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Bioactive Compound Composition and Antioxidant Activity of Aleurone Layers and Whole Grains from Algerian Cultivars of Soft Wheat (*Triticum aestivum)*, Durum Wheat (*Triticum durum*), and Barley (*Hordeum vulgare*)

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ABSTRACT

The outer layers of wheat and barley, especially the aleurone layer (AL), are mostly removed with the bran during white flour production. This study aimed to analyze and compare AL and whole grain (WG) extracts of three cereals; soft wheat (*Triticum aestivum*, Arz), durum wheat (*Triticum durum*, Ofanto), and barley (*Hordeum vulgare*, Rihane) to assess their secondary metabolites, including phenolic compounds, flavonoids, and condensed tannins, as well as their antioxidant activity. The structural/morphological features of the cereal species were examined using scanning electron microscopy. The total polyphenol (TP), flavonoid, (TF) and condensed tannin (CT) contents of the WG and AL of the cereal extracts were determined using colorimetric methods. The antioxidant activity was determined using 2, 2-diphenyl-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Among the WG extracts, 'Rihane' showed the highest TPC (1.788 ± 0.012 mg GAE/g), TF (0.082 ± 0.009 mg QE/g), and CT (0.03 ± 0.01 mg CE/g). In AL extracts, TPC ranged from 0.889 ± 0.012 to 1.397 ± 0.060 mg GAE/g, and TFC from 0.022 ± 0.01 to 0.030 ± 0.01 mg QE/g, with AL 'Ofanto' showing slightly higher values. The highest tannin content in both WG and AL was recorded for 'Rihane' with tannin content of 0.054 ± 0.006 mg CE/g (WG) and 0.043 ± 0.01 mg CE/g (AL). WG 'Rihane' had the most potent antioxidant activity (DPPH IC_{50} = 752 µg/mL). These findings highlight the nutritional potential of the aleurone layer as a valuable source of bioactive compounds including antioxidants.

Keywords: Wheat, Barley, Aleurone layer, Whole Grains, Phenolic compounds, Antioxidant activity.

Introduction

The aleurone layer (AL), typically a single-cell structure located at the periphery of cereal grains, is increasingly recognized as a reservoir of nutrients and bioactive compounds, including macronutrients, micronutrients, and phytochemicals.[1,2,3] It is particularly rich in proteins, minerals, B-group vitamins, dietary fibers, and secondary metabolites such as phenolic acids and flavonoids, which contribute significantly to the nutritional and functional value of cereals.[4,5] Recent studies have highlighted the potential of aleurone concentrates as carriers of phenolic compounds with potent antioxidant properties.[6] In cereals, phenolic compounds exist in both free and bound forms, with bound phenolics being more abundant and influencing bioaccessibility and antioxidant activity.[7,8] In the present study, only total polyphenol content was considered. Since the aleurone fraction is usually removed during conventional milling, targeted recovery of this fraction represents a promising strategy for developing functional ingredients

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Progress has also been made in developing fractionation methods for isolating the aleurone layer and its bioactive constituents. Several reviews have summarized the advantages and limitations of various separation techniques, highlighting their potential to recover phenolic compounds, minerals, and other health-promoting components.[9,10] More recently, advanced wheat bran fractionation has improved the extraction of nutritionally relevant compounds.[11] Earlier studies have examined the structural and functional characteristics of bran,[12] while the effects of cereal processing on the bioavailability of major wheat nutrients have been widely investigated.[13] The variability of phenolic composition among cereal species and genotypes is well documented, with wheat and barley cultivars showing distinct profiles of phenolic acids and antioxidant activity.[14,15] Such differences highlight the importance of comparative studies across species and varieties. Zhou *et al*.[16] reported that phenolic acid composition and antioxidant properties differ markedly between aleurone and whole grain, with aleurone being the richest fraction. In barley, several recent studies have demonstrated that it is an abundant source of phenolic compounds, making it an excellent dietary matrix for natural antioxidants that contribute to disease prevention and health promotion.[17] Moreover, the structural organization of the aleurone layer, revealed by microscopic analyses such as scanning electron microscopy (SEM), influences the accessibility and extractability of phenolic compounds and should therefore be considered alongside biochemical analyses.[6,18] In Algeria, cereals remain central to diets and agricultural systems; however, limited information is available regarding the biochemical potential of fractionated grain tissues such as the aleurone layer. In this study, three locally cultivated cereal species, including soft wheat (*Triticum aestivum*, cv. Arz), durum

wheat (*Triticum durum*, cv. Ofanto), and barley (*Hordeum vulgare*, cv. Rihane) were collected from the Sidi Bel Abbès region in northwestern Algeria. The objective of this research was to isolate and characterize the morphology and structure of the aleurone layer (AL) in these cereals at full maturity using SEM. In addition, the study aims to analyze and compare the biochemical composition of aleurone layers (AL) and whole grains (WG), focusing on secondary metabolites, including total phenolic compounds (TPC), flavonoids, and condensed tannins. Furthermore, the antioxidant potential of these fractions was evaluated using spectrophotometric assays, including the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. This is the first report of the antioxidant potential of the aleurone layers of wheat and barley from Algerian cultivars. It provides evidence of their antioxidant potential by integrating SEM with spectrophotometric assays (TPC, DPPH, FRAP). It also correlated tissue structure with phenolic extractability and antioxidant activity, thereby strengthening the reliability of the results and supporting the valorization of aleurone as a functional food ingredient in Algeria.

Materials and Methods

Plant materials

Three cereal species cultivated in Algeria were used, including one barley variety (*Hordeum vulgare, "*Rihane*"*) and two wheat varieties: soft wheat (*Triticum aestivum*, "Arz") and durum wheat (*Triticum durum*, "Ofanto"), which were supplied by the Technical Institute for Field Crops (ITGC) and the National Institute for Agricultural Research (INRA) in Sidi Bel Abbes, Algeria. These species were grown under standard agronomic conditions, with complete protection of the plants and ears throughout the growing period. The main characteristics of the three varieties are presented in Table 1. After harvest, the grains were stored at 5°C until further analysis.

Sample preparation

Isolation of aleurone layers

The aleurone layer (AL) was manually dissected under a binocular magnifier (ZEISS, Germany). Manual dissection was performed on mature grains to obtain pure and clean aleurone layers, following the procedures described by Laubin *et al*. (2008)[19], Meziani *et al*. (2012)[20], and Meziani *et al*. (2014)[21]. The main characteristics of the wheat and barley grains during dissection are summarized in Table 2. The isolated aleurone fragments were immediately transferred to Eppendorf-type microtubes, excess water was removed using sterile filter paper, and the fragments were ground using a pestle and mortar before storage at -20 °C until analysis.

Preparation of grain powder

Whole grains (wheat and barley samples) were harvested at maturity and manually cleaned to remove all non-grain debris after harvest. The samples (common wheat, durum wheat, and barley) were dried, then ground with a grinder (IKA A11, Germany) to produce whole-meal flours. The powdered samples were wrapped in airtight paper and stored in the laboratory at room temperature.

Observation by Scanning Electron Microscope (SEM)

The aleurone layers were examined using a tabletop scanning electron microscope (Hitachi TM-1000, Japan) operating at 15 kV and equipped with an energy-dispersive X-ray (EDX) spectrometer. This enabled the study of the morphology, detailed observation of delicate structures within the layer, and evaluation of the purity of the recovered tissues, thereby confirming the dissection procedure. Surface morphology images were taken at magnifications ranging from 100× to 800× to obtain detailed surface images of the samples, following the analytical approach of Meziani *et al*. (2019).[22]

Phytochemical analysis

Extraction of phenolic compounds

The total polyphenols were extracted following the method described by Zieliński and Kozłowska (2000),[23] and modified by Oufnac (2006).[24] About 1 g of whole grain (WG) and aleurone layer (AL) fractions from three cereal varieties were added to 4 mL of 90% methanol and vortexed. They were then placed in a water bath at 60°C for 20 minutes, and were vortexed twice during incubation. The liquid and solid phases of each test tube were separated by centrifugation at 2000 rpm for 15 minutes. The supernatant (liquid phase) was transferred. The residue (solid phase) from the ground whole grains and the aleurone layers was mixed with 2 mL of the same solvent for the second extraction. The two supernatants were combined. The tube containing the combined supernatants was placed in a rotary evaporator to remove the solvent. The dry extract was weighed to determine the extraction yield of the samples.

Determination of total polyphenol content

The total polyphenol content of the cereal extracts was determined by the Folin-Ciocalteu colorimetric method as previously described by Ali-Rachedi *et al*. (2018).[25] To each test tube, 200 µL of each extract, 800 µL of sodium carbonate solution (Na_2CO_3), and 1 mL of Folin-Ciocalteu reagent were added. After mixing, the tubes were incubated at room temperature for 30 minutes. Absorbance was measured at 765 nm using a UV–Visible spectrophotometer (Optizen POP/KLAB, South Korea), and the polyphenol content was expressed as micrograms of the standard gallic acid equivalent per milligram of dry extract (mg GAE/mg extract).

Determination of flavonoid content

The flavonoid content of the cereal extracts was determined according to the method described by Viuda-Martos *et al.* (2011).[26] About 2 mL of distilled water, 0.15 mL of 5% $NaNO_2$, and 0.5 mL of extract were combined in a tube. After six minutes, 0.15 mL of 10% $AlCl_3$ was added. Then, 2 mL of 4% NaOH was added to the medium after another 6 minutes, and the volume was adjusted with 0.2 mL of distilled water. After 15 minutes of stirring and incubation, the absorbance was measured at 510 nm using a UV–Visible spectrophotometer (Optizen POP/KLAB, South Korea). A stock solution of quercitrin in methanol was prepared from which dilutions ranging from 100 to 900 µg/mL were made. A calibration curve of absorbance versus concentrations of quercitrin was constructed. The flavonoid content was expressed as micrograms of quercitrin equivalent per milligram of dry extract (mg QE/mg extract).

Determination of condensed tannin content

The condensed tannin content was determined according to the method described by Li *et al*. (2022).[27] Approximately 50 µL of cereal extract was mixed with 3 mL of a 4% (w/v) vanillin solution prepared in absolute methanol, then 1.5 mL of concentrated HCl (37%) was added. After standardization, the mixture was kept at room temperature for 15 minutes. The absorbance was measured at 500 nm using a UV-VIS spectrophotometer (Optizen POP/KLAB, South Korea). Catechin standard (0, 100, 200, 300, 400, and 500 µg/mL) was used to create the calibration curve. Results were expressed as mg catechin equivalents per gram of dry plant material (mg CE/g). All measurements were performed in triplicate.

Determination of antioxidant activity

The antioxidant activity of cereal extracts and their aleurone layers was assessed using two methods: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant power (FRAP) assay.

DPPH free radical scavenging Assay

The DPPH free radical scavenging activity of the cereal extracts was determined according to the method described by Kirby and Schmid (1997).[28] This test measures the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical with a dark violet colour. When reduced by hydrogen from an antioxidant in the extract, the radical turns pale yellow. For the assay, about 500 µL of each extract at different concentrations (15.62, 31.25, 62.5, 125, and 250 µg/mL) were mixed with 500 µL of freshly prepared DPPH solution (0.1 mM in 90% methanol). The mixture was left in the dark at room temperature for 30 minutes. Optical density was measured at 517 nm using a UV-Vis spectrophotometer (Optizen POP/KLAB, South Korea). The percentage of DPPH inhibition was calculated using the following formula (Equation 1):

Inhibition of DPPH % = $\frac{A_C - A_e}{A_C} x\ 100$ …… (Eq. 1)

Where;

Ac: Absorbance of the control (500 µL of 90% methanol and 500 µL of DPPH)

Ae: Absorbance of the sample (500 µL of extract and 500 µL of DPPH)

Ferric reducing antioxidant power (FRAP) assay

The FRAP activity of the cereal extracts was determined according to the method described by Oyaizu (1986).[29] After preparing the extract samples at different concentrations, a volume of 0.1 mL was mixed with 0.25 mL of phosphate buffer solution (0.2 M, pH 6.6) and 0.25 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. After incubation at 50°C for 20 minutes, 0.25 mL of 10% trichloroacetic acid (TCA) was added to stop the reaction. Then, 0.5 mL of each concentration was transferred to new tubes, mixed with 0.5 mL of distilled water, and 0.1 mL of 0.1% iron (iii) chloride ($FeCl_3$) was added. Absorbance was measured at 700 nm against a blank. The 50% effective concentration (EC_{50}) of each extract was determined graphically from absorbance versus concentration plot. Ascorbic acid was used as a reference standard.

Statistical analysis

All tests were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using two-way analysis of variance (ANOVA), followed by Sidak's multiple comparisons test to assess differences in mean values at the 5% significance level ($p \leq 0.05$). The data analysis was performed using GraphPad Prism 8.0.2 (GraphPad Software, USA).

Results and Discussion

Morphological characteristics of the aleurone layer

Understanding the morphology of the aleurone layer (AL) is crucial for characterizing its structure and composition in cereals. Using a previously established manual dissection method for mature cereal grains, the AL was effectively separated from the endosperm without contamination.[10, 19, 21] This method allowed for clear identification of tissue differences between wheat and barley. Notably, barley possessed more aleurone layers than wheat. Scanning electron microscopy confirmed a single aleurone layer in both soft and durum wheat. In contrast, barley had two to three layers (Figure 1), which is consistent with the findings of Brouns *et al*. (2012)[30] and other studies on the peripheral layers of wheat and barley.[31-33] Furthermore, Noticeable differences in cell shape and wall thickness were observed among the studied species during dissection. These structural variations affected the difficulty of tissue dissection: it was most challenging in durum wheat, which has thin aleurone cell walls; somewhat easier in soft wheat; and easiest in barley, which has thicker aleurone cell walls.[10] Microscopic examination of the samples revealed significant tissue and cellular differences. In particular, the AL was markedly thicker in barley, whereas in durum wheat, some areas showed particularly thin aleurone walls.

Total polyphenol content (TPC) of cereal extracts

Extraction of phenolic compounds is essential for their isolation, identification, and use as bioactive molecules. Total polyphenol content of the cereal extracts was measured using the Folin ciocalteu colorimetric assay. The Folin ciocalteu reagent used is a yellow mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PM_{12}O_4$). The method is based on the oxidation of standard phenolic compounds by this reagent, which results in the formation of a blue complex of tungsten and molybdenum oxides. The colour intensity correlates with the amount of polyphenols in the plant extracts. The TPC of the cereal extracts are shown in Figure 2. The results showed that the whole barley flour exhibited higher total polyphenol content than the whole wheat flour. Statistical analysis revealed highly significant differences ($p < 0.05$ to $p < 0.0001$) in TPC across varieties and grain fractions. Each variety–fraction displayed a distinct TPC profile. The highest TPC was observed in the whole grain fraction of the 'Rihane' variety (1.788 ± 0.012 mg GAE/g). This was followed by the aleurone layer of 'Ofanto' (1.397 ± 0.060 mg GAE/g). These two fractions were significantly higher than the others. The whole grain of 'Arz' showed intermediate TPC content of 1.263 ± 0.006 mg GAE/g, followed by 'Rihane' aleurone layer with TPC of 1.163 ± 0.028 mg GAE/g. The whole grain of 'Ofanto' exhibited a lower total polyphenol content (1.038 ± 0.077 mg GAE/g), while the aleurone layer of 'Arz' recorded the lowest total polyphenol content (0.889 ± 0.012 mg GAE/g). These results reveal an uneven distribution of phenolic compounds across different grain layers, emphasizing the richness of the aleurone layer, especially in the 'Ofanto' variety. The higher TPC in 'Rihane's' whole grains indicates its potential for antioxidant-based nutritional products. This aligns with previous research showing barley as a rich source of phenolic compounds.[34] These findings support the targeted valorization of cereal fractions based on their bioactive compound profiles for functional or therapeutic uses. Other studies have also found higher TPC in soft wheat whole grains than in durum wheat whole grains.[35]

The variations in values may be due to the polarity of the compounds in the samples. The present results are consistent with those of López-Perea *et al*. (2019)[36] who studied wheat bran and barley husk, and found a high phenolic concentration in alcohol extracts (ethanol and methanol). Factors affecting the quality and amount of polyphenols in the extract include the part of the plant used, the extraction process, temperature, and solvent selection. Environmental factors such as climate and light also significantly influence phenolic accumulation. Studies have shown that barley has higher phenolic content and antioxidant activity than wheat, and that environmental conditions greatly influence these traits.[37] Similarly, in Northern Algeria, barley showed the highest phenolic content, followed by soft wheat and durum wheat, likely due to Mediterranean climatic conditions.

Total flavonoid content (TFC) of cereal extracts

Quercetin, one of the most abundant and potent natural antioxidants, is a flavonoid found in cereals.[38] Analysis of total flavonoid content (measured in mg QE/g dry matter) showed significant differences among the varieties and fractions studied (Figure 3). In the whole grain (WG) fraction, the 'Rihane' sample had the highest flavonoid content, significantly higher than all other samples, indicating a notable accumulation of these bioactive compounds in this genotype. The other samples (WG: 'Ofanto', WG: 'Arz', AL: 'Arz', AL: 'Ofanto', and AL: 'Rihane') displayed lower and statistically similar flavonoid contents, suggesting no significant differences among these varieties. For the WG fraction, the 'Rihane' variety had a flavonoid content of 0.082 ± 0.009 mg QE/g, significantly higher than that of 'Ofanto' (0.027 ± 0.006 mg QE/g) and 'Arz' (0.015 ± 0.001 mg QE/g), with statistical analysis confirming a highly significant difference ($p < 0.0001$). In contrast, for the aleurone layer (AL) fraction, differences between varieties were less pronounced; values ranged from 0.022 ± 0.01 to 0.030 ± 0.01 mg QE/g, with 'Ofanto' variety having slightly higher value. However, these differences were not statistically significant ($p > 0.05$), indicating a relative uniformity in flavonoid content across varieties in the aleurone layer. Comparing the two fractions, within each variety revealed distinct trends. 'Rihane' displayed significantly higher flavonoid content in the whole grain than in the aleurone layer ($p < 0.0001$), whereas 'Ofanto' and 'Arz' showed comparable flavonoid content between WG and AL fractions, with no statistically significant differences. These results suggest that in 'Rihane', flavonoids are predominantly located in tissues other than the aleurone layer, while in the other varieties, their distribution is more uniform. The findings from the present study are consistent with those of Stuper-Szablewska *et al*. (2019)[39] who reported that many phenolic compounds, including flavonoids, are bound to the cell wall. Conversely, the present results differ from those of Loskutov *et al*. (2021)[38] who classified wheat as the second cereal richest in flavonoids after corn.

Condensed tannin content (CTC) of cereal extracts

Condensed tannins (CT), another name for proanthocyanidins (PAs), are a class of phenolic compounds found in many foods and plants that serve various biological and biochemical functions. CT provides numerous health benefits, including immune support, anti-tumor effects, antioxidant activity, improved blood circulation, and eye protection.[40] The results of the condensed tannin content are shown in Figure 4. No significant differences were observed among most of the samples. The average tannin contents ranged from 0.03 ± 0.01 mg CE/g in the aleurone layer (AL) of 'Arz' to 0.054 ± 0.006 mg CE/g in the whole grain (WG) of 'Rihane'. In whole grain extracts, tannin contents

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Table 1: Characteristics of the different cereal species and varieties grown in Algeria according to INRAA, ITGC, and CCLS

	Characteristics				
Species	**Spike**	**Grain**	**Agronomic characteristics**	**Technological characteristics**	**Adaptation area**
Common wheat (Arz)	Red, elongated, and divergent barbs	Clair and rounded	Yield: High	Excellent Bakery force Swelling is good. Use: corrective wheat	Coastline, interior plains
Durum wheat (Ofanto)	Length excluding barbs: Short. Colour (at maturity): White	Form: Half-Long, Ovoid Colour with phenol: Low.	Yield: High	Semolina quality: Good. Protein content: 15.60%	High plateaus, interior plains.
Barley (Rihane)	Sessile, 6 rows, compact, with long white beards.	Position of the lodicules: lateral. Hairiness of the furrow: absent.	Yield: High	Protein Content: 14.50%	Highlands, inland plains, and coastlines.

Table 2: Morphological characteristics of wheat and barley grains during dissection

	Characteristics				
Species	**Length (mm)**	**Width (mm)**	**Cell Form**	**Grain Form**	**Dissection Mode**
Common wheat (Arz)	5-7	3-4	Rounded	Ovoid and rounded	Less easy (++)
Durum wheat (Ofanto)	6-8	3-4	Large and thick	Long, ovoid	Difficult (+)
Barley (Rihane)	9-13	3-5	Elongated Fine	Elongated and long	Less easy (++)

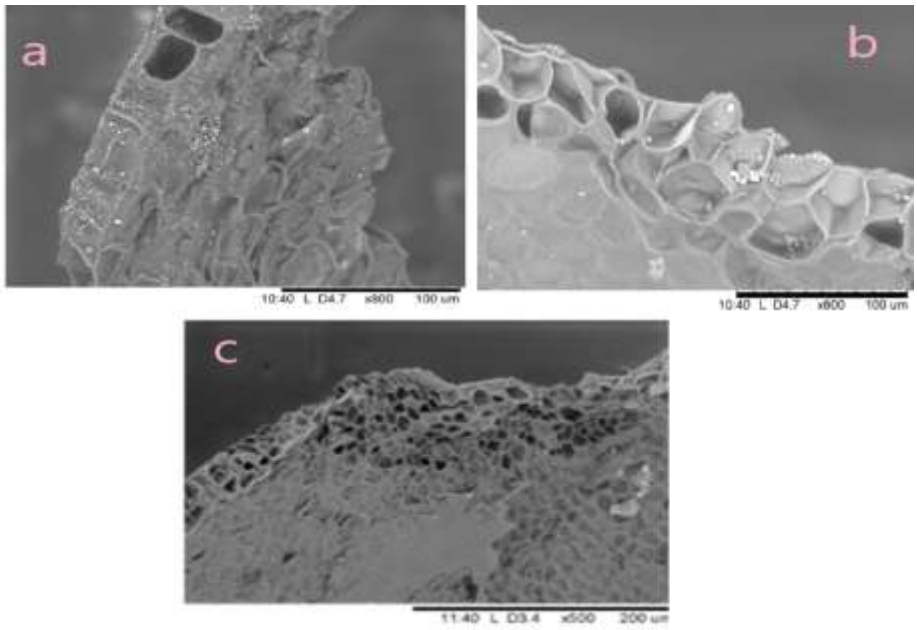

Figure 1: Aleurone layer (AL) observed by scanning electron microscopy, fully separated from the other layers: **(a)** AL of common wheat 'Arz', **(b)** AL of durum wheat 'Ofanto', **(c)** AL of barley 'Rihane'. Magnification: ×100–200; scale bar = 100–200 µm.

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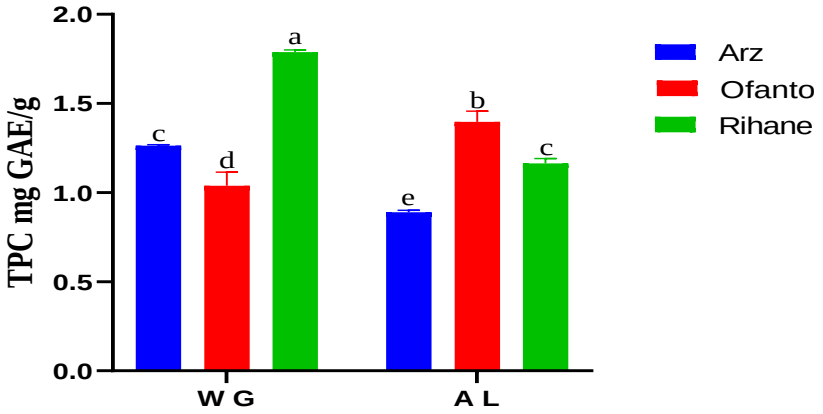

Figure 2: Total polyphenol content (TPC) in whole grains (WG) and aleurone layers (AL) of different cereal varieties
Data represent the mean ± standard deviation (SD), n = 3. The letters a, b, c, d, and e indicate statistically homogeneous groups: Groups with different letters are significantly different at $P < 0.05$.

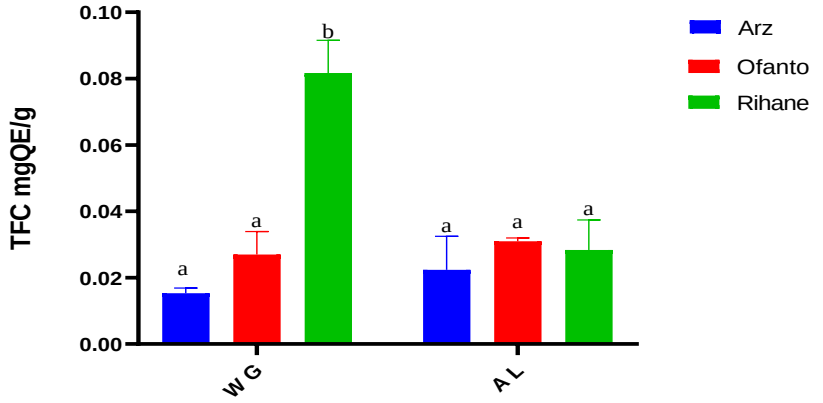

Figure 3: Total flavonoid content of whole grains (WG) and aleurone layers (AL) of different cereal varieties
Data represent the mean ± standard deviation (SD), n = 3. Means with different letters are statistically significant at $p < 0.05$.

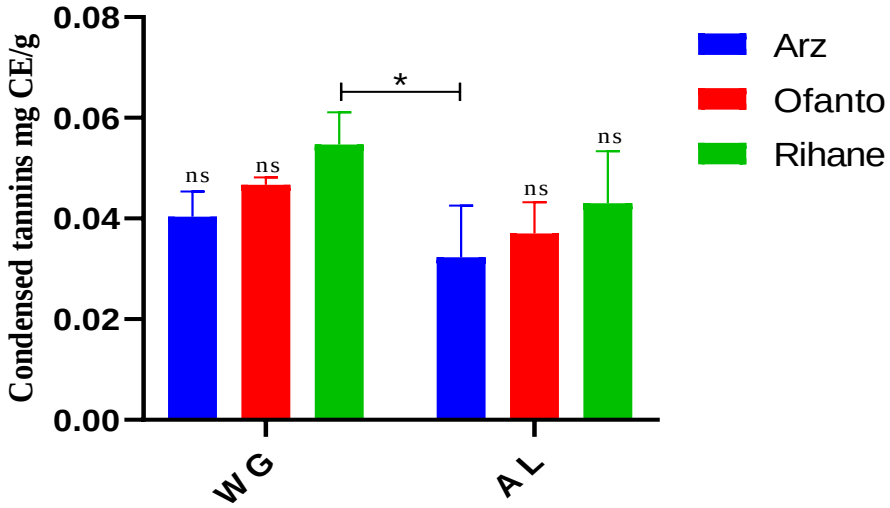

Figure 4: Total tannin content in whole grains (WG) and aleurone layers (AL) of different cereal varieties
Data represent the mean ± standard deviation (SD), n = 3. '*' indicate significant difference at $P < 0.05$), while 'ns' indicate non-significant difference ($P \geq 0.05$).

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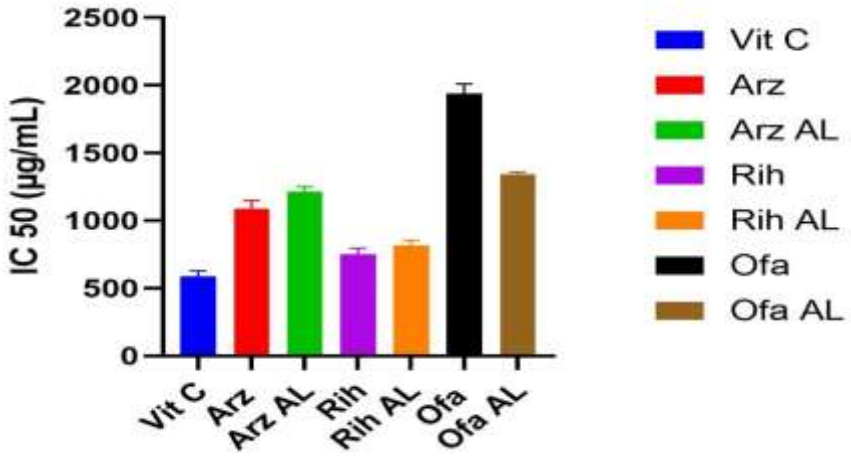

Figure 5: Antioxidant activity (IC_{50} values) of whole grain and aleurone layer fractions of different cereal varieties determined by the DPPH assay.
Vit C: Vitamin C; **Arz:** soft wheat; **Rih:** Rihane (barley); **Ofa:** Ofanto (durum wheat)

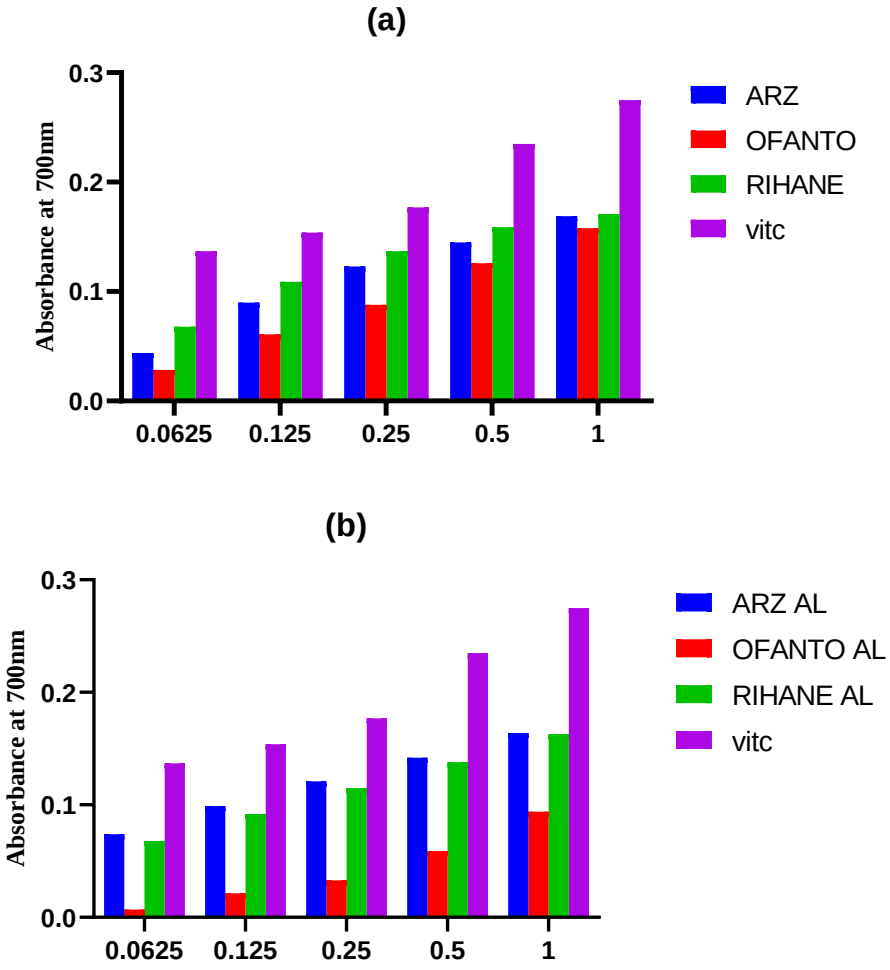

Figure 6: Absorbance as a function of extract concentration measured by the FRAP assay in: **(a)** whole grain extracts of soft wheat, durum wheat, and barley; **(b)** aleurone layer extracts of the same species.

decreased in the order: 'Rihane' (0.054 ± 0.006 mg CE/g) > 'Ofanto' (0.046 ± 0.001 mg CE/g) > 'Arz' (0.040 ± 0.005 mg CE/g). The highest tannin content in aleurone layer extracts was found in barley (0.043 ± 0.01 mg CE/g), followed by durum wheat (0.037 ± 0.006 mg CE/g), with the lowest in 'Arz' (0.03 ± 0.01 mg CE/g). This variation may be due to tannin localization, mainly in the testa layer above the aleurone in wheat.[41] Specific studies on condensed tannin content in the aleurone layers of wheat and barley are limited. The only significant difference observed was between WG 'Rihane' and AL 'Arz', with a mean difference of 0.0223 ($p = 0.0262$), indicating higher tannin contents in 'Rihane'. Overall, tannin contents were fairly consistent across varieties and their aleurone layers. However, comparison with the findings of Triki *et al*. (2018)[42] who studied barley varieties, reveals that condensed tannin content can vary significantly between whole grains of different types.

Antioxidant activity of cereal extracts

DPPH free radical scavenging activity

The antioxidant activity, measured using the DPPH assay and expressed as IC_{50}, was compared across different cereal varieties ('Arz', 'Rihane', and 'Ofanto'), including both whole grains and their aleurone layers, with vitamin C as the reference compound. As expected, vitamin C showed the strongest antioxidant capacity, with the lowest IC_{50} value (588.07 µg/mL) (Figure 5). Among the cereal samples, 'Rihane' demonstrated the highest antioxidant potential, especially in the whole grain (IC_{50} = 752 µg/mL), followed by its aleurone layer (IC_{50} = 817.16 µg/mL), suggesting a relatively even distribution of antioxidant compounds. 'Arz' exhibited moderate activity, with IC_{50} values of 1086.32 µg/mL for the whole grain and 1215.24 µg/mL for the aleurone layer, suggesting a slight decrease in antioxidant content in the aleurone layer. 'Ofanto' recorded the lowest antioxidant activity among the samples, with an IC_{50} of 1940.92 µg/mL in the whole grain, though its aleurone layer showed some improvement (IC_{50} = 1345.22 µg/mL). This aligns with the results of several researchers, who found that barley has a higher antioxidant capacity than wheat.[35] Antioxidant activity depends on the structure and nature of the antioxidants, as well as their concentration, since the phenolic fraction does not contain all antioxidants, and because of synergistic interactions between antioxidants in a mixture.[43] Overall, these results highlight clear differences between varieties and grain fractions, providing insight into how these components could be better utilized for their nutritional and functional benefits. Considering these findings alongside previous research, it is essential to note that while several studies have examined the polyphenol content and antioxidant properties of cereal-based foods or composite flours, [44-46] others have focused on products enriched with the aleurone fraction.[47, 48] To our knowledge, no published work has explored aleurone-enriched foods made from cereals grown in Algeria.

Ferric reducing antioxidant power (FRAP) of the cereal extracts

The antioxidant potential of extracts from the 'Arz', 'Ofanto', and 'Rihane' varieties, as well as their aleurone layers, was evaluated using the FRAP assay. This test measures how well antioxidants reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Results were reported as absorbance values for concentrations ranging from 0.0625 to 1 mg/mL. These were compared to the standard reference, ascorbic acid (vitamin C). All test samples showed stronger antioxidant activity with higher concentrations (Figure 6). At 1 mg/mL, 'Rihane' extract showed the highest absorbance (0.171), closely followed by 'Arz' (0.169) and their respective aleurone layers (AL 'Rihane': 0.163, and AL 'Arz': 0.164). These findings indicate that both the whole grain and aleurone layers of 'Rihane' and 'Arz' exhibit greater antioxidant potential compared to 'Ofanto' and its aleurone layer (AL 'Ofanto': 0.158 and 0.094), which showed much lower absorbance values, especially in the aleurone layer.

Extracts from 'Rihane' and 'Arz' and their aleurone layers retained notable antioxidant activity even at low concentrations. In contrast, AL 'Ofanto' showed a sharp decline in activity below 0.25 mg/mL, as also observed by Ragaee *et al*. (2012).[49] Ascorbic acid (the positive control) had much higher antioxidant activity than the cereal extracts, with absorbance values ranging from 0.275 to 0.137. This confirms the method's sensitivity. These findings suggest that 'Rihane' (barley) has the highest antioxidant activity among the samples, followed by 'Arz' (soft wheat). This is consistent with the results of Holtekjølen *et al*. (2011).[50] The aleurone layer of these varieties also retained much of its antioxidant potential. This underscores their nutritional and functional value for food formulation and health. The link between antioxidant activity and phenolic compound concentration has been established. Several studies have reported a positive relationship between total phenolic content and antioxidant activity in the studied grains. The present study confirms this positive correlation and shows that phenolic compounds mainly drive antioxidant activity in these seed extracts. Extracts with more phenolic content have significant antioxidant activity.

Conclusion

This study demonstrates that the aleurone layer is a particularly rich source of total polyphenols and flavonoids and is moderately rich in tannins, thereby significantly contributing to the antioxidant potential of cereals. Among the analyzed cultivars, barley (Rihane) exhibited the most favorable bioactive profile for both the whole grain and the aleurone layer, with the aleurone layer content sometimes comparable to or surpassing that of durum wheat (Ofanto). Soft wheat (Arz) also displayed noteworthy phenolic content and antioxidant activity in its aleurone fraction. These findings highlight the potential to valorize aleurone fractions (often removed during refining) as functional ingredients to boost dietary intake of natural antioxidants. Overall, this work confirms that both cereal species and cultivar selection are key factors in shaping the bioactive composition and antioxidant capacity of Algerian cereal grains.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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