AR.

AR composition and the ratio of the AR homologues C17/C21 are especially useful in distinguishing cereals species. Our results corroborate those obtained by Chen et al. [33] and Andersson et al. [31] who reported that the C17/C21 ratio in common wheat is 0.1. In contrast, Mattila et al. [3] and Kulawinek et al. [2] obtained a higher C17/C21 ratio for wheat samples, which they attributed to unresolved AR in the HPLC.

Taking into account the higher extraction yield and AR content achieved with fresh bran, it was used for the next experiments performed in this work.

3.2. Effect of process variables on oil extraction yield.

The effect of temperature on the extraction yield was evaluated at 40, 70 and 95 ºC at three different pressures (25, 40 and 55 MPa) and a constant flow of 9 ± 1 kg CO_2/h. The results are shown in Figure 2. At the lowest pressure used in this work, 25 MPa, it appears not to be a significant effect of temperature on the total amount of oil obtained, while at the two other pressures evaluated, 40 and 55 MPa, the extraction yield increases with temperature, which may indicate that, at these pressures, the increase of oil vapor-pressure with temperature is more important than the decrease of SC-CO_2 density. These results suggest a crossover behavior of the isotherms around 25 MPa. This behavior has not been described in the literature for wheat bran oil but it has been generally observed for different oils [34]. Due to the possibility of a crossover region around 25 MPa, influence of a pressure increase seems to be stronger at the highest temperature evaluated in this work.

The extraction yield obtained at 40 MPa and 70 ºC (R6) was 2.9 ± 0.2 g oil/100 g dry bran. This result is similar to that reported by Athukorala et al. [21] who obtained approximately 3.3 ± 0.5 g/100 g wheat bran, when operating at 35 MPa and 70 ºC with pure SC-CO_2. Landberg et al. [20] obtained 4.9 ± 0.5 g oil/100 g dry matter when they extracted wheat bran with SC-CO_2 and ethanol as cosolvent, at 35 MPa and 70 ºC. The higher yield obtained under these extraction conditions is due to the use of a cosolvent that modifies the polarity of the solvent mixture. Francisco et al. [18] also obtained higher yields in the extraction of rye bran when ethanol or methanol were used as cosolvents than when using pure CO_2.

3.3. Effect of process variables on oil quality.

Effect of process variables on oil AR content.

The total AR content of the oils obtained by SFE under the different extraction pressures and temperatures was evaluated using the colorimetric method described in Section 2.4. The results are shown in Figure 3 where it can be observed that the amount of AR in oil slightly increased with extraction temperature when extraction pressure was 40 and 55 MPa. However, at 25 MPa, there was not significantly effect of the extraction temperature on the AR oil content. This suggests a crossover behavior around 25 MPa for AR extraction, similar to that found for oil extraction in section 3.2. Higher temperatures seem to provide not only higher extraction yields but also oil with higher AR content.

Francisco et al. [18] studied the extraction of rye bran with SC-CO_2 + cosolvents at pressures of 8, 15, 30 and 35 MPa and temperatures of 40 and 55 ºC. They found that the extractability of AR slightly decreased with temperature when using extraction pressures of 8 and 15 MPa while it increased when working under pressures of 30 and 35 MPa. They attributed this behavior to the presence of a crossover region between 15 and 30 MPa. In a later work, Francisco et al. [19] concluded that the influence of the extraction temperature on the extraction of AR from rye bran when using SC-CO_2 and ethanol as cosolvent is not significant; nevertheless, according to the results obtained in this work, we might suggest that the extraction temperature in the range of 40 to 95 ºC has a significant influence on wheat bran SFE with pure CO_2.

The total AR content of the oils obtained under the different extraction conditions varied from 37 to 65 mg AR/g oil as evaluated by the colorimetric method (see Fig. 3).

These results are similar to those reported by Landberg et al. [20] who obtained an extract with 62 mg AR/g extract when operating at 35 MPa, 70 ºC and using ethanol as cosolvent. A comparison of both results may indicate that the absence of cosolvent could be offset with higher extraction temperatures and pressures. Francisco et al. [18,19] and Athukorala et al. [21] reported that pure SC-CO_2 could not isolate the AR homologues from rye and wheat bran at 35 MPa and 70 ºC. Nevertheless, the results found in this work showed the viability of AR extraction using pure CO_2.

Effect of process variables on oil TPI.

Wheat bran presents high levels of total phenolic acids (around 4.5 g/kg dry bran) mainly linked to different cell-wall materials being the major phenolic compound ferulic acid [3]. The total polyphenolic content of the extracts was evaluated through the Total Polyphenol Index (TPI) as described in Section 2.4. TPI gives an indication of the amount of polyphenols in the SFE oil. Figure 4 shows the TPI for all the oils evaluated. TPI seems not to be highly affected by extraction pressure or temperature although some slight increase can be observed, mainly with temperature. The influence of a pressure increase seems to be stronger at the highest temperatures evaluated in this work, what may be indicating the presence of a crossover region in phenols solubility. In fact, the solubility of some phenols in pure SC-CO_2 has been previously studied [35-37]. Solubility values for ferulic acid, for instance, present crossover behavior around 20 MPa. This is a fairly common behavior for other phenolic compounds and is probably determining the influence of the extraction parameters on TPI.

Effect of process variables on oil antioxidant capacity.

The antioxidant capacity of the oil obtained under different extraction conditions was measured by the ABTS assay which is applicable to both hydrophilic and lipophilic antioxidants [28]. Fig. 5 reports the antioxidant capacity expressed as Trolox equivalent for the different oils obtained in this work. The influence of extraction temperature and pressure was similar to that reported above for the AR content of the extract. Relationship between the antioxidant capacity of the extract from breakfast cereals and their total amounts of AR was previously reported by Korycińska et al. [38]. Some similarity can be also observed between the effect of pressure and temperature on TPI (Fig. 4) and on antioxidant capacity evaluated by the ABTS method (Fig. 5) indicating some correlation between both parameters. Correlation between total phenolic content and the ABTS scavenging capacity was previously found by Zhou et al. [39] in wheat extracts.

Antioxidant capacity varies from 0.23 to 0.41 mmol Trolox/g oil (5.3-15.6 μmol Trolox/g dry bran) depending on the extraction conditions (see Fig. 5). The highest antioxidant capacity was obtained when the highest temperature and pressure were used. These values were of the same order as those reported for wheat bran extracted with different solvents like acetone and ethanol (2.7-15.2 μmol Trolox/g bran) [13] what indicates that SC-CO_2 used under certain conditions of pressure and temperature could be a good solvent for the extraction of antioxidants from wheat bran. Moore et al. [40] reported higher ABTS scavenging capacity (16.2 to 21.5 μmol Trolox equivalents/g bran) for 20 hard winter wheat varieties grown at two locations extracted with 50% acetone. Antioxidant properties of wheat bran are highly affected by genotype and

environment [39-41] thus, comparison of the antioxidant capacity of our extracts with values reported in the literature is limited.

According to the values obtained in this work, the wheat bran extract obtained by SFE might have an important potential as antioxidant; one gram of oil obtained at 55 MPa and 95 ºC presents an antioxidant capacity (410 µmol Trolox equivalents) which is between that reported for 100 mL of orange juice (249 ± 3 µmol Trolox equivalents) and that reported for 100 mL of tea infusion (631 ± 8 µmol Trolox equivalents) [42].

Effect of process variables on oil fatty acids (FA) content and profile.

The fatty acid analysis of the oils showed that there was no significant difference in the total amount of fatty acids in the oils obtained under the different extraction pressures and temperatures evaluated in this work. The average FA content for runs R2-R10 and standard deviation are given in Table 4 (791 ± 29 mg of fatty acids/g oil). Also the fatty acid profile was similar in all the oils (see Table 4) being linoleic acid the major component (around 58 % of the total fatty acids) followed by palmitic and oleic acids (around 16-17 %). Table 4 also includes the fatty acid profile described in the literature for wheat bran oil obtained by SC-CO_2 [21,43]. These authors have not evaluated the total profile, but just the major FA components what may explain the differences found. Also the different wheat varieties may highly affect the FA profile.

3.4. Fractionation of wheat bran oil by using two-stage supercritical fluid extraction.

Some authors reported the possibility of concentrating the AR components by performing SFE in two stages [18,21], the second one involving the use of cosolvents. In this work we have tried to achieve the same objective but instead of using cosolvents, we have performed a two-stage extraction (R11) using different extraction temperature

and pressure, but pure CO_2. In the first stage, the extraction conditions chosen were those that had previously shown to provide the lowest ratio between the amount of AR and the amount of fatty acids, which were the lowest pressure and temperature used in this work. The second extraction stage was carried out under those conditions that had shown to provide the highest AR/fatty acids ratio, which were the highest pressure and temperature used in this work. The total extraction time was 120 minutes, 60 minutes for each stage.

Table 5 shows the extraction yield and characterization of the corresponding extracts for the first and second extraction stages (Fraction 1 and Fraction 2 respectively). The extraction yield was higher in the first stage while the extract obtained during the second extraction stage was more concentrated in AR and polyphenols, and therefore with a higher antioxidant capacity. Their composition also differed in the amount of fatty acids; around 75% of the first fraction was fatty acids while in the second extraction step only the 40% corresponds to fatty acids. The aspect of the second fraction suggested that it was concentrated in waxes and other lipids which could increase its viscosity resulting in an extract difficult to work with.

Similar fatty acid and AR profiles as those described above for wheat bran oils has been found for the two fractions obtained from the two stage extraction.

Further fractionation studies should be done to optimize the conditions that would provide the highest yield and quality of Fraction 2.

4. Conclusion

Wheat bran oil could be obtained by SFE, using pure CO_2 as solvent, with yields similar to those obtained using conventional organic solvents. The extraction yield presented crossover behavior and as a consequence, the influence of extraction temperature was higher the higher the extraction pressure. Similar trends were found for the extraction of some bioactive compounds found in wheat bran, such as AR and other phenolic compounds. As a result, the highest values of extraction pressure and temperature used in this work (55 MPa and 95 ºC) provided the bran oil with the highest antioxidant capacity.

Supercritical fluid fractionation allowed us to obtain wheat bran oil enriched with AR and other phenols and with higher antioxidant activity.

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Figure Captions

Figure 1. HPLC-DAD chromatogram of AR in wheat bran oil obtained in run R6

Figure 2. Influence of extraction pressure and temperature on total oil yield (R2 – R10). Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.

Figure 3. Influence of extraction pressure and temperature on AR content of wheat bran oils (R2 - R10). Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.

Figure 4. Influence of extraction pressure and temperature on total polyphenol index of wheat bran oil (R2-R10). Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.

Figure 5. Influence of extraction pressure and temperature on $ABTS^{\bullet+}$ scavenging capacity of wheat bran oils expressed as mmol Trolox equivalents. Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.

Table 1. Experimental runs of SFE of wheat bran with pure SC-CO_2. Data are given as mean values ± standard deviation being the number of replicas n = 3

Run	Extraction conditions		
	P (MPa)	T (ºC)	Wheat bran moisture (%)
R1	41 ± 2	70 ± 1	1.6 ± 0.3
R2	25 ± 1	40 ± 1	11.0 ± 0.1
R3	25 ± 1	70 ± 1	11.0 ± 0.1
R4	25 ± 1	95 ± 1	11.0 ± 0.1
R5	40 ± 2	40 ± 1	11.0 ± 0.1
R6	40 ± 2	70 ± 1	11.0 ± 0.1
R7	40 ± 2	95 ± 1	11.0 ± 0.1
R8	55 ± 2	40 ± 1	11.0 ± 0.1
R9	55 ± 2	70 ± 1	11.0 ± 0.1
R10	55 ± 2	95 ± 1	11.0 ± 0.1
R11 (step 1)	25 ± 1	40 ± 1	11.7 ± 0.1
R11 (step 2)	50 ± 2	83 ± 2	-

Table 2. Characterization of the wheat bran oil obtained by SFE at 40 MPa and 70 ºC from dried and fresh wheat bran. Data are given as mean values ± standard deviation being the number of replicas n = 3.

		R1	R6
Wheat bran moisture (%)		1.6 ± 0.3	11.0 ± 0.1
Extraction yield (g oil/100 g dry bran)		1.8 ± 0.1	2.9 ± 0.2
AR content (mg/g oil)	Colorimetric method	31 ± 2	53 ± 3
	HPLC method	62 ± 3	138 ± 5
TPI (uds abs/g oil)		857 ± 35	928 ± 28
ABTS (mmol Trolox/g oil)		0.32 ± 0.01	0.39 ± 0.05
Fatty acids (mg/g oil)		815 ± 15	786 ± 15

Table 3. Alkylresorcinols content in wheat bran oil as determined by HPLC-DAD. Data are given as mean values ± standard deviation being the number of replicas n = 3

Alkyl chain	Oil AR content (mg/g oil)		Oil AR profile (g/100 g AR)		
	R1	R6	R1	R6	[2,32,33]
C15	0.99 ± 0.05	1.40 ± 0.04	1.6 ± 0.1	1.0 ± 0.1	0-1
C17	3.7 ± 0.2	7.6 ± 0.3	6.0 ± 0.4	5.5 ± 0.3	4-9
C19	13.70 ± 0.07	33.6 ± 0.6	22 ± 1	24.5 ± 0.9	31-35
C21	33 ± 3	73 ± 3	54 ± 2	52.4 ± 0.8	45-50
C23	7.0 ± 0.2	16.4 ± 0.7	11.2 ± 0.2	11.8 ± 0.4	8-10.5
C25	3.33 ± 0.07	6.6 ± 0.3	5.3 ± 0.1	4.8 ± 0.1	2-4

Table 4. Average fatty acid profile of wheat bran oil obtained by SFE for the runs R2-R10

Fatty acid	R2-R10 (mg/g oil)	SC-CO_2 [43] (mg/g oil)	SC-CO_2 [21] (mg/g oil)
Caprilic acid (C8:0)	0.3 ± 0.2		
Capric acid (C10:0)	0.13 ± 0.02		
Lauric acid (C12:0)	0.3 ± 0.1		
Myristic acid (C14:0)	1.00 ± 0.09		
Pentadecanoic acid (C15:0)	0.82 ± 0.06		
Palmitic acid (C16:0)	134 ± 3	139 ± 3	157 ± 8
Palmitoleic acid (C16:1 n-7)	1.02 ± 0.07	1.1 ± 0.1	
Heptadecanoic acid (C17:0)	0.87 ± 0.07		
Heptadecenoic acid (C17:1)	0.4 ± 0.2		
Steraric acid (C18:0)	10.0 ± 0.3	7.6 ± 0.7	37 ± 6
Oleic acid (C18:1 n-9)	128 ± 5	192 ± 5	159 ± 14
Vaccenic acid (C18:1 n-7)	6.1 ± 0.1		
Linoleic acid (C18:2 n-6)	457 ± 17	366 ± 8	337 ± 43
γ- linolenic acid (C18:3 n-6)	0.20 ± 0.06		
α- linolenic acid (C18:3 n-3)	40 ± 2	10.4 ± 0.7	
Araquidic acid (C20:0)	1.80 ± 0.08		
Gondoic acid (C20:1 n-9)	4.70 ± 0.06		
Eicosadienoic acid (C20:2 n-6)	0.79 ± 0.05		
Behenic acid (C22:0)	1.4 ± 0.3		
Docosadienoic acid (C22:2 n-6)	0.13 ± 0.07		
Lignoceric acid (C24:0)	1.56 ± 0.06		
Nervonic acid (C24:1)	0.31 ± 0.09		
Saturated fatty acids	152 ± 4	147 ± 4	194 ± 14
Monounsaturated fatty acids	141 ± 6	193 ± 5	159 ± 14
Polyunsaturated fatty acids	498 ± 19	376 ± 9	337 ± 43
Total fatty acids	791 ± 29	716 ± 18	690 ± 71

Table 5. Extraction yield and characterization of wheat bran oil obtained by two stage SFE. Data are given as mean values ± standard deviation being the number of replicas n = 3

		Fraction 1	Fraction 2
Extraction pressure (MPa)		25 ± 1	55 ± 2
Extraction temperature (ºC)		40 ± 1	83 ± 1
Extraction yield (g oil/100 g dry bran)		2.1 ± 0.5	1.1 ± 0.2
AR (mg/g oil)	Colorimetric method	35 ± 5	117 ± 20
	HPLC method	75 ± 9	234 ± 17
TPI (uds abs/g oil)		752 ± 15	1667 ± 263
ABTS (mmol Trolox/g oil)		0.31 ± 0.03	0.58 ± 0.01
Fatty acids (mg/g oil)		751 ± 46	401 ± 42

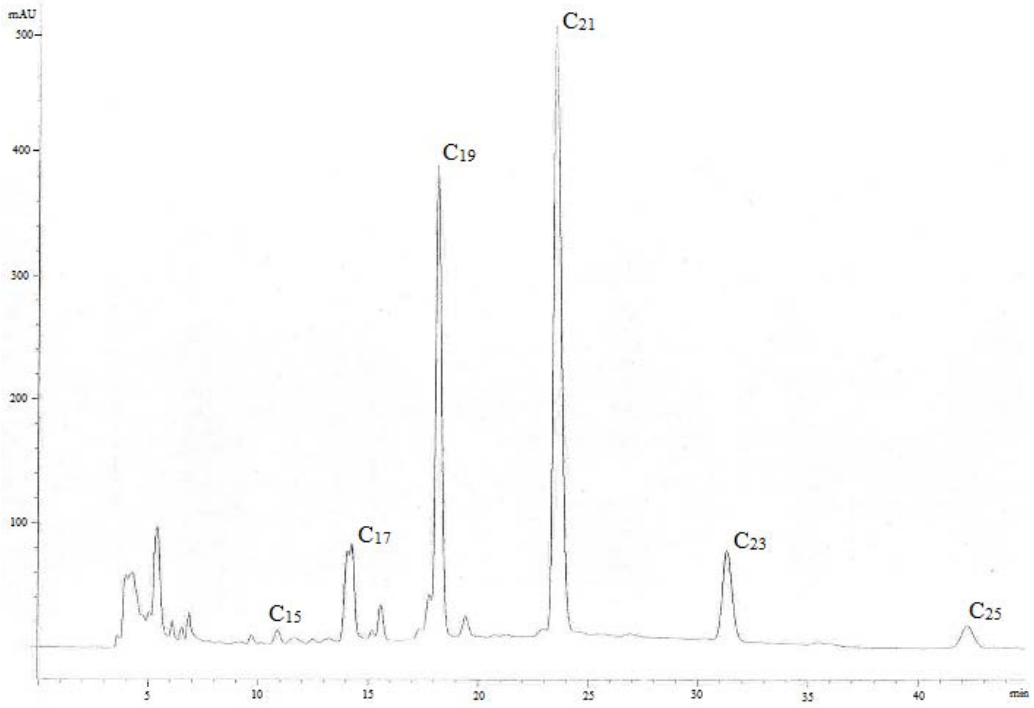


Figure 1. HPLC-DAD chromatogram of AR in wheat bran oil obtained in run R6

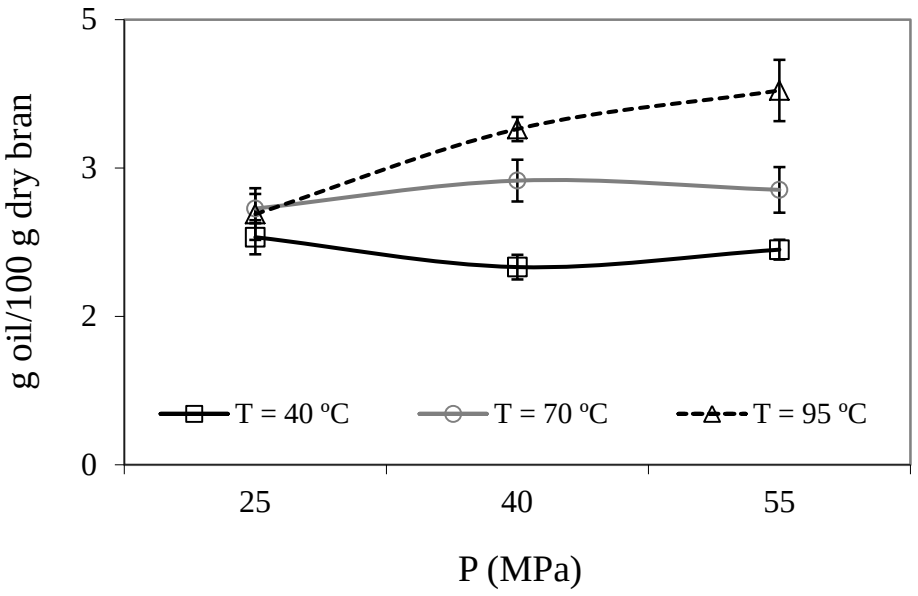


Figure 2. Influence of extraction pressure and temperature on total oil yield (R2 – R10). Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.

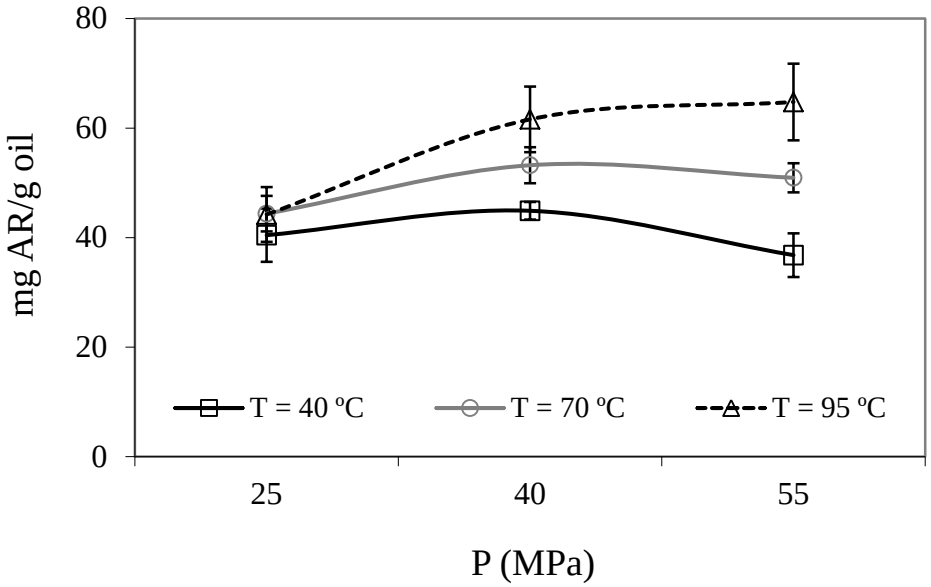


Figure 3. Influence of extraction pressure and temperature on AR content of wheat bran oils (R2 - R10). Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.

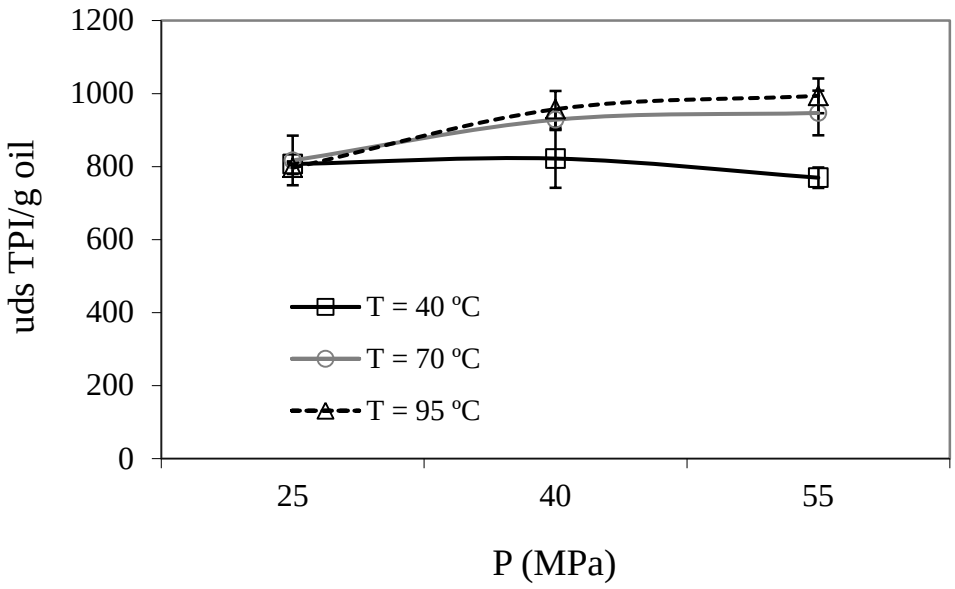
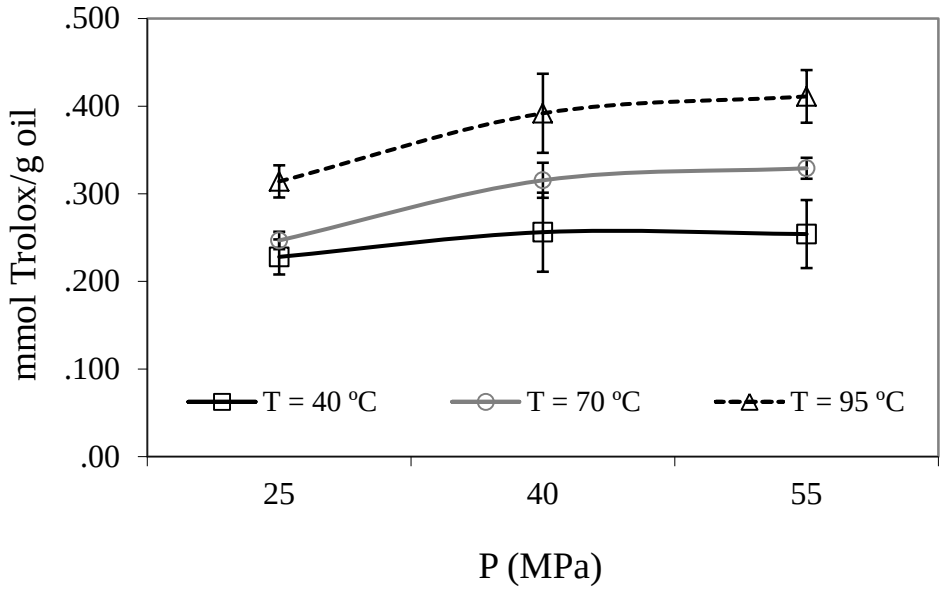


Figure 4. Influence of extraction pressure and temperature on total polyphenol index of wheat bran oil (R2-R10). Points show mean values and bars standard deviation being the number of replicas n = 3. Lines are to guide the eye.



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Graphical abstracts