SUPERCRITICAL FLUID EXTRACTION OF WHEAT BRAN OIL AND CHARACTERIZATION AND FORMULATION OF THE EXTRACTS

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ABSTRACT

Supercritical fluid extraction (SFE) of wheat bran oil with pure supercritical carbon dioxide (SC-CO_2) at different extraction pressures (25, 40 and 55 MPa) and temperatures (40, 70 and 95 ºC) has been studied. Since wheat bran is characterized by having an important content of antioxidant compounds, the content of such compounds in the extracted oil and its antioxidant activity were evaluated. The highest extraction yield was obtained when 55 MPa and 95ºC were used as extraction conditions. These conditions also provided the highest oil content in alkylresorcinols (AR), which are phenolic lipids characteristic of cereal bran.

Characterization of wheat bran oil and evaluation of its antioxidant activity was carried out. Significant content of AR, steryl pherulates, tocopherols and polyunsaturated fatty acids (PUFA) was found in the extracted oil, which could be related to the antioxidant activity of wheat bran oil, as determined by DPPH, FRAP and ABTS methods.

Searching suitability of wheat bran oil for aqueous-based matrix applications, its formulation as oil in water nanoemulsions was studied. The nanoemulsions obtained showed good stability during 60 days storage at 4 and 25ºC and darkness.

INTRODUCTION

Wheat bran is an important source of bioactive compounds, which are related to the health-protective mechanisms of whole-grain cereals [*1*]. Some of these bioactive compounds, such as alkylresorcinols (AR) and tocopherols, and to a lesser extent phenolic compounds, can be extracted by supercritical fluid extraction processes [*2*] However, wheat bran oil extracted with SC-CO_2 has not been widely characterized [*3-5*] and it is known that the solvent system used in the extraction process influences the oil composition and quality [*6*] therefore, new studies on the levels of the different bioactive compounds and the antioxidant activity of supercritical extracted wheat bran oil are necessary in order to evaluate its potential uses in the food industry.

There has been growing interest in the utilization of natural antioxidants in the food, beverage and pharmaceutical industries due to the increasing consumer´s demand for substituting synthetic compounds by natural substances. Due to its lipophilic character, wheat bran oil must be formulated before it can be used for aqueous-based matrix applications. The high stability and low turbidity of nanoemulsions (10-200 nm) make them suitable for incorporating lipophilic active ingredients in aqueous-based food and beverages [*7, 8*].

The aim of the present work was to study the supercritical fluid extraction of wheat bran oil and to evaluate the oil composition and antioxidant activity. Additionally wheat bran oil-in-water nanoemulsions have been formulated for the application of the wheat bran oil in aqueous systems.

MATERIALS AND METHODS

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 [*9*]. In a SFE experiment, 300 g of wheat bran (*Triticum aestivum* L.) 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. 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. Nine runs were carried out by triplicate in order to study the influence of extraction pressure (25, 40 and 55 MPa) and temperature (40, 70 and 95 ºC).

Determination of AR content and profile

Wheat bran oil AR content and profile were evaluated by using previously reported colorimetric and HPLC-DAD methods, respectively [*10*].

Determination of fatty acids content and profile

Fatty acids content and profile was evaluated by GC-FID by the AOAC method, as previously reported by Rebolleda et al. [*10*].

Determination of steryl ferulates content and profile

Steryl ferulates were analyzed by HPLC-DAD according to a previously reported method, with some modifications [*11*]. Separation was carried out in a Zorbax XDB C18 column (150 x 4.6 mm, 5 µm) using isocratic elution with acetonitrile/methanol/isopropanol (50:40:10) at 1 mL/min. Methanolic solutions of wheat bran oil (10 mg/mL) were injected (30 µL). Steryl ferulates were monitored at 330 nm and identified using a standard mixture of steryl ferulates and literature data [*12*].

Determination of tocopherol content and profile

Tocopherol content and profile were evaluate in wheat bran oil by solid phase extraction followed by HPLC-DAD [*13*].

Determination of phenolic content and profile

Phenolic compounds were evaluated by HPLC-DAD according to a previously reported method [*14*].

Evaluation of antioxidant activity

DPPH, FRAP and ABTS methods previously reported were used for the evaluation of the antioxidant activity in wheat bran oil [*10, 15*].

Emulsification procedure

To prepare an emulsion, 1% of wheat bran oil was mixed with 7.3% of a surfactant mixture formed by Span 80 (37.4%) and Tween 80 (62.6%), before water milli-Q was added. Then, emulsification was carried out by using high speed blender (Miccra D9, 29000 rpm, 5 min) followed by ultrasonic processor at 20% of amplitude (Sonics VCX 500, 50 s).

Evaluation of nanoemulsion stability

Stability of wheat bran oil nanoemulsions was measured in terms of their droplet growth ratio during 60 days storage at 4 ºC and darkness (Zetasizer Nano ZS). Also, optical

characterization of creaming stability (Turbiscan Lab Expert equipment) was made for nanoemulsions storage during 60 days at 25ºC and darkness.

RESULTS

Effect of process variables on oil extraction yield and AR content

The effect of temperature on the extraction yield and oil quality 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 of the extraction yield are shown in Figure 1. 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 [*16*]. 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.

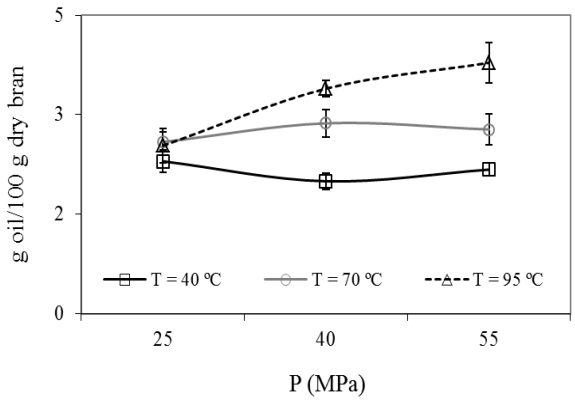

Figure 1: Influence of extraction pressure and temperature on total oil yield

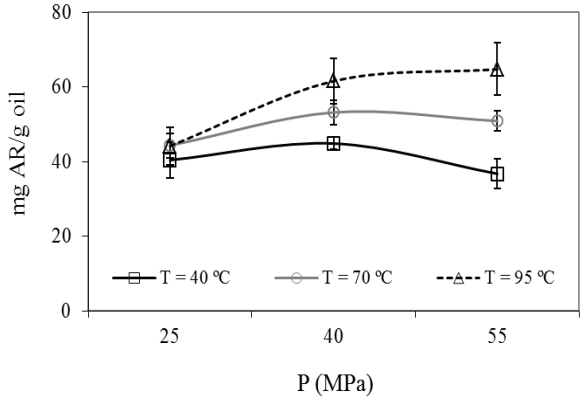

Figure 2: Influence of extraction pressure and temperature on total AR content of wheat bran oil

The total AR content of the oils obtained by SFE under the different extraction pressures and temperatures are shown in Figure 2, 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 the extraction yield. Higher temperatures seem to provide not only higher extraction yields but also oil with higher AR content.

Evaluation of oil composition and antioxidant activity

Analysis of the composition and antioxidant activity of wheat bran oil extracted under specific conditions (25 MPa and 40ºC) was evaluated. Table 1 shows the total amount and profile of some bioactive compounds such as alkylresorcinols, tocopherols, steryl ferulates, phenolic compounds and fatty acids.

Table 1. Bioactive compounds evaluated in supercritical extracted wheat bran oil

Fatty acids (mg/g oil)	712 ± 19
Palmitic acid (C16:0)	118 ± 2
Stearic acid (C18:0)	7.9 ± 0.1
Oleic acid (C18:1)	114 ± 3
Linoleic acid (C18:2)	410 ± 10
α- linolenic acid (C18:3)	37.3 ± 0.8
Others	24.6 ± 4
Alkylresorcinols (mg/g oil)	46.8 ± 0.7
C15- AR	0.52 ± 0.01
C17- AR	3.3 ± 0.2
C19-AR	14.3 ± 0.3
C21- AR	22.4 ± 0.2
C23- AR	4.40 ± 0.03
C25- AR	1.97 ± 0.06
Steryl ferulates (mg/g oil wrt γ-oryzanol)	18.2 ± 0.2
Campesteryl ferulate	2.4 ± 0.1
Sitosteryl ferulate + campestanyl ferulate	9.9 ± 0.5
Sitostanyl ferulate	5.9 ± 0.4
Tocopherols (mg/g oil)	6.8 ± 0.1
α- T	3.84 ± 0.01
β- T	0.195 ± 0.002
γ-T	2.67 ± 0.08
δ-T	0.087 ± 0.003
Phenolic compounds (ppm)	25 ± 2
Vanillin	13.8 ± 0.1
Vanillic acid	3.5 ± 0.5
Syringic aldehyde	3.4 ± 0.8
Ferulic acid	1.8 ± 0.3
Syringic acid	1.6 ± 0.4
p-OH-benzaldehyde	0.7 ± 0.1

Values are mean ± standard deviation of three replicates (n=3). wrt with respect to

The fatty acid profile reveals that most of the fatty acids are polyunsaturated fatty acids (PUFA), around 63%, with a low amount of saturated fatty acids (around 18%). Linoleic acid (LA, C18:2ω6) was the major PUFA detected (around 58%), and significant quantities of α-linolenic acid (ALA, C18:3ω3) were also quantified. Both compounds are essential PUFA, precursors of the omega-6 and omega-3 families respectively, and therefore, very important in the human diet. Its large PUFA content makes wheat bran oil to be considered of higher quality than some of the most commonly used oils, which usually have very low levels of PUFA (e.g. palm oil) and often show very low levels of ALA (e.g. sunflower oil, sesame oil, and palm oil) [*17*].

Alkylresorcinols have been described to have a wide range of biological activities such as antibacterial, antifungical, anticancer and enzyme inhibitor activities, among others [*18*]. The intensity of these activities is different for each AR homologue [*19*] which is probably due to the different length of the alkyl chain. The AR profile of the bran oil under study (Table 1) is similar to that previously reported for wheat bran [*20*], being C19 and C21 homologues the major ones (around 30 and 48% respectively).

Steryl ferulates, which are esters of ferulic acid with sterols, have been widely described for rice bran oil and they are considered to be potential antioxidants because of the hydrogen-donating ability of the phenolic group of ferulic acid [*21*]. Data in Table 1 show significant levels of steryl ferulates, higher than those described for hexane extracted oils [*22*]. The steryl ferulate profile obtained (Table 1) was similar to that reported in acetone

extracts of wheat bran [*23*] corresponding 54% to campestanyl + sitosteryl ferulates, 32% to sitostanyl ferulate and 13% to campesteryl ferulate.

Tocopherol content of wheat bran oils extracted by supercritical fluid extraction has been scarcely studied. The mean tocopherol content of the wheat bran oil here under study was 6.8 ± 0.1 mg/g which is a much higher value than that described for other wheat bran oil also obtained by SFE but from *Triticum durum* (4.3 ± 0.7 mg/g oil) [*5*] what could be explained by the different wheat variety [*24*] and extraction conditions used. The main tocopherols of the wheat bran oil here studied were α-tocopherol (57%) and γ-tocopherol (39%), showing a similar α-tocopherol proportion than that described for wheat germ and cottonseed oils and a higher proportion of γ-tocopherol than that reported for sunflower and olive oils [*25*]. These results could be of interest to the food industry because it has been described that α-tocopherol presents high biological activity, and γ-tocopherol has been reported as the most effective tocopherol isomer to inhibit the oxidation of fats and oils [*26*] although in recent years different results suggest that the antioxidant activity of each tocopherol homologue depends, among other factors, on the food system where they are evaluated [*27*].

Wheat bran has been described as a rich source of phenolic compounds being the main phenolic acids reported ferulic, vanillic and syringic acids [*28*]. However, due to the low solubility that these compounds present in supercritical CO_2 [*29*], reduced phenol content was found in the SFE wheat bran oil here under study (Table 1). Vanillic acid and aldehyde were the main phenols detected what could be explained by their higher solubility in SC-CO_2 regarding other phenols reported for wheat bran [*29*].

Antioxidant activity of wheat bran oil was evaluated by the DPPH, FRAP and ABTS methods (Table 2). The DPPH value was higher than that reported for supercritical wheat bran oil from *Triticum durum* [*5*]. This result is concordant with the lower levels of tocopherols, strong antioxidants of oils, of the wheat bran oil obtained in that work, as it has been previously discussed. Similarly, the antioxidant capacity evaluated by the ABTS method (270 μmol Trolox/g) was higher than that reported for other vegetable oils such as olive and sunflower oils [*30*]. These differences could be explained considering the different antioxidant composition of the different oils. The tocopherol content found for wheat bran oil was higher than that reported for olive and sunflower oils. Also, the presence of alkylresorcinols and steryl ferulates in wheat bran oil could contribute to obtaining a higher antioxidant activity for this oil.

Table 2. Antioxidant activity of wheat bran oil evaluated by different methods

Antioxidant method	Standard compound	Units	Value
DPPH	Trolox	μmol Trolox/g	26 ± 2
FRAP	$FeSO_4$	μmol Fe (II)/g	228 ± 12
ABTS	Trolox	μmol Trolox/g	270 ± 6

Formulation of wheat bran oil: nanoemulsification

Wheat bran oil-in-water nanoemulsions were formulated for the application of wheat bran oil in water systems. Optimization of oil concentration, surfactant type and concentration and emulsification process was carried out to obtain nanoemulsions with the minimum droplet size and the maximum stability (data not shown). Optimal nanoemulsion, with 40 nm of

droplet size, was formulated and emulsified, according to the process described in materials and methods section, and stability during storage was evaluated.

There was no significant change in droplet size for the optimal nanoemulsions after 15 and 60 days of storage at 4ºC (Fig. 3), with no noticeable changes on visual emulsion stability. However, creaming stability measurements along 60 days at 25ºC, using the Turbiscan Lab Expert apparatus, showed that there was a slight backscattering increase along time for the middle zone of the measurement cell, which indicates an increase in droplet size caused by the coalescence of oil droplets. The formation of a sedimentation front at the bottom of the sample (about 3 mm of cell height) was also observed during the last days, indicating a tiny emulsion destabilization at the end of the storage period.

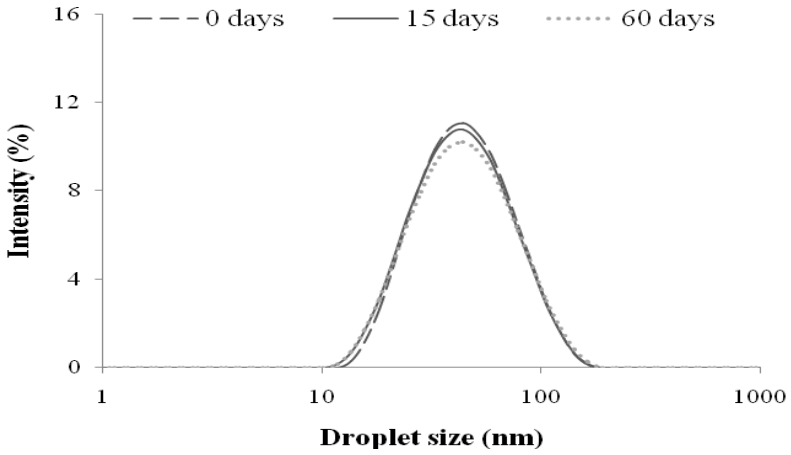

Figure 3. Droplet size distribution of wheat bran oil nanoemulsion after 0, 15 and 60 days of storage at 4 ºC and darkness

CONCLUSION

Wheat bran oil could be obtained by SFE, using pure CO_2 as solvent. 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 alkylresorcinols.

Characterization of the extracted oil showed an important content of bioactive compounds such as AR, steryl ferulates, tocopherols and fatty acids. Furthermore, a low content of other phenolic compounds was found. Wheat bran oil also showed a high antioxidant activity probably related to its content in the previously mentioned compounds.

Good stability during storage was determined for wheat bran oil in water nanoemulsions, what indicates the possibility of using these delivery systems for the incorporation of wheat bran oil in aqueous medium.

Acknowledgments

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