Bioactive compounds and antioxidant activity of wheat bran and barley husk in the extracts with different polarity

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ABSTRACT

Extracts of wheat bran and barley husk with solvents of different polarity were analyzed to determine the concentration of bioactive compounds (phytic acid, saponins, flavonoids, total phenolic compounds and phenolic profile) and their antioxidant activity by DPPH and ABTS assays. The results showed that the phytic acid concentration in wheat bran was higher than the phytic acid concentration in barley husk. The concentration of saponins was inverse with higher concentrations in barley husk than wheat bran. The solvents with the highest extraction of phytochemicals were 80% ethanol in wheat bran and 50% acetone in barley husk. The chromatograph profile showed that wheat bran presented a greater concentration of caffeic acid and barley husk contained kampferol. The properties of extraction solvents significantly affected the concentration of flavonoids, phenolic compounds, and antioxidant activity. The addition of water to the solvents increased the extraction efficiency of bioactive compounds and antioxidant activity. The results for barley husk are among the first reported because there are few studies on this cereal.

ARTICLE HISTORY

Received 9 November 2018 Revised 14 March 2019 Accepted 22 March 2019

KEYWORDS

Wheat bran; barley husk; saponins; phytic acid; phenolic compounds; antioxidant activity

Introduction

Phytochemicals are known for helping to prevent oxidative stress and thus maintain a balance between oxidants and antioxidants. The strong antioxidant activity of flavonoids, phenolic compounds, and saponins, as well as their ability to eliminate free radicals have special attention. Phenolic compounds are bioactive compounds possessing antioxidant properties that promote health by protecting against oxidation. Some studies have demonstrated that grain fractions (bran, bread flour, reduction flour, shorts) possess different antioxidant capacities. Wheat bran has been reported to show a concentration of phenolic acids in the aleurone layer and cell wall. Studies have shown that barley contains phenolic compounds in free and bound forms of the bran fraction’s outer layer. Nevertheless, the concentration of phenolic compounds in bran and husk is not well known due to few studies to draw upon for more information. The amount of phenolic compounds extracted depends on the polarity and the structure of the phenols that are present in the sample. In addition to phenolic compounds, phytic acid is considered an antinutritional compound
because phytic acid affects the bioavailability of magnesium, zinc, iron and calcium.\cite{9} More than 80% of phytic acid is located in the aleurone layer and the rest of the germ.\cite{10} In sorghum fractions obtained by traditional milling, the phosphorus concentration observed in bran is higher than observed in the whole grain, with minimal amounts in the husked grain. This suggests that grain bran and aleurone layers are not only limited to the concentration of phenolic compounds but there is also a significant deposit of phytate and total phosphorus in sorghum.\cite{11} Few studies exist on the bioactive compounds of cereals such as bran and husk. Therefore, this research aims to determine the presence and concentration of bioactive compounds (phytic acid, saponins, flavonoids, total phenolic compounds and the phenolic profile) in wheat bran and barley husk. In addition, solvents with different polarity are evaluated to obtain the highest extraction yield of phenolic compounds and flavonoids, and to determine antioxidant activity.

Materials and methods

Wheat bran and barley husks from whole grains used in the study were obtained from producers in the Apan region of Hidalgo, Mexico. Both grains were cultivated with rainfall in summer, of average precipitation of the surface of the region was from 105- to 110 mm.

To obtain the husk and bran, Seedburo Barley Pearler I7810 was used with a Direct Drive Fan Motor 4K638C, using a No. 8 and 7 mesh. The peeling time was 1 minute.

Phytic acid

By following the method of Vaintraub and Lapteva\cite{12} and Latta and Eskin,\cite{13} the concentration of phytate was determined. First, 0.5 g flour was weighed. Then 10 mL 3.5% HCl was added and continuously stirred for 1 h, followed by centrifugation at 10 000 rpm for 10 min. The reaction was carried out with 200 μL extract, 2800 μL distilled water and 1 mL Wade’s reagent (30 mg FeCl₃⋅6H₂O + 300 mg sulfosalicylic acid and 100 mL distilled water). The absorbance was measured at a wavelength of 500 nm. The concentration was calculated based on the calibration curve of sodium phytate from 0 to 160 μg/mL, and the results were expressed in mg of sodium phytate equivalent per 100 g of sample dry basis (db).

Saponins

By modifying the method employed by Hiai et al.,\cite{14} the concentration of saponins were determined. Firstly, 0.5 g of each of sample was weighed and 10 mL 80% methanol was added and stirred for 16 h. The samples were centrifuged at 10 000 rpm for 10 min and the supernatants were collected. Subsequently, 5 mL 80% methanol was added and centrifuged again at 10000 rpm for 10 min and the supernatant was collected again. This procedure was repeated twice. The reaction was made using 200 μL of extract and 50 μL of 80% methanol in an ice bath with 0.25 mL of the vanillin reagent (C₈H₈O₃, purum ≥ 98%) added afterwards. The samples were removed from the ice bath and 2.5 mL H₂SO₄ were added to each. The mixture was heated in a bath at 60°C for 10 min. The tubes were cooled in an ice bath and absorbance was measured at a wavelength of 520 nm. The concentration was calculated based on the diosgenin calibration curve of 0–125 μg/mL. The results were expressed in mg diosgenin equivalent (QD) per 100 g of sample db.

Total flavonoid content

Flavonoids were determined by following the method used by Žilić et al.,\cite{15} and Eberhardt et al.,\cite{16} The extraction was made with 1 g of each sample in 10 mL of different solvents. The solvents used were absolute methanol, 80% methanol, absolute ethanol, 80% ethanol, and 50% acetone. Each sample mixed with a solvent was shaken for 30 min at room temperature. Then 5% NaNO₂...
(0.075 mL) was mixed with 0.5 mL of the sample (supernatant diluted with 1 mL of water). After 6 min passed, 10% AlCl₃ solution (0.15 mL) was added and the mixture was allowed to stand for another 5 min. Then, 1 M NaOH (0.5 mL) was added and the volume was brought up to 2.5 mL using distilled water. The absorbance was measured at 510 nm immediately after mixing. The final results were expressed as mg of quercetin equivalents (QE) per 100 grams db.

**Total phenolic compounds**

The extraction of total phenolic compounds (TPC) was performed by following the method described by Dueñas et al.\textsuperscript{[17]} with modifications to the sample amount (0.5 g) for the extraction and 10 mL of different solvents (absolute methanol, 80% methanol, absolute ethanol, 80% ethanol, and 50% acetone). The solutions were made and rested for 16 h at room temperature. Subsequently, the samples were centrifuged at 10,000 rpm and the supernatant was collected. The TPC was determined using Folin-Ciocalteu’s phenol reagent.\textsuperscript{[18]} Briefly 20 μL extract, 1.58 mL distilled water, 100 μL Folin-Ciocalteu reagent diluted at a ratio of 1:2, and 300 μL 10% sodium carbonate, were combined, mixed, and stored away from sources of light. The absorbance was read at a wavelength of 765 nm. Calculations were performed based on the calibration curve with gallic acid at a concentration range of 0–0.5 mg/mL. The final results were expressed as mg of gallic acid equivalents (GAE) per 100 grams db.

**Identification and quantification of phenolic compounds using HPLC chromatographic conditions**

HPLC analyses were performed using the same method as Harbaum et al.\textsuperscript{[19]} with modifications. An HPLC-diode array detector (Agilent Technologies 1200, Santa Clara, CA, U.S.A.) was equipped with an Atlantis C18 column (5 μm, 4.6 mm × 150 mm) for chromatographic separations. The UV–vis absorption spectra were monitored by the DAD from 200 to 600 nm, and the phenolic compounds were detected at 280, 325, and 360 nm. The flow rate was 1 mL/min. The mobile phases comprised 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 100% acetonitrile (solvent B). The injection volume was 5 μL and the mobile phase gradient was as follows: at start, A:B = 90:10; from 0 to 0.1 min, A:B = 90:10; from 0.1 to 50 min, A:B = 60:40; and from 50 to 60 min, A:B = 55:45. The contents of individual phenolics were evaluated using the calibration curves of the different standards.

**Antioxidant activity**

**DPPH assay**: The assay was performed following the method described by Brand-Williams et al.\textsuperscript{[20]} 2.5 mg of the radical (DPPH\textsuperscript{•}) was dissolved in 40 mL of methanol. The solution was diluted to 50 mL with methanol and protected from light. Its absorbance was read at 520 nm and adjusted to 0.5. Each extract (200 μL) was mixed with 2000 μL of the DPPH\textsuperscript{•}. A reading of each sample was taken at 60 min of reaction. The inhibition percent was calculated with Equation (1)

\[
\text{Antiradical Activity (\%) } = 1 - \frac{\text{Abs sample}}{\text{Abs blank}} \times 100
\]

Antioxidant activity was expressed in \textmu mol Trolox/g sample db.

**ABTS assay\textsuperscript{•+}:** The total phenolic extracts were used for antioxidant tests. Antioxidant activities were determined using ABTS radical according to the method described by Pastrana-Bonilla et al.\textsuperscript{[21]} with slight modifications. ABTS was dissolved in water to 7 mM and allowed to react with a 2.45 mM potassium persulfate solution for 16 h in darkness. The volumes used in this analysis to generate the colorimetric reaction were: 990 μL ABTS (adjusted to an absorbance of 0.7) and
10 μL of each extract. The absorbance was measured at 734 nm each minute for 5 min. Percent inhibition was calculated using Equation (2) and plotted as a function of content of antioxidants and Trolox for the standard reference data.

\[
Inhibition \, (\%) = \left( \frac{Abs \, sample \, t_0 - Abs \, sample \, t_5}{Abs \, sample \, t_0 - Abs \, solvent \, t_0} \right) \times 100
\]

where “Abs sample” is the absorbance of the extract at \( t_0 \) (initial) or at \( t_5 \) (5 minutes) and “Abs solvent” is the absorbance of the extraction solvent at \( t_0 \) (initial) or at \( t_5 \) (5 minutes).

Antioxidant activity was expressed in μmol Trolox/g sample db.

**Statistical analysis**

The results were expressed as the average of three determinations ± their standard deviation. Data were analyzed by one-way analysis of variance (ANOVA). A comparison of means was performed using the Tukey’s test with a 95% confidence level. All of these analyses were performed using Minitab statistical software version 17.1.0. 2013.

**Results and discussion**

**Phytic acid**

Table 1 shows the values of phytic acid in wheat bran and barley husk. Wheat bran (3.68 mg/g) showed a significant and higher concentration than barley husk (2.32 mg/g). It has been reported that phytic acid is found in bran layers of cereal grains.\(^{[22]}\) Wheat bran consists of cuticle, epicarp, endocarp, test, and aleurone and there may be small concentrations of phytic acid that contribute to its total quantification. In contrast, barley husk only represents the external part of the grain. Additionally, enzymes play an important role in the degradation of phytic acid; physiologically, seeds have different levels of intrinsic phytase activity through their maturation, which may assist in explaining the difference in phytic acid levels present in the samples. On the other hand, the distribution of the phytases inside the grains differs, although studies on this are limited. There is variation in reports on phytic acid concentration in cereals where some researchers have reported higher values in wheat than those obtained in this study. Dost and Tokul\(^{[23]}\) found a phytic acid concentration of 21.5 mg/g in the grain of barley; however, there are too few studies on the concentration of this component in the husk. The difference in phytic acid content may be due to various factors such as: type of soil; location; plantation irrigation conditions; use of fertilizers; amount of rainfall; and fluctuations in other environmental variables. The concentrations of phytic acid found in the analyzed samples were lower than those found in other whole grain cereals, which favors the quality of wheat bran and barley husk. Dai et al.\(^{[24]}\) explained that the formation of phytic acid complexes with proteins and minerals make them less available for absorption in the body.

| Sample         | Phytic acid* (mg/g of sample) | Saponins* (mg DE/g of sample) |
|----------------|--------------------------------|-------------------------------|
| Wheat bran     | 3.68 ± 0.03\(^a\)             | 1.16 ± 0.10\(^b\)             |
| Barley husk    | 2.32 ± 0.05\(^b\)             | 1.96 ± 0.10\(^b\)             |

DE = Diosgenin equivalent.
The results are the average of three determinations ± the standard deviation.
\(^a\)Different letters in the same column indicate significant difference \((P< 0.05)\).
\(^b\) Dry basis
Saponins

The concentration of saponins found in barley husk were significantly higher \((P < 0.05)\) than in wheat bran (Table 1). Saponins are more frequently found in the leaves and roots of plants. This may be the reason for the low concentration of saponins reported in barley husk and wheat bran. These results are similar to results reported by Gómez-Caravaca et al.\(^{25}\) who found low concentrations of saponins in quinoa (2.44 mg/g). On the other hand, two different families of saponins synthesized in oats have been reported (steroids avenacosides and triterpenoid avenacin) and are also found in leaves and roots, respectively.\(^{26}\) It is important to note that limited research exists on the content of saponins in other cereals.

Total flavonoids content

The concentration of total flavonoids is presented in Table 2. The concentration of wheat bran in the sample was between 8.67 and 24.98 mg QE/100 g. The 80% methanol extract had the lowest concentration of total flavonoids, which was significantly different from the other samples \((P < 0.05)\). The extract with absolute ethanol showed the highest concentration, presenting a significant difference \((P < 0.05)\) compared to samples with the other solvents, followed by 80% ethanol and 50% acetone. The high concentration of flavonoids in wheat bran with ethanol, may be associated with flavonoid aglycone structure, which are easier to extract in ethanol. In alcohols, hydroxyl groups can develop hydrogen bonds with oxygen atoms of the compounds, which may contribute to their being more affine to ethanol or methanol than to acetone.\(^{27}\)

In the sample of barley husk, the concentration of total flavonoids was between 5.73 and 31.31 mg QE/100 g. The sample with 50% acetone extract was significantly higher than the other solvents \((P < 0.05)\) while the lowest concentration of total flavonoids was observed in the sample with absolute methanol. The extraction efficiency of 50% acetone can be attributed to the ability of the mixture (water/acetone) to degrade complexes present in the sample and therefore release the flavonol compounds.\(^{28}\) When water is added to acetone, the polarity of the solvent increases, therefore the system is capable of extracting compounds of low, medium and high polarity, which represents a higher concentration of total flavonoids. Mixtures of acetone and methanol with water have shown to be more efficient in the extraction of phenolic components compared to the monocomponent solvent system.\(^{29}\)

When compared, samples with 50% acetone, 80% ethanol, and 80% methanol showed higher extraction efficiency in barley husk than in wheat bran. The difference between the values of each sample and solvent can be associated to the polarity degree of the compounds present in the samples, since the polarities of acetone (0.355), ethanol (0.654) and methanol (0.762) are different, as well as the viscosity and vapor pressure.\(^{30,31}\) When the solvent has a higher molecular weight, the lower polarity allows for easier extraction of other substances with approximately the same molecular

| Solvent   | Wheat bran* | Barley husk* |
|-----------|-------------|--------------|
| Methanol  | 10.08 ± 0.59\(^{CDa}\) | 5.73 ± 0.22\(^{Db}\) |
| 80% Methanol | 8.67 ± 0.58\(^{Db}\) | 14.60 ± 0.4\(^{Ba}\) |
| Ethanol   | 24.98 ± 2.01\(^{Aa}\) | 9.89 ± 0.16\(^{Cb}\) |
| 80% Ethanol | 10.90 ± 0.66\(^{BCb}\) | 14.63 ± 0.39\(^{Ba}\) |
| 50% Acetone | 12.19 ± 0.59\(^{Bb}\) | 31.31 ± 0.49\(^{Aa}\) |

*Dray basis

The results are the average of three determinations ± the standard deviation.
A-D Upper case letters indicate comparison of means between solvents of the same sample.
a-b Lower case letters mean comparison of means between samples with the same solvent.
Samples with the same letter did not present significant difference using Tukey’s test \((P < 0.05)\).
weight. Therefore, there is no single solvent capable of simultaneously extracting all classes of phenolic compounds from a sample.\textsuperscript{32}

Abozed et al.\textsuperscript{33} reported higher concentrations of flavonoids in wheat bran with 70% ethanol, a concentration of 22.39 mg/100 g in common wheat variety (Gemiza-9), and 25.80 mg/100 g in durum wheat variety (Beni-Suef-3). Studies on bran and rice husks have reported values higher than those found in this research study. Wanyo et al.\textsuperscript{34} reported values of 388 and 266 mg/100 g in bran and husk respectively; however, the type of extraction was different (distilled water at 70°C for 2 h). The concentration of flavonoids in barley husk has not been widely studied, yet some reports indicate that the concentration of these compounds in barley flour ranges from 87.73 to 247 mg/100 g.\textsuperscript{35} The concentrations of flavonoids in barley husk are among the first reported studies.

**Total phenolic compounds**

Results of phenolic compounds showed that concentrations varied between the different solvents (Table 3). The wheat bran sample had a concentration between 99.11 and 188.61 mg GAE/100 g. The solvents 80% ethanol and 50% acetone extracted the highest concentrations of phenolic compounds (179.79 and 188.61 mg GAE/100 g, respectively). These concentrations were statistically different from results obtained with the other solvents ($P < 0.05$). However, 50% acetone presented no significant difference ($P > 0.05$) with respect to 80% methanol. In contrast, absolute methanol and ethanol were the solvents that extracted the lowest quantity of phenolic compounds (112.79 and 99.11 mg GAE/100 g, respectively). Some lipid components were also extracted, which may have a limiting effect on the extraction of phenolics.\textsuperscript{36} The extraction efficiency also depends on the polarity of certain phenolic compounds; not all are extracted efficiently with organic solvents. The addition of water in solvents generates a medium polarity that facilitates the extraction of other components soluble in organic solvents and/or water as compared to absolute solvents.\textsuperscript{37} The tendency to solubility can be associated with the stereochimistry of the phenols (the polar and the nonpolar fragment within their molecules) and the intermolecular forces of hydrogen bonds that occur between the phenolic compounds and the solvents.\textsuperscript{27}

The barley husk sample presented values of 82.86–340.72 mg GAE/100 g. The 50% acetone and 80% methanol solvents showed high efficiency in the extraction (340.72 and 324.38 mg GAE/100 g, respectively), which presented a significant difference ($P < 0.05$) with respect to the other solvents. Absolute methanol exhibited the statistically lowest extraction yield (76.38 mg GAE/100 g) while 50% acetone was the best extraction solvent for both analyzed samples. The constant high amount of phenols found with acetone, can be understood by how this type of solvent diffuses easily in the pores of vegetal materials allowing it to leach bioactive compounds,\textsuperscript{38} which contributes to greater concentrations.

**Table 3.** Total phenolic content in wheat bran and barley husk extracted with different solvents (mgGAE/100g of sample).

| Solvent      | Wheat bran*               | Barley husk*               |
|--------------|---------------------------|----------------------------|
| Methanol     | 112.79 ± 7.70 Ca          | 82.86 ± 2.03 Db            |
| 80% Methanol | 170.85 ± 5.79 Bb          | 324.38 ± 4.82 Aa           |
| Ethanol      | 99.11 ± 9.08 Cb           | 261.17 ± 12.57 Ra          |
| 80% Ethanol  | 188.61 ± 3.08 Aa          | 133.25 ± 1.57 Cb           |
| 50% Acetone  | 179.79 ± 1.15 Abb         | 340.72 ± 12.98 Aa          |

GAE = Gallic acid equivalents.
The results are the average of three determinations ± the standard deviation.
A-D Upper case letters indicate comparison of means between solvents of the same sample.
a-b Lower case letters mean comparison of means between samples with the same solvent.
Samples with the same letter did not present significant difference using Tukey’s test ($P < 0.05$).
*Dray basis
The extraction trend with the different solvents was similar to that reported by [39], who showed that the content of total phenols was affected by the solvent, with acetone being the highest extraction, followed by ethanol and methanol. Abozed et al. [33] also found a greater extraction efficiency with 50% acetone in wheat bran. However, the concentrations reported by these authors (466 and 388 mg/100 g) were higher than those obtained and reported in this study.

Wang et al. [34] reported that the most efficient phenolic compounds extraction was obtained with 64% ethanol at a concentration of 312 mg/100 g. Similar concentrations were reported by Kim et al. [40] in two types of white and red bran (336.2–396.7 mg/100 g). Brewer et al. [41] reported concentrations from 518 to 736 mg/100 g in wheat bran with 80% methanol. Jonnala et al. [42] studied wheat bran from different growing lines and found a concentration value of about 225 mg/100 g. Vaher et al. [43] reported similar concentrations (125.8–315.7 mg/100 g); however, the solvent used was absolute methanol. Barley husk studies are few, but there are studies conducted using flours from this cereal. Liu and Yao [8] studied different extraction solvents: 70% acetone, 70% ethanol, and 70% methanol with barley flour. The highest concentration of phenolic compounds extracted was obtained with 70% acetone (196 mg/100 g). Djordjevic et al. [44] reported higher concentrations with 70% ethanol in barley grain (Hordeum vulgare).

Phenolic compounds are mainly concentrated in the cell walls of the outer layer. They are mostly esterified to the arabinoxyl side groups of arabinoxylans. Then, the highest proportion is located in the bran and germ fractions of wheat, being eliminated when wheat is milled into flour. [43] The content of phenolic acids depends on genetic components, as well as genetic interactions with the environment, which results in variations between the species of cereals and cultivars of the same family.

Identification and quantification of phenolic compounds

Table 4 shows the phenols found in wheat bran and barley husk. In wheat bran, only gallic, caffeic, p-coumaric, and ferulic acids were identified. Caffeic acid with 50% acetone showed the highest

| Phenolic compounds in wheat bran and barley husk with different solvents. |
|-----------------------------|--------|--------|--------|--------|--------|
| **Phenolics** (mg/100g)     | Methanol | 80% Methanol | Ethanol | 80% Ethanol | 50% Acetone |
| Gallic acid                 | 18.12 ± 2.01a | 17.16 ± 0.15a | 16.57 ± 0.16a | 16.95 ± 0.56a | 17.15 ± 0.62a |
| Chlorogenic acid            | nd      | nd      | nd      | nd      | nd      |
| Vanillic acid               | nd      | nd      | nd      | nd      | nd      |
| Caffeic acid                | 29.76 ± 0.97c | 64.29 ± 0.25b | 0.86 ± 0.08d | 53.55 ± 2.27b | 97.61 ± 9.60a |
| p-coumaric acid             | 19.68 ± 0.33b | 20.63 ± 0.15b | nd      | 20.57 ± 0.24b | 23.40 ± 0.64a |
| Ferulic acid                | 9.06 ± 0.15a | 9.73 ± 0.09a | nd      | 9.12 ± 0.02a | 9.70 ± 0.33a |
| Benzoic acid                | nd      | nd      | nd      | nd      | nd      |
| Quercetin                   | nd      | nd      | nd      | nd      | nd      |
| Kaempferol                  | nd      | nd      | nd      | nd      | nd      |

| Phenolics (mg/100g)         | Methanol | 80% Methanol | Ethanol | 80% Ethanol | 50% Acetone |
|-----------------------------|--------|--------|--------|--------|--------|
| Gallic acid                 | 21.21 ± 2.0b | 41.80 ± 4.1a | 22.93 ± 2.7b | 22.87 ± 1.6b | 22.97 ± 2.0b |
| Chlorogenic acid            | 43.12 ± 3.73b | 75.92 ± 0.06c | 13.85 ± 0.24c | 43.96 ± 3.82b | 51.23 ± 2.57b |
| Vanillic acid               | nd      | nd      | nd      | nd      | nd      |
| Caffeic acid                | 21.71 ± 0.74a | 22.24 ± 0.71a | nd      | 20.20 ± 0.41a | 20.82 ± 0.32a |
| Benzoic acid                | 9.89 ± 0.24c | 10.59 ± 0.14d | nd      | 11.00 ± 0.02b | 11.57 ± 0.02a |
| Quercetin                   | 19.40 ± 0.44b | 20.52 ± 0.75ab | nd      | 19.84 ± 0.68b | 22.53 ± 0.1b  |
| Kaempferol                  | 24.98 ± 0.37a | 28.22 ± 2.49a | nd      | 26.24 ± 2.39a | 27.21 ± 1.73a |

nd = not detected
The results are the average of three determinations ± the standard deviation.
a-b Upper case letters indicate comparison of means between solvents of the same sample.
Samples with the same letter did not present significant difference using Tukey’s test (P < 0.05).
*Dray basis
concentration of phenolic acid. Similarly, p-coumaric acid showed a significantly higher concentration ($P < 0.05$) with 50% acetone compared to the other solvents. Gallic and ferulic acid, did not present any significant difference ($P > 0.05$) with respect to the different solvents used. The concentration was 16.57–18.12 mg/100 g for gallic acid and 9.06–9.73 mg/100 g for ferulic acid. Chlorogenic, vanillic, and benzoic acids, as well as kaempferol and quercetin were not identified with any solvent. Furthermore, p-coumaric and ferulic acid were not detected with absolute ethanol; this last solvent was the least efficient for identifying phenols. The extraction efficiency of the different solvents was similar to that observed in the quantification of total phenols. Kim et al.\cite{40} observed lower concentrations in red and white wheat bran. Pasha et al., 2012 also reported lower concentrations of caffeic acid, p-coumaric acid and ferulic acid in wheat bran of different varieties compared with those found in this study. Vaher et al.\cite{43} reported a higher concentration of ferulic acid in winter wheat bran (53.2 mg/100 g) than in springer (26.89 mg/100 g). Both concentrations were higher than those reported in this study (Table 4). They also reported p-coumaric acid; however, this was lower in winter bran than in springer bran, and both were lower values than the data reported in Table 4.

The profile of phenols in barley husk revealed the presence of gallic, chlorogenic, caffeic, p-coumaric, ferulic, and benzoic acids, as well as kaempferol and quercetin. The highest concentration found was kaempferol with absolute methanol, 80% methanol, and 50% acetone. Gallic acid presented a significantly higher concentration ($P < 0.05$) with 80% methanol while the other extraction solvents did not show any significant difference ($P > 0.05$). Chlorogenic acid also presented a significantly higher concentration ($P < 0.05$) with 80% methanol and the lowest concentration was observed with ethanol. Additionally, p-coumaric acid and quercetin did not present a significant difference ($P > 0.05$), as compared with any solvent: the concentrations ranged from 20.20 to 22.24 and from 24.98 to 28.22 mg/100 g, respectively. Ferulic acid and benzoic acid showed higher concentration with 50% acetone; however, benzoic acid was observed in a high concentration with 80% methanol. It was observed that 80% methanol and 50% acetone yielded better extraction efficiency and phenolic identification; a similar behavior was observed in the quantification of total phenols.\cite{45,46} have reported that flavonols are the most abundant phenolic fraction, which concurs with data reported for barley husk. Studies of phenolic compounds in barley husk are limited. Concentrations obtained in the husk are higher than values reported in barley flours.\cite{47} In bran and rice husk, lower concentrations of phenolic compounds have also been reported than those in barley husk and wheat bran. Wanyo et al.\cite{34} reported on rice bran, the concentrations of vanillic acid, p-coumaric, ferulic and kaempferol (0.41 mg/100 g, 1.1 mg/100 g and 0.2 mg/100 g respectively). For rice husk, they reported vanillic, p-coumaric, and ferulic acids (0.413 mg/100 g, 0.15 mg/100 g, and 0.99 mg/100 g, respectively); nevertheless, kaempferol was not detected. The variation of concentrations may be associated with the different type of varieties and differences in growing time.\cite{43} The phenolic profile concentration was higher in barley husk than in wheat bran with 50% acetone, which suggests that barley husk can be considered an ingredient that acts as a source of natural antioxidants with health potential for the prevention of certain diseases.

**Antioxidant activity**

The antioxidant activity measured by DPPH showed that the extracts with 80% methanol and absolute ethanol exhibited the lowest antioxidant activity in both samples (Table 5). Antioxidant activity values from 5.24 to 9.49 μmol TE/g were obtained in wheat bran. The highest concentration was observed in the extracts with methanol, 80% ethanol, and 50% acetone.

In barley husk samples, the antioxidant activity was from 2.11 to 36.08 μmol TE/g. All barley husk extracts presented a significant difference ($P < 0.05$) between them. The highest concentration was obtained with 50% acetone, the second highest with ethanol at 80%, followed by absolute methanol, and finally 80% methanol and absolute ethanol. Furthermore, 50% acetone exhibited the highest antioxidant activity values in both samples. The data concurs with results reported by Zhou and Yu.\cite{48} who found the highest concentration of phenolic compounds and antioxidant activity in wheat bran, using 50% acetone.
Comparing the two analyzed samples, barley husk was the most active in all extraction solvents, except for the extract with 80% methanol that had no significant difference (P > 0.05) with respect to wheat bran. The data found for wheat bran agree with findings from Abozed et al.\textsuperscript{33} who observed that the percentage of free radical inhibition was higher with 70% ethanol in two different varieties of wheat bran. Also, antioxidant activity of 5.074 μmol TE/g has been reported in white rice bran, which is similar to antioxidant activity found in wheat bran and barley husk with 80% methanol.\textsuperscript{49}

Liu and Yao\textsuperscript{8} reported greater efficiency in the percentage of inhibition of free radicals in extracts with 70% acetone in barley extracts using DPPH\textsuperscript{*}. ABTS\textsuperscript{+} method produced higher concentrations of antioxidant activity than DPPH\textsuperscript{*} method. It is known that DPPH reacts better with polyphenols but not with phenolic acids and sugars. While ABTS has higher reactivity, it has an increased ability to react with a wide range of antioxidants,\textsuperscript{50,51} which may be the reason for the difference in concentrations.

With ABTS\textsuperscript{+} method, concentrations from 112.15 to 172.62 μmol TE/g were observed in wheat bran, and the highest activity was obtained with absolute methanol and 80% ethanol with a significant difference (P < 0.05) when compared against the other extracts, while the lowest concentration was observed with absolute ethanol. Higher ranges were observed in barley husks when compared to wheat bran with values from 134.71 to 494.44 μmol TE/g. The highest antioxidant activity was observed with the extract of 50% acetone, which also showed a significant difference (P < 0.05) as compared to the other extracts. In contrast, 80% ethanol and absolute ethanol extracts showed the lowest values. The high concentration of antioxidant activity in barley husk is related to the concentration of phenols, flavonoids, and saponins; the latter which also demonstrate antioxidant activity. All of these components were found to be higher in barley husk than in wheat bran.

The variation in the contents of antioxidant activity besides depending on the polarity of the phenolic compounds and flavonoids. It also depends on selectivity and action mechanism of each radical used.\textsuperscript{52} Phenolic compounds exhibit different behaviors with radicals, according to their chemical structures.\textsuperscript{53} In addition, barley usually contains a higher concentration of sugars that can influence greater antioxidant activity. Carbohydrates that are soluble can form a synergistic effect with phenolic compounds and increase antioxidant activity.\textsuperscript{54} They donate hydrogen from their activated reducing ends to oxidized phenolic compounds. In addition, soluble polysaccharides can improve the chemical stability of phenolic compounds due to the steric hindrance that protects them from oxidation.\textsuperscript{55,56} It is possible that these conjugated compounds are contributing in an important way to the elimination of free radicals\textsuperscript{57} and therefore the increase in antioxidant activity. To test this hypothesis, it is necessary to conduct more studies.

| Table 5. Antioxidant activity in wheat bran and barley husk extracted with different solvents (μmol TE/g of sample). |
|---------------------------------------------------------------|
| **Solvent**                                             | Wheat bran | Barley husk |
|---------------------------------------------------------------|
| DPPH\textsuperscript{*}                                     |            |             |
| Methanol                                                 | 9.49 ± 0.12<sup>Ab</sup> | 19.160 ± 0.39<sup>Ca</sup> |
| 80% Methanol                                             | 5.24 ± 0.49<sup>Ba</sup> | 5.73 ± 0.03<sup>Da</sup> |
| Ethanol                                                  | 6.14 ± 0.17<sup>Ba</sup> | 2.11 ± 0.13<sup>Eb</sup> |
| 80% Ethanol                                              | 8.76 ± 0.74<sup>Ab</sup> | 25.70 ± 0.28<sup>Ba</sup> |
| 50% Acetone                                              | 8.44 ± 0.40<sup>Ab</sup> | 36.08 ± 0.17<sup>Ca</sup> |
| ABTS\textsuperscript{+}                                  |            |             |
| Methanol                                                 | 172.62 ± 3.19<sup>Aa</sup> | 170.81 ± 9.25<sup>BCa</sup> |
| 80% Methanol                                             | 116.36 ± 9.12<sup>Cb</sup> | 191.97 ± 3.64<sup>Ba</sup> |
| Ethanol                                                  | 112.15 ± 1.62<sup>Db</sup> | 149.32 ± 6.40<sup>Cb</sup> |
| 80% Ethanol                                              | 163.24 ± 2.18<sup>Da</sup> | 134.71 ± 10.88<sup>Db</sup> |
| 50% Acetone                                              | 132.91 ± 3.85<sup>Bb</sup> | 494.44 ± 13.28<sup>Aa</sup> |

The results are the average of three determinations ± the standard deviation.

A-D Upper case letters indicate comparison of means between solvents of the same sample.

a-b Lower case letters mean comparison of means between samples with the same solvent.

Samples with the same letter did not present significant difference using Tukey’s test (P < 0.05).

\*Dray basis
The results indicate that the phenolic compounds, flavonoids and antioxidant activity can be highly extracted with 80% ethanol and 50% acetone in wheat bran and barley husk respectively. Studies in barley husk are still limited. The use of bran and husk in food may be restricted by antinutritional compounds and high level of ash, however the extracts obtained from each sample are viable to be applied in food through their incorporation as additives, which can contribute to the benefit of human health.

Conclusion

The results of this study reveal that wheat bran has a higher concentration of phytic acid and a lower concentration of saponins when compared to barley husk. In wheat bran, 80% ethanol was the solvent with the highest extraction of phenols and antioxidant activity and the solvent with the lowest efficiency was absolute ethanol. In barley husk, acetone at 50% was the most efficient solvent, and was most efficient for the extraction of caffeic and p-coumaric acid. The gallic and ferulic acid were extracted with the same efficiency for all solvents. Chlorogenic, vanillic, benzoic acid, quercetin and kamferol were not detected in the wheat bran sample. In barley husk, vanillic and caffeic acid were not detected, with the last one only identified with 50% acetone. The addition of water to the solvents used permitted an increase in the extraction efficiency of bioactive compounds and antioxidant activity. Synergistic interactions between phenols and other compounds such as carbohydrates and protein could contribute to increased antioxidant activity. Studies on barley husk are limited and are rarely used in the industry. More studies are necessary to understand the recommended uses of barley husk as a functional ingredient, due to possible biological contaminants such as fungi, mycotoxins, as well as non-nutritional compounds.

Acknowledgments

This research manuscript was supported by Catedras CONACyT project 1232. In addition, the authors gratefully acknowledge María Yolanda Mercedes Rodríguez Aza from CINVESTAV- Irapuato Campus for her assistance with HPLC analysis. All authors possess no conflicts of interest.

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