

## COMPARISON OF THE NUTRITIONAL AND ANTIOXIDANT VALUES OF THE PERIPHERAL LAYERS IN TWO SPECIES OF WHEAT (SOFT AND HARD) GROWN IN ALGERIA

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### ABSTRACT

A study was carried out to determine the content of secondary metabolites and their antioxidant activity, and compare the chemical composition of the peripheral layers of two varieties of wheat, soft wheat (*Triticum aestivum*) Ziden and durum wheat (*Triticum durum*) Chen's, cultivated in the western region of Algeria. The infusion of the powder from the outer layers of both varieties was carried out with methanol. Antioxidant activity was evaluated by the DPPH (2,2-diphényl 1-picrylhydrazyle) and FRAP (fluorescence recovery after photo bleaching) methods. The phenolic compounds in the crude extracts of the two varieties were analyzed by HPLC. Contents of calcium, iron, magnesium, copper, and manganese were analyzed by atomic absorption spectrophotometry. The ethyl acetate extract ( $EC_{50} \text{ mg mL}^{-1} = 0.24 \pm 0.001$ ) for Ziden ( $EC_{50} \text{ mg mL}^{-1} = 0.03 \pm 0.004$ ) for Chen's showed antioxidant potential. Three phenolic acids were detected for the Chen's variety (Gallic acid, 4- (p)-hydroxybenzoic and Syringic acid), and a phenolic acid (Syringic acid) was detected for the Ziden variety. Protein determination by the Bradford method showed that the Chen's variety has a higher concentration of proteins (0.005 mg / mL), with a 1.5% fiber content. Regarding mineral content, the most abundant element was calcium. Images of the peripheral surface layers of husked wheat grains obtained by scanning electron microscopy revealed almost similar structures for the two species. It can be concluded that Chen's (durum wheat) has more nutritional value than Ziden (durum wheat).

Keywords: HPLC, Polyphenol, wheat, peripheral layer, minerals, fibers, proteins.

## INTRODUCTION

Wheat (Gramineae or Poaceae family) is a cereal belonging to the *Triticum* genus. Its processing has many advantages for human nutrition. Several works have studied the different stages, varieties and parts of the wheat seed, allowing for a good understanding of their health benefits (Viol, 2013; Sennous et al., 2020). In fact, wheat has many beneficial characteristics because it is rich in proteins, fiber, vitamins, minerals (Antoine et al., 2002; Amrein et al., 2003), and secondary metabolites (Anson, 2010; Chiboub et al., 2017). Wheat bran is collected as a co-product of milling. It represents about 15% (w / w) of the total grain, being composed of several so-called peripheral cellular layers: aleurone layer (AL), integument or hyaline nucellus, testa (derived from the inner wall of the ovary) and the layer of pericarp, which is divided into two parts (outer and inner pericarp), with an adherent starch remaining in the endosperm (FAO, 1970).

The consumption of whole grain is very beneficial for health because of its high content of secondary metabolites, which are polyphenols (mainly flavonoids and phenolic acids) (Viol, 2013). These compounds help protect against cardiovascular disease, diabetes, and colorectal cancer (Tayyem et al., 2016), while high levels of vitamin C (in barley) support the immune system. Liyana-Pathirana et al. (2006) showed that most of phenolic acids are present in the aleurone layer, being closely associated with cell wall materials, such as polysaccharides and lignins.

The objective of the present work was to determine the content of secondary metabolites and their antioxidant activity in order to compare the chemical composition of the peripheral layers of two varieties of wheat, soft wheat Ziden (*Triticum aestivum*) and durum wheat Chen's (*Triticum durum*), cultivated in the western region of Algeria. Scanning electron microscope imaging was used to study the peripheral layers, followed by the study of the cytological appearance.

## MATERIALS AND METHODS

### Plant material and extraction

Two varieties of wheat, soft wheat (*Triticum aestivum*) Ziden and durum wheat (*Triticum durum*) Chen's, were used. The different characteristics of these two varieties are included in Table 1.

Dissection and isolation of the peripheral layers was the first experimental part of this study. The seeds were harvested at a mature stage and washed with distilled water. The peripheral layers (PL) were separated manually under a binocular magnifying glass and kept until analysis to be powdered. An amount of 10g of the PL was extracted twice with 200 mL of methanol by the infusion method. The extracts were filtered and concentrated to dryness using a rotary evaporator; then distilled water was added. A second extraction was conducted using a decanting funnel with chloroform (CHCl<sub>3</sub>), followed by ethyl acetate (EA) and N-butanol to select bioactive molecules with solvents of different polarity.

### Scanning electron microscopy

Scanning electron microscopy (SEM), which is commonly used in the study of biological materials to determine the morphology and structure of tissues (Farber and Gallant, 1976), was used. After the manual dissection of the peripheral layers (PLs) of each variety, kernels were cleaned and dried. Surface morphology was determined using SEM with magnifications ranging from 400 to 1600 in order to obtain high resolution images on the surface of a sample (Thornley and Cartz, 1962).

### Nutritional Value

**Protein content.** To determine the concentration of proteins in the PL of the two wheat varieties under study (Ziden and Chen's), the Bradford's method (1976) was used. The method is based on the change in absorbance at 595 nm observed after the addition of 2 mL of an acidic solution of Coomassie Brilliant Blue G-250.

**Table 1. Botanical characteristics of the two species: Chen's (durum wheat) and Ziden (soft wheat).**

Characteristics	Chen's	Ziden
Plant	Short	Short
Epi	Rosse, pyramidal	White, pyramidal
Grain	Half elongate	Ovoid, reddish color
Rod	Low hairiness	No to very low hairiness
Variety type	Pure line	Pure line
Variety	Durum wheat	Soft wheat

After homogenization and incubation from 5 to 15 minutes in the dark, absorbance of the protein-dye complex at 595 nm allows a precise quantification of the protein content of a sample. Concentrations were calculated from the linear curve using BSA as standard at 1 g/L expressed in mg/mL (Snyder and Desborough, 1978; Berges et al., 1993).

**Mineral content by atomic absorption spectrophotometry.** The mineral assay protocol was performed using an atomic absorption spectrophotometer (Shimadzu, model AA7000) (Mahood et al., 2021). The peripheral layers were brought into contact with 65% nitric acid, adding 30% hydrogen peroxide  $H_2O_2$ , followed by heating from 90 °C to 120 °C to remove all the solvent. Subsequently, cooling hydrolysis and filtration were conducted and the samples were atomized by acetylene-airflame. Absorbance was read at a specific wavelength for each chemical and the results were expressed in mg / kg.

**Raw fiber content (Weende).** The content of raw fiber was determined according to the protocol described by Kitcherside et al. (2000). This method is based on the solubilization of non-cellulose compounds in solutions of sulfuric acid and potassium hydroxide. An amount of 1g of each sample was mixed with 150 mL of 1.25% sulfuric acid; then 3-5 drops of the anti-foaming agent n-octanol were added and the mixture was boiled for 30 min. After draining, the crucibles were washed 3 times with hot distilled water. The same steps were repeated using 1.25% potassium hydroxide, except for the last wash in which cold deionized water was used, followed by 3 washes with 25mL of acetone. After drying at 105 °C for 1 h, the crucibles were removed and weighed, which corresponded to raw fiber and ash content (F1). Subsequently, the crucibles were placed in a muffle furnace (NABER D-2804, Germany) at 550 °C for 3 h and weighed again after cooling in a desiccator (F2). The results were expressed in% (Eq. 1).

$$\% \text{ raw fibers} = (F1 - F2 / F0) \times 100 \quad \text{Eq. 1}$$

F1: raw fiber weight + ash

F2: ash weight

F0: weight after grinding

### Biological studies

**Total phenolic content.** The total phenolic content was determined using the Folin-Ciocalteu method (Vermarius and Nicholson, 2006), while absorbance was measured using a spectrophotometer at a wavelength of 725nm. A

volume of 0.1 mL of each extract was mixed with 2 mL of 2% sodium carbonate solution. After 5 min, a volume of 0.1 mL of Folin-Ciocalteu (0.2 N) reagent was added; the mixture was incubated at room temperature for 30 min and protected from light.

The calibration curve was performed using gallic acid as a standard, expressed in  $\mu\text{g GAE mg}^{-1}$  of extract.

### Total Flavonoid Content

For the determination of total flavonoids, the complex reaction between  $Al^{3+}$  and the flavonoids was used (Zhishen et al., 1999), using quercetin as a standard; the rate was expressed as  $\mu\text{g QE mg}^{-1}$  of extract.

A volume of 50  $\mu\text{L}$  of each extract was mixed with 130  $\mu\text{L}$  of methanol and 10  $\mu\text{L}$  of potassium acetate solution (1M) ( $CH_3COOK$ ). Then 10  $\mu\text{L}$  of aluminum nitrate ( $Al(NO_3)_3$ ) (10%) were added to the mixture. After a 40-min incubation at room temperature, the absorbance was read at 415 nm against a blank using a spectrophotometer.

### Antioxidant Activity

**DPPH Radical Scavenging Activity.** The scavenging free radical activity of the raw extracts and the different fractions from two wheat varieties (Chen's and Ziden) was measured by the DPPH method (Samarth et al., 2008). A volume of 1950  $\mu\text{L}$  of DPPH radical solution was prepared in methanol and added to 50  $\mu\text{L}$  of different concentrations of each extract. A negative control was prepared by adding 50  $\mu\text{L}$  of methanol to 1950  $\mu\text{L}$  of DPPH solution. After 30 min of incubation at room temperature in the dark, absorbance was read at 517 nm against a blank using a spectrophotometer. BHA (3-tertiobutyl-4-hydroxyanisole) and ascorbic acid were used as standards (positive control). The lower absorbance of the reaction mixture indicated a high free radical scavenging activity. DPPH radical scavenging activity (%) was calculated as follows (Eq. 2).

$$DPPH \% = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} * 100 \quad \text{Eq. 2}$$

DPPH (%): the reducing percentage of DPPH.

Acontrol: the absorbance of the negative control.

Asample: the absorbance of the sample.

The results were expressed as the average of three measurements obtained for each sample. The  $IC_{50}$  value is the concentration of the sample that can scavenge 50% of the DPPH free radical; it was revealed graphically for each extract of the curve of the reducing percentage of DPPH as a function of the concentration.

**Ferric reducing power (FRAP).** This method was determined according to the protocol of Karagözler et al. (2008). After preparing the extracts at different concentrations, a volume of 0.1 mL was mixed with 0.25 mL of the phosphate buffer solution (0.2 M, pH = 6.6) and 0.25 mL of potassium ferricyanide ( $K_3Fe(CN)_6$ ) at 1%. After incubation at 50 °C for 20 min, 0.25 mL of 10% trichloroacetic acid (TCA) was added to the tubes in order to stop the reaction. Then a volume of 0.5 mL of each concentration was introduced into new tubes, and then mixed with 0.5 mL of distilled water and 0.1 mL of iron chloride ( $FeCl_3$ ) at 0.1%. Absorbance was measured at 700 nm against a blank. The determination of the effective concentration ( $EC_{50}$ ) of each extract was calculated graphically using linear regression curves, representing absorbance depending on concentration. Ascorbic acid was used as a standard.

#### Characterization of Phenolic Compounds by RP-HPLC-LC-2030C 3D Analysis

The phenolic content in the crude extract of the two varieties (Chen's and Ziden) was determined using high-performance liquid chromatography coupled with UV- vis detector RP-HPLC-LC-2030C 3D (Shimadzu, Prominence-i; LC-2030C 3D) (Annaet al., 2020), using four modes of identification. A processing data system was used for the analysis. The separation was achieved on a Supelco C18 column (25 cm x 4.6 mm, 5  $\mu$ m) at room temperature. The mobile phase consisted of water / acetic acid (pH = 3), phase B consisted of methanol / acetic acid (pH = 3). The

gradient elution system was as follows: 0.01- 2 min, 5% solvent B concentration; 2 – 40 min, from 5% to 100% solvent B concentration; 40–45 min, 100% solvent B concentration at a wavelength of 280 nm. Phytochemicals were identified from a combination of retention time and spectral matching.

**Statistical analysis.** Data were analyzed by an analysis of variance (ANOVA) using statistical software. The validation of the results of the different activities was carried out by three analyzes of each sample and each experiment was carried out in triplicate (n = 3). The mean value and standard deviation were calculated from the data obtained.

## RESULTS AND DISCUSSION

#### Scanning electron microscopy

Fig. 1 shows tissue images of the peripheral surface layers of husked wheat kernels observed by scanning electron microscopy (SEM). Upon dissection, pronounced differences in hardness were observed between the two wheat varieties (Chen's and Ziden). On the longitudinal section of the kernels, the layer structure was obtained from an irregular surface structure characterized by a more elongated thin pericarp (seed coat). As described by Surget and Barron (2005), it was very fibrous in nature and mainly composed of cellulose, hemicelluloses (pentosans) and lignin.

In general, the peripheral tissues of the grains were less severely damaged. From a

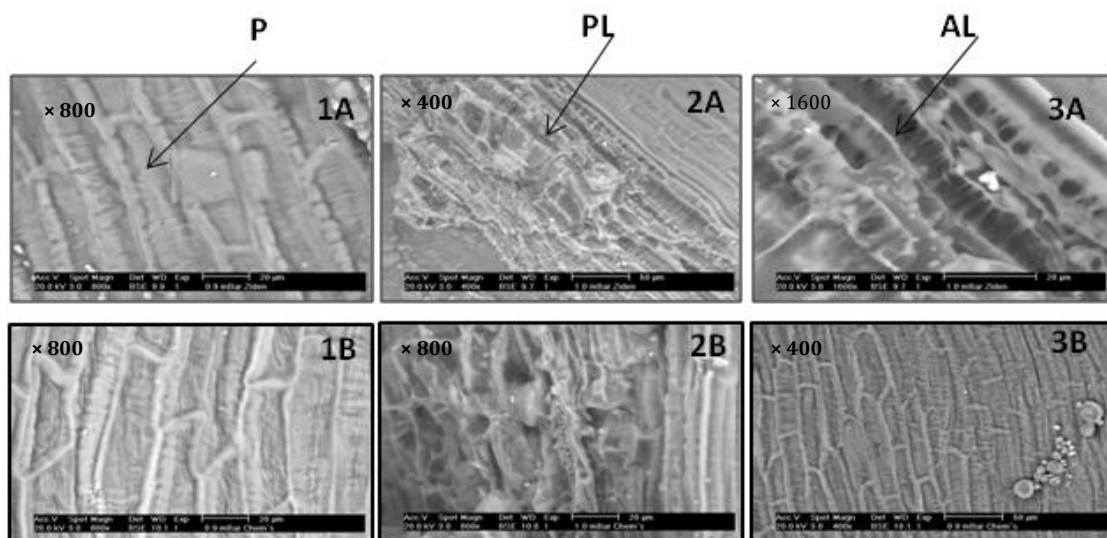


Fig. 1. Observation of the peripheral layer of the two wheat varieties in the mature stage using a scanning electron microscope. Scale 20  $\mu$ m. P: Pericarp (1A, 1B, 3B); PL: Peripheral layer (2A, 2B); AL: Aleurone layer (3A).



nutritional point of view, this is particularly important because the main amino acids, fibers and minerals of the grain are found in the aleurone layer. For both soft and durum wheat, the microscopic images observed revealed superposition of different tissues from outside to the inner surface.

The histology of the peripheral layers consists of the pericarp, the testa, the aleurone layer and the epidermal nucellus. The aleurone layer is the innermost layer attached to the pericarp tissue along with the hyaline layer and the testa layer (Meziani et al., 2021). Images 1A, 1B and 3B show the pericarp in a continuous band, wrapped around caryopses, which begin to form on the inner side of the outer layers of wheat caryopses. The single-celled tissue forms the aleurone (3A) layer surrounding the endosperm.

### Nutritional value results

**Protein content.** The protein content of the peripheral layers of the extracts was 0.005 mg / mL for Chen's and 0.001 mg / mL for Ziden by the Bradford method (Table 2).

The protein content obtained seems low but agrees with previous studies that have reported values of 9.60% and 18.6% in durum wheat bran (Curti et al., 2013). In addition, Pinckney (1961) indicated that durum wheat protein is more resistant to extraction than other varieties, which confirms our results for the Chen's variety. Most proteins from the peripheral layers of wheat grains are present in the first stage of development because of metabolic activity, photosynthesis, defense against oxidative stress and pathogenesis. These proteins accumulate in early stages of development and decline towards maturity with decreased oxygen availability (Tasleem-Tahir et al., 2011; Mechin et al., 2007).

**Mineral content.** The calibration curves for calcium are shown in Table 2. The highest Ca

concentration was observed in the Chen's variety with a value of 0.5329 mg / kg, while Ziden reached lower levels of 0.4396 mg / kg. However, Ziden recorded higher contents of Fe, Cu and Mn, with values of 0.0623 mg / kg; 0.0057 mg / kg; and 0.0216 mg / mL, respectively. Conversely, Chen's variety recorded lower values of 0.0356 mg / kg; 0.0048 mg / kg; and 0.0090 mg / kg for Fe, Cu and Mn, respectively. With respect to magnesium, concentrations were relatively similar for both varieties, with values of 0.1071 mg / kg for Ziden and 0.1066 mg / kg for Chen's ( $p < 0.05$ ).

Chemical studies on the distribution of minerals in cereal grains have shown that most of mineral reserves are contained in the bran and germ fractions of the grains (O'Dell et al., 1972). It occurs as phytate, a mixed salt of potassium and magnesium myo-inositol hexaphosphate, which accounts for 70-90% of the total phosphorus reserve of wheat (O'Dell et al., 1972; Frolich and Nyman, 1988).

Our results agree with those reported by Svetlana and Özcan (2016) in a study on barley grains. The authors concluded that Ca, K, Mg, P and S are the main macro-elements, and reported contents of 487mg / kg for Ca; 1724mg / kg for Mg 6.7mg / kg for Cu; 57.7mg / kg for Fe, and 18.8mg / kg for Mn. The higher values they obtained compared to our results can be explained by the fact that our study focused on the peripheral layers of the grains, while the extraction method could also account for this situation.

**Fiber content.** Wheat bran is a source of insoluble fiber. The highest percentage of crude fiber (1.5%) was obtained with the Chen's variety (durum wheat), where as Ziden (soft wheat) had a slight difference of 0.3%, recording 1.2% ( $p < 0.05$ ).

In terms of health benefits, fiber can reduce the risk of certain chronic diseases such as cardiovascular disease, type 2 diabetes and certain cancers, especially colorectal cancers

**Table 2. Contents of protein, minerals and crude fiber of the two species of wheat (Ziden and Chen's).**

Sample	Soft wheat (Ziden)	Durum wheat (Chen's)
Yield (%)	0.864	0.013
Mineral		
Calcium (mg/mL)	0.4396 ± 0.07a	0.5329 ± 0.04a
Iron (mg/mL)	0.0623 ± 0.017a	0.0356 ± 0.015a
Mg (mg/mL)	0.1071 ± 0.03a	0.1066 ± 0.006a
Cu (mg/mL)	0.0057 ± 0.005a	0.0048 ± 0.001a
Mn (mg/mL)	0.0216 ± 0.014a	0.0090 ± 0.008a
Protein Content(mg/mL)	0.001 ± 0.0008a	0.005 ± 0.001a
Raw fibers (%)	1.2 ± 0.07a	1.5 ± 0.03a

<sup>a</sup>Values expressed as the mean ± S.D. of three parallel measurements.

(Fung et al., 2002; Koh-Banerjee et al., 2004; Sayhoun et al., 2006; Seal et al., 2006; De Munter et al., 2007; Schatzkin et al., 2007; Mellen et al., 2009). According to Decosse (1989), supplements of cereal fiber of more than 11 g/ day inhibit benign neoplasia of the large intestine. These results agree with those of Mellen et al. (2009), who confirmed that fibers of food grains reduce the risk of neoplasia in the large bowel.

#### Content of polyphenols and flavonoids.

Cereals are a rich source of total polyphenols. In wheat, these compounds are mainly concentrated in the bran (Slavin et al., 1999). The polyphenol values for the two varieties are presented in Table 3. For the Ziden variety, the maximum polyphenol concentration was obtained by the butanol fraction, with a value of 590 ( $\mu\text{g GAE mg}^{-1}\text{E}$ ), followed by the ethyl acetate fraction (468  $\mu\text{g GAE mg}^{-1}\text{E}$ ), the crude extract (427  $\mu\text{g GAE mg}^{-1}\text{E}$ ), and the chloroform extract (208  $\mu\text{g GAE mg}^{-1}\text{E}$ ). For the Chen's variety, the crude extract had the highest value (550  $\mu\text{g GAE mg}^{-1}\text{E}$ ), followed by the butanol fraction (510  $\mu\text{g GAE mg}^{-1}\text{E}$ ), chloroform (420  $\mu\text{g GAE mg}^{-1}\text{E}$ ), and ethyl acetate (390  $\mu\text{g GAE mg}^{-1}\text{E}$ ) ( $p < 0.05$ ).

Regarding flavonoids, concentrations varied between the different solvents. The highest value in total flavonoids was recorded in the crude extract, with a similar value of 619  $\mu\text{g QE mg}^{-1}\text{E}$  for both varieties, followed by the butanol fraction with values of 543 and 520,  $\mu\text{g QE mg}^{-1}$ , ethyl acetate with values of 502 and 490  $\mu\text{g QE mg}^{-1}\text{E}$ , and the chloroform extract with values of 218 and 454  $\mu\text{g QE mg}^{-1}\text{E}$  for Ziden and Chen's, respectively ( $p < 0.05$ ).

The difference between the values may be related to the polarity of the compounds present in the samples. Our results fully agree with Lopez et al. (2019), who worked on wheat bran and barley husk. The authors found high concentrations of flavonoids in alcoholic extracts (ethanol, methanol), which was possibly associated with the aglycone structure of flavonoids; they also confirmed that absolute methanol exhibited the

statistically lowest extraction yield of 76.38 mg GAE / 100 g. A study conducted by Abozed et al. (2014) on wheat bran showed that the highest concentration of flavonoids was 22.39 mg / 100 g in soft wheat (Gemiza-9 variety) and 25.80 mg / 100 g in durum wheat obtained with 70% ethanol. In addition, Vaher et al. (2010) reported that 80% methanol extract from bran layers had the highest content of total phenolic compounds, which are removed when milling wheat into white flour. The authors measured the polyphenol content of 20 different wheat genotypes, reporting values that varied from 2.7 to 3.5 mg GAE / mg. The values are lower than those obtained in the present study.

A study conducted by Wojdylo and Oszmainski (2007) showed that it was important for the hydrolysis process to be adopted in order to obtain a maximum yield of phenolic acids from cereals. The parameters that affect the quality and quantity of an extract are due to the plant part used as the starting material, and the extraction process adopted. Temperature and solvent type play an important role in determining biologically active compounds (Ncube et al., 2008). Changes in concentrations between the results obtained from peripheral pure layers in the present study and other studies conducted on wheat bran, may be associated with the presence of a small amount of starch, different types of varieties, growth time differences, growing area (region or country) and climatic conditions.

**Antioxidant Activity.** The antioxidant capacity of the extracts was measured by the DPPH and FRAP methods by calculating their  $\text{IC}_{50}$  and  $\text{EC}_{50}$ , which are inversely proportional to the efficiency of the extracts. The results obtained ( $\text{IC}_{50}$  and  $\text{EC}_{50}$ ) are provided in Table 4.

The tested extracts are less active than standard antioxidants (ascorbic acid). The most effective fraction was recorded with the ethyl acetate extract in both varieties  $\text{EC}_{50} = 0.240\text{ mg mL}^{-1}$  (Ziden) and  $\text{EC}_{50} = 0.03\text{ mg mL}^{-1}$  (Chen's). Regarding the Ziden variety, the results revealed

**Table 3. Total polyphenols (TPC) and flavonoids (TFC) of the two species of wheat (Chen's and Ziden varieties). TPC: Total phenolic compound; TFC: total flavonoid content.**

Settings Samples	aTPC ( $\mu\text{g GAE mg}^{-1}\text{E}$ )		aTFC ( $\mu\text{g QE mg}^{-1}\text{E}$ )	
	Ziden	Chen's	Ziden	Chen's
Crude extract	427 $\pm$ 1.2b	550 $\pm$ 0.34b	619 $\pm$ 0.5b	619 $\pm$ 1.5b
Chloroform extract	208 $\pm$ 0.7b	420 $\pm$ 0.48b	218 $\pm$ 0.5b	454 $\pm$ 1.8b
Ethyl acetate extract	468 $\pm$ 1.02b	390 $\pm$ 0.8b	502 $\pm$ 1.41b	490 $\pm$ 0.9b
Butanol extract	590 $\pm$ 0.64b	510 $\pm$ 0.5b	543 $\pm$ 0.7b	520 $\pm$ 1b

<sup>a</sup>Values expressed as the mean  $\pm$  S.D. of three parallel measurements.

<sup>b</sup>  $p < 0.05$ .

**Table 4. Analysis of the antioxidant activity (FRAP and DPPH) of the two wheat varieties.**

Settings	DPPH (IC <sub>50</sub> ) (mg mL <sup>-1</sup> )		Reducing power assay EC <sub>50</sub> (mg mL <sup>-1</sup> )	
	Ziden	Chen's	Ziden	Chen's
	Crude extract	1.7 ± 0.48	0.74 ± 1.73	0.670 ± 0.06
Chloroform extract	0.71 ± 0.26	1.210 ± 0.1	0.280 ± 0.002	0.620 ± 0.001
Ethyl acetate extract	> 4	0.19 ± 0.094	0.240 ± 0.001	0.030 ± 0.004
Butanol extract	0.47 ± 0.8	2.430 ± 1.2	0.960 ± 0.008	0.090 ± 0.01
Ascorbic acid mg mL <sup>-1</sup>	0.00293 ± 1.08	0.00293 ± 1.08	0.01533 ± 0.08	0.01533 ± 0.08

that the crude extract has a weak antiradical activity with IC<sub>50</sub> = 1.700 mg mL<sup>-1</sup> and EC<sub>50</sub> = 0.670 mg mL<sup>-1</sup>, followed by the chloroform, butanol and ethyl acetate extracts, with values of IC<sub>50</sub> = >4 mg mL<sup>-1</sup>, EC<sub>50</sub> = 0.24 mg; IC<sub>50</sub> = 0.71 mg mL<sup>-1</sup>, EC<sub>50</sub> = 0.28 mg and IC<sub>50</sub> = 0.47 mg mL<sup>-1</sup> and EC<sub>50</sub> = 0.96 mg mL<sup>-1</sup>, respectively. The highest antiradical value for Chen's variety was detected in the ethyl acetate extract, with a value of IC<sub>50</sub> = 0.19 mg mL<sup>-1</sup>, EC<sub>50</sub> = 0.03 mg mL<sup>-1</sup>, followed by the crude, chloroform and butanol extracts with values of IC<sub>50</sub> = 0.74 mg mL<sup>-1</sup>, EC<sub>50</sub> = 0.72 mg mL<sup>-1</sup>; IC<sub>50</sub> = 1.210 mg mL<sup>-1</sup>, EC<sub>50</sub> = 0.62 mg mL<sup>-1</sup>; IC<sub>50</sub> = 2.430 mg mL<sup>-1</sup>, EC<sub>50</sub> = 0.09 mg mL<sup>-1</sup>, respectively. According to the study, the results are significantly different (p < 0.05).

In terms of antiradical activity (EC<sub>50</sub>) (Fig. 2), it can be deduced that the ethyl acetate extract has a good activity for both varieties, while the butanol extract presents the lowest activity for Chen's compared to Ziden. This can be explained by solvent polarity, intervention of another component or phenomenon that prevents the activity, or by differences in phenolic content between the two varieties.

A study conducted by Abozed (2014) revealed that the percentage of free radical inhibition was higher with 70% ethanol in two different varieties of wheat bran, showing that phenolic compounds, and particularly flavonoids, are the main contributors to the antioxidant activity of different extracts of food grains (Donkor et al., 2012; Vaher et al., 2010; Beta et al., 2005; Verma et al., 2009). The content of total polyphenols is closely related to antioxidant activity (Ehala et al., 2005). This was confirmed since the extracts with the highest content of phenolic acid exhibited the most remarkable antioxidant activity (lowest IC<sub>50</sub>), which also agrees with Vaher et al. (2010).

**Analysis of total polyphenols.** Analysis of crude extracts on the peripheral layers of two varieties of wheat grains (Chen's and Ziden) was achieved by RP-HPLC-LC-2030C 3D method using four modes of identification. The

chromatograms obtained (Figs. 3 and 4) showed a relatively low peak. Three peaks were detected for Chen's and one peak was detected for Ziden selected at 280 nm.

The syringic acid peak was more intense, followed by gallic acid and 4-(p)-hydroxybenzoic acid with methanol. This agrees with López-Perea et al. (2019), who reported a high concentration of benzoic acid with 80% methanol in the barley husk. The variation in peaks may be associated with different types of varieties (soft wheat and durum wheat), differences in growth time (Vaher et al., 2010) or the plant part under study (peripheral layer).

In the rice bran and rice husk analyzed by Pitchaporn et al. (2016), it was possible to identify 4 hydroxybenzoic acid (HBA): gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanilic acid and 6 hydrocinnamic acid (HCA): chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid. Ferulic acid was principally concentrated in the outer pericarp of wheat bran, while p-coumaric acid was mainly present in the aleurone layer (Antoine et al., 2004). The extraction method and the solvent used play a very important role on the content of phenolic compounds. Ethanol-water mixtures have been used as an extraction solvent, which improved extraction efficiency i.e., when the concentration of ethanol increases the total phenolic content of the bran extracts decrease (Wang et al., 2008).

## CONCLUSIONS

This study evaluated and compared the nutritional values of durum (Chen's variety) and soft wheat (Ziden variety) by analyzing the extracts of peripheral layers at different polarities. The ethyl acetate and n-butanol extracts had a good antioxidant activity, indicating the presence of bioactive molecules that are related to the prevention of certain diseases. According to the results obtained by the HPLC method, Chen's has a higher content of phenolic compounds

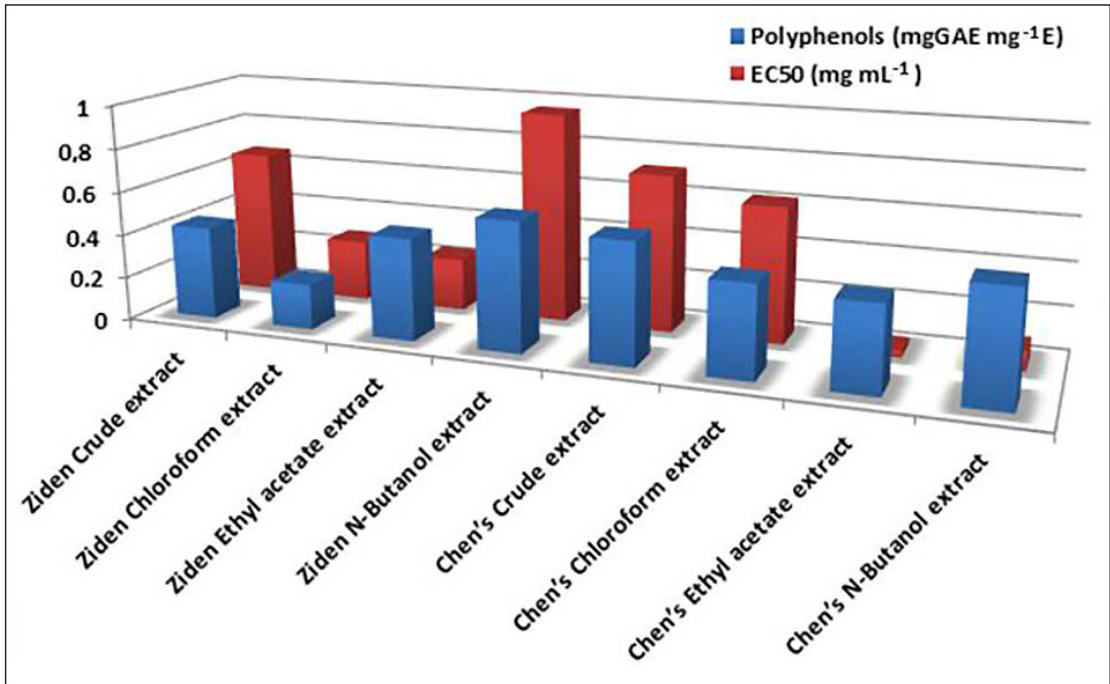


Fig. 2. Total phenolic content (mg GAE /mg E) (blue) and antioxidant activity EC50 (red) of different extracts of the peripheral layers of wheat varieties (Chen's and Ziden).

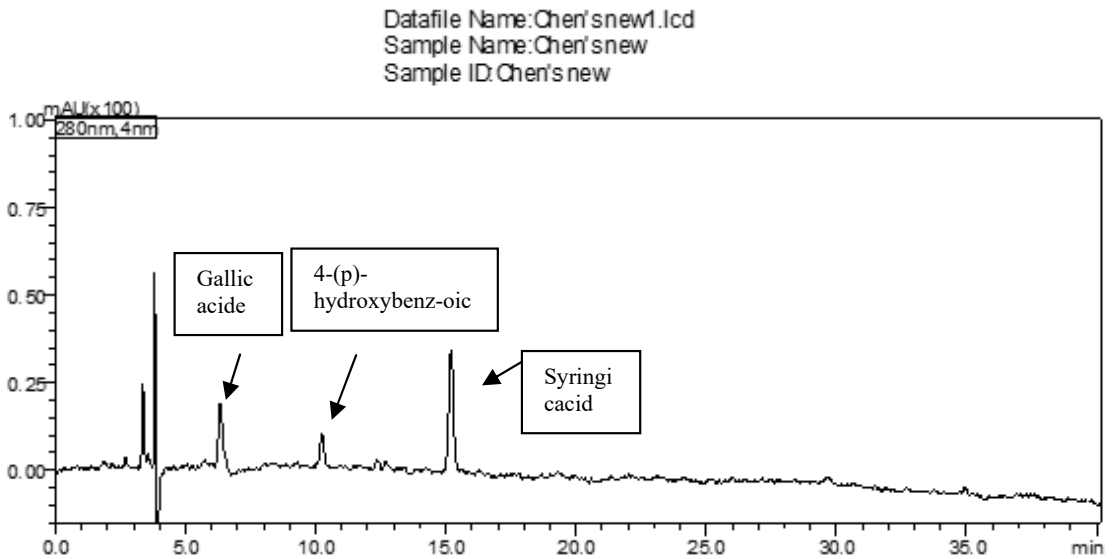


Fig. 3. HPLC chromatogram of the crude extract of the peripheral layer (Chen's variety).



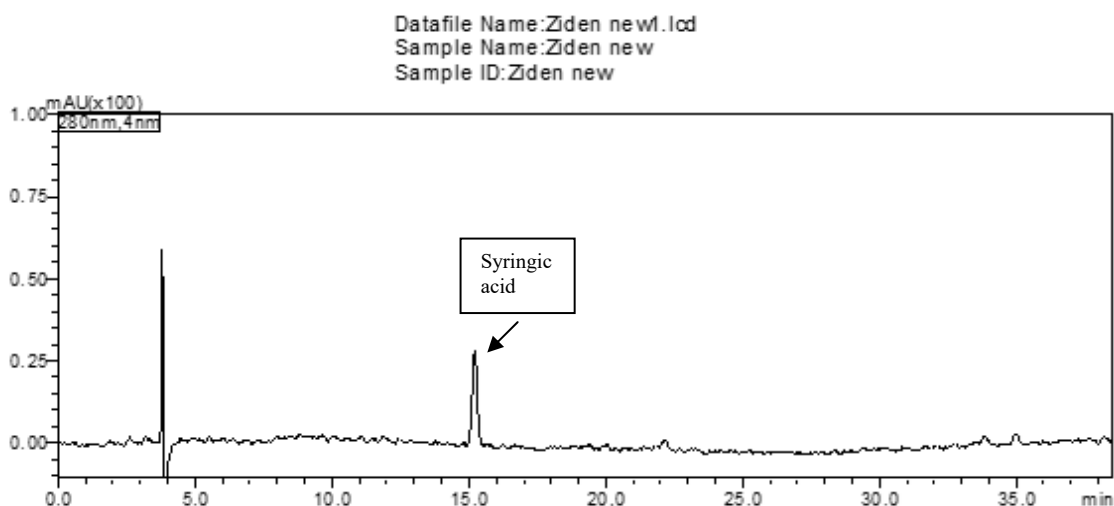


Fig. 4. HPLC chromatogram of the crude extract of the peripheral layer (Ziden variety).

than Ziden. The peripheral surface layers of the wheat grains observed by scanning electron microscopy showed almost similar structures for the two species. Based on the results obtained, it can be concluded that, despite being cultivated in the same region and period of time, the Chen's variety (durum wheat) has more nutritional value than the Ziden variety (soft wheat). To enrich this comparison, it is necessary to broaden the research and perform other more in-depth analyzes such as genetic analysis of the nutritional value of wheat, identification of proteins at different stages of development.

#### ACKNOWLEDGEMENTS

The authors thank the University of Sidi Bel Abbes, laboratory of Biototoxicology for the financial support of this research.

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