Bioactive Compounds in the Peripheral Layers of Barley and Triticale Species in the Mature Grain Cultivated in Algeria

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ABSTRACT

The present study designed to determine in compositions of peripheral layer (PL) from barley and triticale. The peripheral layer is a co-product of the grain mill, it represents with the flour and the germ one of the fractions of the milling, it is used for the chemical protection of the endosperm and the germ. Phytochemicals (phenolic compounds, vitamins and minerals) are beneficial for the health of consumers and are found abundantly in the peripheral layer of cereals. The objective of our work consists an evaluation of the phytochemical value for peripheral layers, the evaluation of the antioxidant content and the antioxidant activity of two varieties of two species of cereal in mature grain: triticale (Ksar Shahi, Beni Haroun) and barley (Fouara, Saidia); from two different regions (Sidi Bel Abbès and Constantine). Finally a comparative study was found in this work. The results obtained show that the variety of each species Triticale (Ksar Shahi), barley (Fouara) have the highest content of polyphenol and flavonoid (0.027 mg (EAG)/g; 0.019 mg EC/g) and (0.012 mg (EAG)/g; 0.013 mg EC/g), respectively, for the antioxidant activity barley Fouara 1.91 mg/ml shows the best activity against the DPPH radical, a high level of minerals has been found for the triticale variety of barley 33.78 mg/l. We are planning additional studies to better characterize the nature of the polyphenolic compounds existing in different histological parts of the wheat grain.

Keywords: Barley, Triticale, Peripheral layer, Polyphenols, Antioxidant activity,

INTRODUCTION

In cereals, study for grains tissus, is observed in different species, in barley (Hordeum vulgare) and Triticale (× Triticosecale Wittm.). Whole-grain barley and triticale and some dry-milled bran grain products. Whole-grain barley and triticale flours contain occupies an important place among forage species. About 65% of the barley grain is used for animal feed, 33% for malting, while only 2% is used directly for human consumption. In barley, the epidemiological studies have consistently shown that regular consumption of whole grain barley reduces the risk of developing chronic diseases. Whole grain barley also contains phytochemicals, including phenolic acids, flavonoids, lignans, tocols, phytosterols and folicale. These phytochemicals have strong antioxidant capacities, useful for reducing the risk of certain diseases.

The peripheral layer is a co-product of the flour mill, it represents with the flour and the germ one of the three fractions of the milling, it is used for the physico-chemical protection of the endosperm and the germ. The main phytochemicals in barley that have shown health benefits are phenols, flavonoids, vitamin (tocols), sterols and vitamins. Its compounds provide defenses against pathogens and contribute to the color of plants. Although in barley (Hordeum vulgare L.) it comprises about three cell layers, the AL surrounds the endosperm tissue of grass seeds and is morphologically and biochemically distinct from it. Regarding the morphology, barley showed the thickest...
The plant material used in our study includes four samples, two of each species: two varieties of triticale Ksar Shahi (TKS) and Triticale Beni Haroun (TBH); two varieties of barley: Barley Fouara (BF) and barley Saida (BS). Dissection or isolation of the peripheral layers is the first experimental part carried out in this study, the grains were harvested at the mature stage, then washed with distilled water to moisten the seed. The peripheral layers were separate manually under a binocular microscopic; finally, the layers obtained are stored in the freezing at -80 °C until analysis to be reduced to powder.

**Extraction of soluble phenolic compounds**

In our study, extraction is performed by maceration in an organic solvent. Samples were defatted twice by stirring in hexane for 1 h at ambient temperature. Then macerated in...
100 ml of Ethanol (70%). After 24 hours of agitation for release both bound and free phenolic compounds, the mixtures were separated by filtration. The extracts are then evaporated to dryness using a rotary evaporator at a temperature of around 45°C. A weighing at this stage makes it possible to calculate the yield of extracts 25.

**Determination of total phenolic content**

The determination of the total polyphenols of the extract is determined by the use of the Folin-Ciocalteu reagent and which is described by the method 21. After addition of the Folin-Ciocalteu reagent (0.25 ml) and 20% aqueous sodium carbonate solution (1.25 ml), tubes were vortexed. After 40 min, the absorbance of the resulting blue colored mixtures was recorded at 765 nm against a blank containing only an extraction solvent (0.2 ml). During the oxidation of phenols, the blue coloration produced has a maximum absorption around, with reference to a standard range obtained with a phenolic acid (gallic acid), to determine the amount of total polyphenols present in an extract. It is expressed in mg of gallic acid equivalent per g of dry matter.

**Determination of flavonoid content**

Flavonoid content was determined using a colorimetric method described previously 22. Briefly, 0.5 ml of the ethanol extract was diluted with 1 ml of distilled water. Then, 0.075 ml of a 5% NaNO₂ solution was added to the mixture. After 6 min, 0.15 ml of a 10% AlCl₃ × 6H₂O solution was added, and the mixture was allowed to stand for another 5 min. Half of a milliliter of 1 M NaOH was added and the volume was made up to 2.5 ml with distilled water. The solution was well mixed, and the absorbance was measured immediately against the blank (containing the extraction solvent instead of a sample) at 510 nm. The flavonoids are quantified using a calibration curve obtained by measuring the absorbance of the known concentrations of the quercitine spread solutions and the results are expressed in microgram equivalents of quercitine per milligram of dry extract (mgEQ / g).

**Determination of total tannins**

Tannins are polyphenolic compounds found in the plant kingdom. Their main characteristic is the capability of binding and precipitating proteins. Tannins have molecular weights ranging from 500 to 3,000 23.

The tannins are hardly soluble in cold water but soluble in hot water (colloidal solutions), they are soluble in alcohol and acetone, insoluble in organic apolar solvents (ether). Their solubility varies according to their degree of polymerization. 0.1 ml of each extract was placed in tubes to which 3 ml of 4% (w/v) vanillin methanol solution was added. Absorbance was measured at 500 nm after incubation for 20 minutes. The calibration curve was prepared under the same conditions using catechin as the standard and the results were expressed in catechin mg equivalent / g dry matter (mg EC / g MS). 24.

**Antioxidant Activity**

**DPPH Radical Scavenging Activity**

The free radical scavenging activity of peripheral layers extracts was measured by DPPH method 25. A volume of 0.025 mg mL⁻¹ of DPPH radical solution was prepared in methanol and 1,950 μL of this solution was added to 50 μL of different concentrations of each extract. A negative control was prepared by adding 50 μL of methanol to 1,950 μL of DPPH solution. After incubation for 30 min in dark at room temperature, the absorbance was read at 517 nm using a spectrophotometer, against a blank. The positive control was BHA and ascorbic acid. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging in percentage was calculated as follows:

\[
\text{DPPH} \% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Where DPPH (%) is the reducing percentage of DPPH, Acontrol is the absorbance of negative control, and Asample is the absorbance of sample. The results were expressed as the average of three measurements obtained for each sample. The IC₅₀ value that determined the concentration which reduced 50% of DPPH radical was revealed graphically for each extract from the curve of the reducing percentage of DPPH depending on the concentration.

**Total Antioxidant Capacity**

The total antioxidant capacity (TAC) of the extracts was evaluated with phosphomolybdenum technique based on the reduction of molybdenum(VI) to molybdenum(V) by the plant extracts, which produced a green phosphomolybdenum(V) complex under acid conditions. 26 An aliquot 0.2 mL of different concentrations of extract was combined with 2 mL of reagent solution (0.6 M sulfuric acid, 29 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 min. The absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed in milligram equivalent of ascorbic acid per gram of extract (mg EAA g⁻¹ E). The calibration curve of ascorbic acid range was 0.10–0.80 mg mL⁻¹.

**Determination of minerals (determination of potassium and sodium)**

For the screening of the mineral salts 0.5g of the sample powder is taken in 10 ml of distilled water, shaken for 1 hour and then centrifuged for 10 min. 2.42g of KCl are taken in 100 ml of distilled water for the calibration curve of K⁺, but for the determination of sodium, 1.95g of NaCl are taken in 100 ml of distilled water. Absorbance was measured by flame photometry.

**RESULTS AND DISCUSSIONS**

**Yield Extraction**

Extraction is a very important step in the isolation, identification, use of phenolic compounds. Extraction methods depend on the extraction yield of phenolic compounds 27. The extraction of different cereals for barley and triticale varieties allowed us to calculate the yield of each water / ethanol extract. The yield, which was determined relative to 5 g of dry and ground vegetable material, is expressed as a percentage. The results obtained are shown in Table 2.

**Result of total phenolic, flavonoid and tannin**

The phenolic content was determined via the Folin-Ciocalteu test, this test was chosen to measure the phenolic compounds for the following reasons: It is a method which satisfies the criteria of feasibility and reproducibility. The availability of the Folin-Ciocalteu reagent. The method is well standardized. The long absorption wavelength (765nm) of the chromophore minimizes interference with the sample matrix, which is often colored. The results obtained from the extracts of the peripheral layers (PL) analyzed reveal that the variety (BS) of the barley species is the richest in total phenolic compounds 0.058 ± 0.004 mg (EAG)/g followed by TGH secondly with a content of 0.045 ± 0.0029 mg (EAG)/g. TKS with a content of 0.027 ± 0.0025 mg (EAG) / g and finally the variety (BF) registers a content of 0.012 ± 0.0019 mg (EAG) / g (Table 2).
So, the results clearly show that the amount of polyphenols is high in the extracts of BS and TBH followed by the extracts of TKS and then the CP and BF which have the least important level of polyphenols. According to Fadet et al. 2010, the polyphenole level represents 1.1 mg / 100g in cereal bran. In wheat bran, phenolic compounds are the main contributors to total antioxidant capacity and therefore a high TPC reflects a high total antioxidant activity. It should be noted that during the extraction process, it is possible to have a slight hydrolysis of polyphenolic compounds due to the acid extraction conditions. Nevertheless, it has been reported that consumption of bran has increased phenols and antioxidant capacity in bran to a level comparable to some extent with other phenol-rich foods according to 28. The lowest content is observed for rye where in the composition of triticale at 54.14 µg/g the highest rate is recorded for barley 149.38 µg/g, according to the results obtained by 29. In addition to better justify our results, The nutritional value of triticale is close to that of wheat and rye 10, 11. Numerous studies reported that phenolic compounds in wheat grains are mostly in the bound form and exist in bran associated with cell wall materials 20, 31, 32. Triticale and waxy wheat by-products such as bran may serve as sources of valuable phenolics for food.

However, it has higher TPC values in triticals (TBH). The TPC of triticale was found to be 940 mg/g of grain as reported by 33. A study by 34 reported 2849 mg/g bran for TPC of total phenolics of triticale. Our results the same below the results by the different works and this may be due to also the extraction method we employed to release these compounds. Regarding the flavonoids, the main reason for choosing this class of polyphenols is that flavonoids are the most important polyphenolic class, with more than 5,000 compounds already described. Flavanols, anthocyanins, and proanthocyanidins (polymers of flavonoids) are the major types of flavonoids found in barley grains. Flavanols and anthocyanins are located in the pericarp of barley grains. 36, 37, studied the flavonoid content of 127 lines of hulled and unhulled colored barley wherein the total flavonoid content was found to range between 62.0 and 300.8 mg/g.

Note from the results in (Table 2) below, that the amount of flavonoids in the extract of TBH and BS are greater: are represent respectively the following (0.12 ± 0.04 mg EC/g) and (0.08 ± 0.005 mg EC/g) followed by extracts of triticale TKS (0.019 ± 0.001 mg EC/g), BF (0.013 ± 0.0016 mg EC/g) represents the lowest rate, respectively. The phenolic compounds found in cereal are the phenolic acids and flavonoids located in the outer layer of grain. Most phenolic compounds in bran are related with carbohydrates, and can survive gastrointestinal digestion reaching the colon intact.

For tannin measurements, The quantification of the tannins was carried out by a method adapted by 39. A calibration curve is carried out using catechin. These assays reveal that the extracts of the varieties TBH, BF, contain the highest levels of condensed tannins. Their concentration reached 0.37 ± 0.05 mg EAG/g, 0.29 ± 0.07 mg EAG/g, respectively, when it was less than 0.1 mg EC/g in the other varieties BS,TKS. Comparing with the results of 40, who study on barley varieties and show that the content of condensed tannins varies considerably between different varieties. The highest content is revealed during severe stress with an average value of 0.93 ± 0.18 mg EC / g MF, these results are a little close to our results and the small difference it may be due to the solvent used.

### Table 2: yield of hydro-ethanolic in percentage (%), concentration of polyphenol in mg (EAG)/g, flavonoids in mg EC/g and of tannins in mg EAG/g, of peripheral layers extracts in different variety for two species (Barley and Triticale).

| Parameters      | Variety | Yield in % | Total polyphenoles mg (EAG)/g | Flavonoids mg EC/g | Tannins mg EAG/g |
|-----------------|---------|------------|--------------------------------|-------------------|-----------------|
| Variety         |         |            |                                |                   |                 |
|                 | BS      | 08         | 0.058 ± 0.004                  | 0.08 ± 0.005      | <0.1            |
|                 | BF      | 5.55       | 0.012 ± 0.0019                 | 0.013 ± 0.0016    | 0.29 ± 0.07     |
|                 | TKS     | 08         | 0.027 ± 0.0025                 | 0.019 ± 0.001     | <0.1            |
|                 | TBH     | 0.145      | 0.045 ± 0.0029                 | 0.12 ± 0.004      | 0.37 ± 0.05     |

BS: Saida Barley; BF : Feouara Barley; TKS: Triticale Kser Sbahi; TBH: Triticale Benni Haroun

**Iron reduction: FRAP (Ferric reducing antioxidant power)**

It is a method of measuring the ability of the substances in our extracts to reduce ferric iron Fe³⁺ to ferrous iron Fe²⁺. It is a fast, easy and reproducible technique. The reducing capacity of a compound can serve as a significant indicator of its potential antioxidant activity. Previous work has indicated that there is a direct relationship between antioxidant activities and the reducing power of components in some plants. In our work, we tested by the FRAP method different extracts of PL from different varieties of wheat and the results obtained allowed us to draw curves for each extract. We note that the iron reduction capacity is proportional to the increase in the concentration of the samples. The results obtained show that the capacity of our extracts to reduce iron is lower than that of ascorbic acid for the four varieties, but it is variable between the varieties of the peripheral layers studied. This reduction is much greater in the TBH variety (DO = 1.165) which is much greater than TKS (DO = 0.67).

We note that the variety of the peripheral layer OS showed a great activity of reduction of iron with a maximum optical density of 0.7285 at a concentration of 1 mg / ml compared to BF, which showed an OD of the order of 0.288 for the same concentration. We can deduce that all Triticium varieties have the capacity to reduce iron but it is still less than that of ascorbic acid (OD = 1.375). If we classify our varieties according to the reduction power of iron compared to ascorbic acid, we will obtain the following order: ascorbic acid< BS > TBH> TKS> BF.
The comparison of the antioxidant power of our extracts between them reveals that that of barley after TKS are the most active. This is in agreement with the results of several researchers [46, 47]; a work from [48] in which barley has a higher antioxidant power than that of wheat. This result is in agreement with the highest contents (in phenolic compounds) obtained from the outer layers of barley compared to other wheat extracts. According to [49], proanthocyanidins (dimers and trimers of tannins) are the main contributors to the antioxidant activity of barley. All of our tested extracts are less active than standard antioxidants; gallic acid and quercetin.

The antioxidant activity of a compound corresponds to its capacity to resist oxidation [50]. Many methods are currently used to assess this activity. The DPPH radical has been widely used for the study of the anti-radical activity of different plant extracts [51]. The absorbance (or optical density DO) measurement was performed by spectrophotometry at 517 nm. From the values obtained, we calculated the inhibition percentages using the formula given in the materials and methods section. The values obtained made it possible to draw the curves represented in Figures [2], which represent the variation in the percentage of inhibition as a function of the concentrations of our extracts. We have graphically determined the concentration corresponding to 50% inhibition (EC50).

Our results were expressed by measuring the effective concentration (EC50). The latter is the concentration of the sample which is necessary to inhibit the concentration (or absorbance) of DPPH ° at 50%. Knowing that, the EC50 value is inversely proportional to the antioxidant power [52]. The EC50 values found for all the varieties studied are shown in Figure 1 as histograms. By comparing the IC50 of different studied varieties of cereals against that of ascorbic acid, we note that the anti-radical activity of all our varieties is lower than the trapping capacity of the DPPH radical of the reference substance. This capacity is greater in the Fouara barley (BF) variety (1.91). The BS, triticale (TKS) and (TBH) varieties do not represent antioxidant activity, compared to ascorbic acid even at higher concentrations. To be noted that there is a growing interest in natural antioxidants of plant origin because of their promising biological capacities to protect the human body against the harmful effects of free radicals, to delay the progression of several chronic diseases and avoid the randity of food by 1 lipid oxidation. In the present study, the DPPH ° method was developed to evaluate the antioxidant power of our extracts prepared from the peripheral layers of some varieties of cereals. Based on work done by [53], antioxidants from food grain extracts, all appear to act as scavenger agents of the DPPH radical. Barley bran is ranked first followed by others bran and cereal germ extracts. The ethyl acetate extract of barley bran is the most active with the lowest EC50 and is equal to 1.77 mg / ml which are correlated with our results and she deduced that the extracts of medicinal plants appear more powerful than extracts from food grains. Numerous studies indicate, The antioxidative activity investigated for 80% methanolic extracts originating from different whole grains were in the hierarchy: barley > oat > wheat > rye [54]. This high antioxidative activity of phytochemicals present in barley makes it a useful natural means for the prevention of diabetes and obesity [55]. In addition to barley phytochemicals’ antioxidative properties, barley phytochemical compounds have potent anti-inflammatory actions and could thereby moderate diabetes and obesity risk by this mechanism [56].

![Figure 1: Histogram of Ic50 of extracts studied by FRAP according to different varieties. BS: Saida Barley; BF: Feouara Barley; TKS: Triticale Kser Shahi; TBH: Triticale Benni Haroun](image1)

![Figure 2: Histogram of the values of the inhibitory concentrations 50 of the different varieties in mg/ml BS: Saida Barley; BF : Feouara Barley; TKS: Triticale Kser Shahi; TBH: Triticale Benni Haroun](image2)
Determination of minerals (NA, K) by flame spectrophotometer

The concentration of the NA⁺ and K⁺ ions was calculated from the regression equation for the calibration ranges established with sodium and potassium. We note from the results obtained in the different varieties of PL have a low sodium content unlike potassium which are rich, something which is in agreement with the results of British Nutrition Foundation. The maximum potassium concentrations can be found in the TKS variety (329.33 mg / l). Compared with triticale, barley has low potassium content (a concentration of 79.44 mg / l for BF against a concentration of 24.29 mg / l for “very weak” BS). On the other hand, barley has the highest sodium content with a concentration of 33.78 mg / l for BF and 15.28 mg / l for BS. The sodium content is almost identical for the two triticale varieties (TKS, TBH). In barley grains, the peripheral layer is the major storage site for phosphate, magnesium, potassium, sodium and calcium, accumulating over 70% (97% for magnesium) of the endosperm stores of these minerals. It has been revealed through the specialized documentation a large variability in the contents of mineral matter from one sample of peripheral layer to another, these differences are under the influence of environmental factors which characterize the culture of the grain and the varietal effect, the wheat grain transformation process is also a source of variation in the mineral concentration of PL.

Table 3: concentration of potassium and sodium in mg content in peripheral layers extracts of different variety for two species (Barley and Triticale).

| Variety | Potassium (K) in (mg/l) | Sodium (Na) in (mg) |
|---------|------------------------|---------------------|
| BS      | 24.29                  | 33.78               |
| BF      | 79.44                  | 15.28               |
| TKS     | 195.04                 | 4.77                |
| TBH     | 329.33                 | 9.4                 |

BS: Saida Barley; BF: Feouara Barley; TKS: Triticale Kser Shali; TBH: Triticale Benni Haroun

CONCLUSION

Barley and triticale has an assortment of phytochemicals with the potential antioxidant to impact human health. These benefits are due to inherent properties present in phytochemicals, such as high antioxidative activity against different free radicals, however, there is a need for more systemic and detailed study on barley, triticale and others varieties and species of cereals remains studied. Although there are variations among barley and triticale samples for phenolics, further research is required to confirm the presence in peripheral layer in different conditions environmental and research others bioactive natural.

Conflict of interest

The authors have no conflict of interest to declare.

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