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Bioactive Compounds and Antioxidant Potential of Soft Wheat and Oat Bran on the Algerian Market

Samira MEZIANI *, Souad SAIDANI, Lahouaria LABGA, Rawda BENGUELLA, Imene BEKHALED

Laboratory of Biotoxicology, Djillali Liabes University, Faculty of Life and Natural Sciences, Department of Biology, Algeria.

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* **Corresponding author info:**
Dr. Samira MEZIANI
Email: meziani_samira@yahoo.fr
Tel. +213 56 03 37 210

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ABSTRACT

BACKGROUND: Rich in dietary fibers and beneficial to health, wheat and oats have been a popular part of the human diet. The whole grain is rich in protein, lipid, starch and phenolic compounds concentrated at the level of the peripheral layer of the bran. **AIMS**: The natural compounds and the antioxidant potential of two different species of soft wheat and oat bran on the Algerian market have been studied in this work. Wheat bran was furnished by Azzouz's Cereal and Dried Vegetable Cooperative (CCLS) being the most commercialized oat bran in Algeria. **MATERIALS AND METHODS**: Some parameters and bran biochemical compounds such as proteins, cellulose, ash content, phenolics, and antioxidant potential (DPPH) were determined using different techniques and methods (infra-red approach spectrophotometer, and flame spectrophotometer). **RESULTS**: The results obtained showed that studied soft wheat bran was rich in protein (17.36%). Concerning cellulose, a high value was recorded for this bran variety 11.3%, which was lower for oat bran (2.7%). The maximum concentration of potassium and sodium was observed in the soft wheat bran variety; 3.16 mg/L, 30.36 mg/L respectively. The levels of the phenolic compounds were 0.720 ± 0.050 mg EAG / g and 1.101 ± 0.01mg EAG / g for the oat bran and the soft wheat bran respectively. These results underline that both studied brans contain significant levels of compounds essential for consumer needs. **CONCLUSIONS**: The studied soft wheat bran variety is considered to be an important source of phytonutrients.

KEYWORDS: Wheat, oat, antioxidants, radical scavenging, phenolic contents.

1. INTRODUCTION

Wheat and oats constitute a valuable source of dietary fiber in the human diet showing various health benefits. The cultivation of wheat and oats is well known worldwide representing more than 50% of cereals [1]. the grains of both species share several similarities in the tissue composition at the level of the endosperm and the embryo during plant development. Sieving, separation, and grinding are the different milling processes used for grain to prepare flour and by-products. Their nutrient content after grinding will belong to the rate extracted from the eliminated bran where vitamins, minerals, and fibers are concentrated [1,2]. The whole grain of cereals is found with higher amount at the level of the peripheral grain part (i.e. the envelopes, AL and germ). Their quantities decrease concomitantly with the separation processes used to isolate the starchy endosperm [3] that are rich in natural compounds including phenolics, carotenoids, vitamins especially vitamin E being beneficial in whole grains [4]. Cereal bran, such as that obtained from wheat and oats, was used in animal and poultry feed [5]. Oat (*Avena sativa L.*) is a well-known annual crop in temperate climates. Oat is rich in protein, lipid, starch and phenolic compounds concentrated in the peripheral layer of bran [6]. This cereal species is recognized to be a

healthy food containing significant amounts of soluble dietary fiber, β-glucans, fat-soluble vitamin E and polyunsaturated fatty acids. Oats have various health benefits, cholesterol-lowering and anti-cancer drugs, recommended for celiac patients. Furthermore, it could be added to certain food products such as bread, biscuits and baby foods [7]. On the other hand, wheat bran (*Triticum aestivum*) is composed of the outer pericarp, the intermediate layers, and the inner pericarp [8]. Wheat bran is generally considered as a major source of proteins, lipids, fiber, minerals, and phenolic compounds that are important for human health [4]. The total phenolic and total flavonoid contents are higher in wheat bran that may act as antioxidants to preventing heart disease and lowering colon cancer incidence [9].

The objective of the current study was to determine the specific compounds of bran in two cereal species, mature grain (wheat and oats). This paper provides an overview of the nutritional and health benefits, to compare the biochemical and phytochemical content particularly of total phenolic content (TPC) between the two species. This will contribute to consider and reiterate the nutritional benefits of wheat and oats for consumers and the Algerian market.

2. MATERIAL AND METHODS

The present study is devoted to the separation, analysis, and comparison of two species in bran cereals: commercialized oat bran (OB) and imported wheat bran Azzouz (WBAZ) traded in the Northwestern region of Algeria. The samples were tested, analyzed and compared to determine the biochemical compounds (moisture-ash-starch rate-rate of cellulose-protein level) and to assess secondary metabolites (phenolic compounds, DPPH).

2.1. Determination of chemicals parameters

Analysis in the infrared approach (NIRS): NIRS: Near-infrared spectroscopy Near-infrared spectrometry is an increasingly sophisticated analytical technique for the rapid control of grain quality. This technique allows determining the rate of different parameters such as moisture-ash-starch rate-rate of cellulose-protein. The principle is the absorption of organic matter by infrared light [10]. The technique is based on the measurement of the reflectance of radiation emitted at a given wavelength in the visible or the infrared, the different chemical bonds of the tested product (OH, NH or CH) absorb at lengths of specific wave equal to their vibration frequency and thus go from a ground state to an excited state [11].

2.2. Determination of minerals (Potassium and Sodium)

A quantity of 0.5 g of wheat bran and oats was sampled to be diluted in 10 ml of distilled water, then centrifuged and stirred several times for 10 min. Quantities were prepared at known concentrations to draw the calibration curve. To perform it we took 1.94 g of NaCl for the NA^+ and 2.43 g for the solution of K^+ in 100 ml of distilled water, the solutions were made apart.

2.3. Phytochemical study

2.3.1. Extraction of phenolic compounds

The extraction was carried out by maceration according to the protocol of Diouf *et al.* [12]. A mass of 10 g of each wheat and oat bran samples was macerated in 100 ml of ethanol (70%). After 24 hours, the mixtures were separated by filtration. The extracts were then evaporated to dryness using the rotary evaporator at approximately 45°C.

2.3.2. Determination of total polyphenols

Folin-Ciocalteu reagent has the ability to oxidize the phenolate ions that result from the formation of the added sodium carbonate complex to the extract solution. A blue color is obtained, and the intensity reflects the concentration of the phenolic compound in a given extract. The Folin-Ciocalteu method [13], simple and sensitive, is used to measure the amount of the total phenol. In 100 µl of the extract, we added 250 µl of Folin reagent (Sigma-Aldrich, Germany) diluted (50% v / v). After 5 min of incubation at 25°C, 250 µl of 20% (w / v) sodium carbonate was added in the tubes and the whole was brought to 2000µl with distilled water. The absorbance was read at 760 nm after 60 min. The blanks were prepared for each variety by replacing the Folin reagent with distilled water. Gallic acid (Sigma-Aldrich, Germany) was utilized as standard and the results were expressed in mg gallic acid equivalent per 100 mg of material dried. Despite the sensitivity and simplicity of the Folin method, it is not specific to polyphenols. Indeed, the reagent can react with proteins, reducing sugars, ascorbic acid and sulfur compounds [13].

2.3.3. Determination of flavonoids

The flavonoids represent a subclass of phenolic compounds and their presence is an indicator of their richness in those products. The usefulness of this method is determined according to the protocol described by Djeridane *et al.* [15] using a colorimetric method described previously [16]. A reaction with the hydroxyl groups of flavonoids to form a yellow complex with $ALCL_3$ chelating metals into AL_3 [14].

The absorbance was measured immediately against the blank at 510 nm. Flavonoids were dosed first by the use of

the calibration curve with quercitrin, carried out by measuring the absorbance at known concentrations. the results were expressed in microgram equivalents of quercitrin per milligram of dry extract (mgEQ / g).

2.3.4. Determination of antioxidant activity

In order to evaluate the antioxidant activity, the reducing power, radical scavenging DPPH were measured in wheat and oat bran samples extract.

2.3.5. DPPH radical scavenging activity

The DPPH gives a purple color and rapidly disappears when reacting with compounds displaying anti-free radical properties at 517 nm of absorbance. The intensity of the reaction is inversely proportional [17]. Antioxidant activity was measured by the DPPH method =1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich, Germany) [18]. 10 ml of a hydroacetone solution (80%: 80 ml of acetone and 20 ml of water) was poured into tubes containing 0.5 g of each variety. The mixture was vortexed every 10 minutes during 2 h and centrifuged at 1700 g for 10 min. The supernatant was recovered for dosages. Ascorbic acid (Sigma-Aldrich, Germany) was used as a standard.

2.3.6. Antioxidant activity measurement

This activity was determined by the method of Awika *et al.*[19]. The radical DPPH is dissolved in methanol at a concentration of 6.10^{-5} mol.L^{-1} and kept at -20°C protected from light before use. To each extract of 0.3 ml, 2.7 ml of DPPH solution was added and the absorbance was measured after 8 am at 517 nm. The results were compared with a standard using ascorbic acid as the antioxidant of the better performance of this analysis. IC50 was calculated that provides 50% inhibition of DPPH.

3. RESULTS AND DISCUSSION

3.1. Result of chemicals parameters

Separation and evaluation of phytochemicals in bran samples were conducted following the protocol of Liyana-Pathirana & Shahidi [20]. The extraction methods vary and depend on the use of organic solvents widely used and accepted to extract a significant rate of phenolic compounds (methanol, ethanol, and acetone). In our experiment, ethanol was the most appropriate solvent for extracting the entire phenols from bran samples.

The biochemical analyses carried out at the level of quality control laboratory of the "Habbour" mills by the INFRANEO infra-red spectrophotometer allowed us to study various parameters. The obtained results are expressed in percentage as shown in Table 1. The starch content was low for wheat bran (SWBAZ) 16.4%, and high 38.9% for oat bran (OB) sample. This difference could result from the poor handling of the milling process. This makes the bran containing fragments of the outer extern layer of the starchy albumin and the starch grains remain attached to the different particles of the bran [21]. The protein content in the oat sample was the lowest with a rate of 13.8%. Protein values are consistent with the study on soft wheat bran [22-24].

Table 1: Results in (%) of biochemical analyzes of the two varieties of soft wheat bran and oat bran

Parameter / Species	Origin	Humidity (%)	Ashes (%)	Proteins (%)	Starch (%)	Cellulose (%)
SWBAZ	French	13.09	6.42	17.36	16.4	11.3
(OB)	Local	17.8	4.42	13. 8	38.9	2.7

Table 2: Concentration of Potassium and Sodium in mg content in the two bran samples

Parameters / Species	Potassium (K) in (mg)	Sodium (NA) in (mg)
SWBAZ	3.16	30.36
OB	2.35	37.21

Table 3: Extraction yield in % of wheat bran by ethanol/methanol and total phenolic compound in EAG/g and flavonoid in mg EC/g

Parameters / Species	Methanol	Ethanol	Phenolic compounds	Flavonoids
SWBAZ	11.21	14.14	1.10	0.2
OB	12.34	54.19	0.72	0.17

SWBAZ: Soft wheat bran AZZOUZ, OB: Oat bran

3.2. Results of minerals (Na, K)

The concentration of Na^+ and K^+ ions was calculated from the regression equation of the calibration ranges established with Sodium and Potassium. As shown in Table 2, we noticed that sodium was more abundant than potassium in the bran of both varieties. The maximum concentration of potassium was reported in the bran soft weight variety (SWBAZ) sample with a concentration of 3.16 mg/L and the highest concentration of sodium was observed for oat bran (OB); 37.21 g/L. The high mineral content in both samples of bran is explained by the richness of the cereals in minerals, especially the aleurone layer and the pericarp [25]. A significant variability in the mineral content of one sample of cereals bran is noted in the literature. This difference is due to environmental factors that characterize, the genotypes and species [26];

the wheat grain transformation process could be a source of variation in the mineral concentration [27].

3.3. Results of phytochemical analyzes

Research has focused on the extraction of phenolic compounds from new inexpensive or residual vegetable sources in the agri-foods industries. The results of several studies confirm that phytochemicals of interest that are beneficial to consumer health are mainly found in bran and wheat germ [28,29]. The starchy endosperm is produced by grinding the grain by separating the germ and the bran made up of peripheral tissues (Testa, haylin and aleurone layer) which are separated, removed and intended for animal feed. The presence of the phenolics compounds in wheat bran is mainly covalently cross-linked with cell wall polymers [31].

3.3.1. Efficiency of ethanol/methanol extraction

Extraction is a crucial step in isolation and identification. The extraction yield depends on the method and the solvent used [28]. The extraction of phenolic compounds with ethanol in our samples allowed us to calculate the yield of each extract, determined by 10 g of plant material expressed as a percentage. The obtained results are illustrated in Table 3. Ethanol extraction showed that the (OB) extract displays a strong yield at a value of 54.19%, other more or less considerable yield were observed in the extracts of oat bran.

3.3.2. Determination of total polyphenols

The results of the colorimetric assay provide an overview or an idea of the content of phenols and flavonoids. The phenolic extracts thus obtained generally have a honey-colored, slightly caramelized pasty appearance for (OB) and (SWBAZ) extracts. Folin-Ciocalteu was selected to determine the phenolic compounds. In this method, we used the gallic acid standard to plot the calibration curve at known concentrations with the absorbance measured. These parameters are used to determine the concentrations of polyphenols expressed in milligrams per gram present in the bran. The values of compound phenolic different cultivars of wheat bran and oat bran of extraction conditions are summarized in Table 3. The results drawn diagrammatically show that the number of polyphenols is high in extracts (SWBAZ) with a content of 1.101±0.01mg EAG/g, compared with oat bran extract (OB) with a content of 0.720±0.05 mg EAG/g. This variation can be explained in the differences that exist in the chemical composition between plant tissues.

The experimental values of PC were 0.921 mg GAE/g bran according to a descriptive study by Singh *et al.* [30]. From a comparative point of view, the genetic variation between varieties must be taken into consideration in order to evaluate phenolic compounds, because its compounds depend on the structure and genetic composition of the wheat raw material and also the method of extraction and produce wheat bran which causes a great variation between the cultivars. The wheat bran layers contain several tissues (aleurone layer, intermediate layers, and seed coat) and every tissue contains essential nutrients, namely total phenolics which are mainly related to cell wall components [31,32]. Other studies showed that the aleurone layer (wheat bran fraction) in relation to other tissues that consistently possess the highest antioxidant capacity among wheat fractions [33,34].

3.3.3. Flavonoid dosage

More than 5000 flavonoids have been classified and described in the group of polyphenols [36]. In our study, the flavonoid assay was performed according to the $AlCl_3$ method using quercetin as standard (Y=4.6153x-0.0118). The results obtained are expressed in milligrams per gram of dry matter in the equivalent of quercetin. The flavonoid levels are expressed in Table 3. It can be seen, from the results, that a significant rate of flavonoids was found in oat bran with 0.200 ± 0.004mg ER / g. SWBAZ wheat bran was 0.174 ± 0.04mg ER / g of the same compound. Onipe *et al.* [24] and Keegstra *et al.* [25] estimated a flavonoid content comprise between 3000-4300 micrograms. Flavonoids are a class of beneficial antioxidant substances of total phenolic compounds and some studies suggest that this class is more effective as antioxidants than Vitamin C. According to Žilić *et al.* [4], the flavonoids were detected at the level of the bound to the cell wall of wheat. Total flavonoids recorded in durum wheat bran 259.31 mg CE/kg.

3.3.4. Scavenger power of the radical DPPH

In this test, the highest antiradical power is vitamin C with a percentage of (76.522%) followed by common wheat (68.007%) and the lowest value is attributed to oats (62.325%) (Figure 1). According to a study described by Turkmen *et al.* [37], who indicated that this class is an important and effective donor of hydrogen from this radical, due to their ideal chemical structural composition. The phenolic fraction does not incorporate all the antioxidants and the synergistic interactions between the antioxidants in a mixture makes that the antioxidant activity depends not only on the concentration but also on the structure and nature of the antioxidants [38]. On the other hand, ultrafine milling of wheat bran increased the surface, which increased its antioxidant capacity.

The first point found is that the milling gold or mechanical grinding is applied in the husks of the grains, the phenolic components which are attached by the walls or the

peripheral tissues with the glycans contribute to increasing the antioxidant potential. The same observation was noticed by Keegstra *et al.* [25] where the significant increase, during the grain crushing stage with reduction of the tiller, rendered the phenolic components more accessible in the bran.

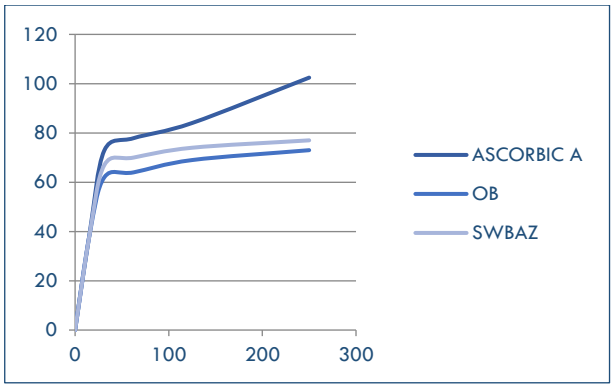

Figure 1: Antioxidant activity of oat bran and soft wheat bran Azzouz

Figure 2: inhibition concentration of two bran varieties of cereals.
SWBAZ: Soft wheat bran AZZOUZ, OB: Oat bran

Our results show that wheat and oat bran extracts have significant antioxidant potential with IC50 varying between 40 and 122 µg / ml (Fig 2). The IC50 expresses the quantity of antioxidants necessary for the reduction of DPPH to 50%. In the case where the index is important, the antioxidant activity is weak [39]. The genotypes and the different species used in our case explain this difference. Furthermore, the extraction method, the choice of solvent used and also the dosage methods, the presence of hydroxyl groups as hydrogen donor constitute essential factors of these variations.

4. CONCLUSION

Bran is a co-product of the flour mill representing with flour and germ one of the three fractions of the milling. Bran is used for physical or chemical protection for the endosperm and the germ. Several studies confirm that phytochemicals (phenolic compounds, vitamins, carotenoids, and minerals) mainly found in the bran and germ of cereals have a beneficial interest in consumers' health. Bran plays a key role in the overall health benefits of whole grains. The assessment of bioactive compounds revealed the presence of a considerable quantity of polyphenols and flavonoids qualitatively and quantitatively. Our study showed that soft wheat bran and oats are an important source of antioxidants. Varietal differences, under the influence of environmental factors characterized by grain cultivation and the varietal effect of wheat grain and oats, are also considered.

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