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Physicochemical characterization, antioxidant activity and phenolic acids profile of Tunisian whole cereal grains

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ABSTRACT

Six varieties of Tunisian durum wheat, barley and oat cultivars namely Maali, Karim, Rihane, Manel, Meliane and Ghzella were analyzed for their physicochemical properties, antioxidant activities and phenolic acids profile. Results showed that there are significant ($p < 0.05$) differences in moisture (11.8-12.8%), proteins (10.98-12.09%), neutral detergent fibre (NDF) (47.67-67.39%), ash (2.4-3.93%), fat (2.6- 6.75%), and carbohydrates (64.78-70.95%) contents between all studied varieties. Cereal grains were also a good source of potassium, sodium, calcium, zinc and iron. The results revealed that oat Meliane (5.9%) and barley Rihane (5.4%) varieties are good sources of dietary fiber β-glucan compared to the other varieties. Rihane, Meliane and Maali varieties had the highest total phenolic, total flavonoids and total condensed tannin contents. Antioxidant activity of whole grain extracts was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test and revealed that barley Rihane had the best radical scavenging activity compared to oat and durum wheat varieties. The RP-HPLC analysis revealed that Tunisian barley, oat and durum wheat varieties are a good source of phenolic components and natural antioxidants.

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1. Introduction

Cereal cultivars such as wheat, rice, corn and barley are primary sources of the human diet worldwide (1).

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The grass family Poaceae (Gramineae) includes many crops such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare*

L.), oat (*Avena sativa* L.) and rye (*Secale cereale* L.).
These cereal grains have been one of the most important human foods since ancient times. In general, the main cultivated wheat species are *Triticum aestivum* L. and *Triticum durum* L. However, cultivated oat, as well as wheat, exists in many different genomes *(Avena sativa, Avena byzantine…)* (2). The common oat (*Avena sativa*) is mainly grown for its utilization for human consumption as oatmeal as well as for livestock feed. Barley is one of the first and antiquely domesticated crops among wheat and pea dating from about 10,000 years ago in the Fertile Crescent of the Middle East (3). This latter has a long history of use as a source for animal feed and poorly for human nutrition, except for the production of alcoholic beverages such as beer. Approximately 65% of cultivated barley is used for animal feed, 33% for malting, whereas only 2% is used directly for human consumption (4).
It has been recommended that in vegetable-based foods the health benefits are attributed to the additive and synergistic effects of bioactive phytochemicals (1). Cereal whole grains are considered the most abundant source of bioactive compounds (5). In recent times, several epidemiological studies have associated the regular consumption of whole grain cereals with its potential to reduce the risks of various diseases such as chronic cardiovascular disease (6), colonic cancer, high blood pressure and diabetes. These therapeutic potentials are attributed to the functional and bioactive components present in whole cereals such as dietary fibers, minerals, vitamins, fatty acids, phytochemicals and antioxidants.
Phenolic acids and flavonoids represent the most common form of phenolic compounds found in whole grains. These valuable nutritional constituents are in general influenced by grain genotypes, varieties and environmental conditions (7). The most interesting soluble fiber that is extracted from cereal grains, notably oats and barley, is β-Glucan. The β-Glucan content varied according to the cereals species. Its content ranges from 3% to 7% in oats and 5% to 11% in barley (8). The latter has also many health benefits, including decreasing the risk of heart disease, blood glucose serum concentrations and colon cancer (9).
In Tunisia, barley, wheat and oat are grown in regions characterized by low rainfall or drought, high temperature and low soil fertility. The environment and climate are characterized by a wide range of climatic conditions from the humid and sub-humid of the northeast and northwest, to the semi-arid of the southwestern and north-central parts of the country (10). To the best of our knowledge, the nutritional composition of these cereal grains has rarely been reported. The present study aimed to evaluate the physicochemical properties, antioxidant activities and phenolic acids profile of Tunisian small cereal varieties in order to bring some information on their nutritional characteristics.

2. Materials and Methods

2.1. Sample collection and preparation

The six varieties of grain cereals, including Maali and Karim (durum wheat), Rihane and Manel (barley) and Ghzella and Meliane (oat), were provided by the Field Crop Lab. of INRA-Tunisia during the crop season 2013-2014. Whole flour cereals were obtained by milling grains on a Cyclotec 1093 Sample mill (FOSS TECATOR) and were kept in a refrigerator for further analysis.

2.2. Physical measurements

The grain samples were analysed for various characteristics related to grain quality. Specific weight (SW), expressed as kg hl^{-1}, was determined as of the AACC method 55-10 AACC. (11). Thousand grain weight (TGW), was determined using an electronic seed counter. Color measurements were executed using a colorimeter (Lovibond PFX-195, UK) and color parameters were presented as L* (lightness), a* (redness), and b* (yellowness) according to the Hunter color scale. All the analyses were conducted in triplicate.

2.3. Chemical analysis

Moisture (44-15) and ash (08-01) were determined on the whole grain samples using the AACC standard methods (11). Ash was obtained after mineralization of the whole grain samples at 550°C for 3 h in a muffle furnace. Ash contents were expressed as a percent of dry matter. The lipid content was measured according to AOAC official method 932.06 (12) with a Soxhlet extractor apparatus with 250 mL of petroleum ether at 60°C for 8 h.

Total nitrogen of whole grain samples was determined by the Kjeldahl method according to the AOAC official method 930.29 (12). Total protein content was calculated from the nitrogen content multiplied by a factor depending on the type of cereal (5.7 for wheat flour, 5.83 for barley and oat whole grains). Total carbohydrate was calculated by difference (100 – a sum of protein, fat, ash and moisture). Mineral elements were analyzed using an Atomic Absorption Spectrophotometer (Hitachi Z-6100, Japan) according to the method described by the AOAC (3). The content of dietary fibers, hemicellulose, cellulose, neutral detergent fibre (NDF) and lignin (ADL) was determined in the whole grain samples by the Van Soest detergent method modified by Mertens (14) using the Fibertec system 2010 (Foods, Sweden). The β-glucans were extracted and purified from barley and oat flours according to the protocol described by Lazaridou *et al*. (15). All the analyses were conducted triplicate.

2.4. Determination of total polyphenols (TPC), flavonoids (TFC) and condensed tannins content (CTC)

TPC was determined by the Folin–Ciocalteu spectrophotometric method (UV–VIS) as described by Dewanto *et al*. (16). TPC was expressed as mg gallic acid equivalent per gram of dry matter (mg EAG/g DW) through the calibration curve with gallic acid. For the determination of TFC, 250 µL of the methanolic extract was combined with 75 µL $NaNO_2$ (5%). TFC levels are expressed in mg quercetin equivalent per gram of dry matter (mg EC/g DW). The protocol followed in the extraction of CTC is that recommended by Sun *et al*.

(17). CTC was expressed in mg of catechin equivalent per gram of extract (mg CE/g DW).

2.5. Anti-radical activity

An amount of 1000 µL of methanol extract was added to 500 µL of DPPH (0.2 mM) (18). After vigorous stirring, the mixture was kept at room temperature for 30 min in the dark and then the absorbance was measured at 517 nm. The anti-radical activity was calculated as described by Zarroug *et al*. (19).

2.6. Identification of phenolic compounds using RP-HPLC

Methanol (40 mL) containing BHT (1 g L^{-1}) were added to 0.5 g of a dried sample. Then 10 mL of 6 M HCL was added. The mixture was stirred carefully and then sonicated for 15 min and refluxed in a water bath at 90°C for 2 h. For the determination of the phenolic compound acids, the obtained extract was injected to a reverse-phase-HPLC system (RP-HPLC, Agilent Technologies 1100 series liquid chromatograph coupled with a UV–Vis multi-wavelength detector, Santa Clara, California, USA) as described by Zarroug *et al* (20).

2.7. Statistical analysis

The results of the physicochemical and phytochemical composition of cereal whole grains were statistically analysed. Analytical values were determined, using three independent determinations. The values of the different parameters were expressed as mean ± standard deviation (x ± SD). Statistical analyses were performed with the STATISTICA software.

The Duncan's test was used to evaluate the significance of differences between mean values at ($p \leq 0.05$)

3. Results

3.1. Physical characteristics

The physical characteristics of durum wheat, barley and oat whole grains are presented in Table 1. On average, durum wheat whole grains had the highest SW and TGW values (81.7 kg hl^{-1} and 55.15 g, respectively) followed by barley (65.6 kg hl^{-1} and 49.95 g, respectively) and oat (51.03 kg hl^{-1} and 36.82g, respectively) whole grains. A significant difference ($p \leq 0.05$) was observed in SW and TGW for all studied species. TGW is a characteristic that provides guidance on the grain size distribution, as well as on the milling value of cereals, particularly about milling yield (21). Having the high TGW in durum wheat genotypes supports the observations of high SW values. Among the tested cereal varieties, the highest SW and TGW values were detected in the durum wheat Maali variety (82.9 kg hl^{-1} and 57.61 g). In barley varieties, Rihane had higher SW and TGW values (64.7 kg hl^{-1} and 49.37g) than Manel (66.5 kg hl^{-1} and 50.54 g). However, in oat varieties, the highest SW and TGW values (55.32 kg hl^{-1} and 39.33 g) were detected in Meliane variety. Concerning whole grain color, the results are shown in Table 1. Among all studied cereals, a significant difference was found between L*, a* and b* values. Results revealed that the highest L* values were found in durum wheat, followed by barley and oat varieties. However, among these cereal grains, the lowest a* and b* values were observed in Ghzella and Rihane varieties.

Table 1. Physicochemical composition of small grain cereals grown in Tunisia.

Species		TGW (g)	S W (kg hl-1)	Moisture (%)	Ash (%)	Lipids (%)	Proteins (%)	Carbohydrates (%)	L*	a*	b*
Durum Wheat	Karim	52.7±0.1e	80.5±0.1 e	11.8±0.5 ab	2.76±1.08 ab	4.8±0.3 c	11.76±0.01 d	68.74±1c	85.3±0.28a	0.22±0.03e	11.33±0.23 d
	Maali	57.61±0.01f	82.9±0.01f	11.73±0.4a	2.04±0.5a	3.2±0.1 b	12.43±0.01f	70.58±0.64 d	85.19±0.65b	0.61±0.19f	10.86±0.1e
Barley	Rihane	49.37±0.01c	64.7±0.1c	12.66±0.11cd	2.52±0.56a	2.7±0.1 a	11.53±0.01c	70.57±0.53 d	82.27±0.55c	0.3±0.01 d	7.91±0.38f
	Manel	50.54±0.01d	66.5±0.1d	12.93±0.32d	2.97±0.55ab	2.5±0.01a	10.43±0,01a	71.33±0.83 d	86.49±0.71d	0.32±0.01a	8.21±0.32c
Oat	Meliane	39.33±0.01b	55.32±0.01 b	11.5 ±0.2a	3.93±0.2b	7±0.1e	12.3±0.1 e	63.66±0.08 a	78.76±0.62e	0.85±0.13b	14.10±0.29 b
	Ghzella	34.32±0.01a	46.75±0.01 a	12.33±0.1bc	3.93±0.2b	6.5±0.1 d	11.33±0.01b	65.90±0.47 b	80.40±0.73f	0.18±0.03c	11.08±0.31a

TGW: Thousand grain weight, SW: specific weight. Values with the same letter within the same column are not significantly different (p ≤ 0.05). Values are means±Standard Deviations (SD) of three determinations.

Table 2. Mineral contents of small grain cereals grown in Tunisia.

	Mineral contents (mg/100 g DM)					
Species	**Durum wheat**		**barley**		**Oat**	
	Karim	**Maali**	**Rihane**	**Manel**	**Melaine**	**Ghzella**
Cr	0,05±0.01ab	0.06±0.01b	0.04±0.1 a	0.07±0.1c	0.65±0.01a	0.25±0.01f
Co	0.06±0.1b	0.03±0.1 a	0.05±0.1b	0.06±0.01b	0.75±0.01c	0.55±0.01c
Cu	0.02±0.01a	0.01±0.05a	0.01±0.1a	0.02±0.1a	0.1±0.01f	0.11±0.01b
Mn	0.26±0.1c	0.24±0.01b	0.13±0.1a	0.14±0.1a	4.2±0.02d	3.95±0.01a
Ni	0.1±0.01	0.05±0.1a	0.1±0.02b	0.15±0.01	0.1±0.03c	0.2±0.01d
Cd	0.05±0.01	0.05±0.01b	0.05±0.03a	0.05±0.03a	0.1±0.02b	0.1±0.02f
Zn	0.1±0.01b	0.24±0.1 d	0 a	0.13±0.1c	1±0.01a	1.8±0.02c
Pb	0.87±0.1 b	0.86±0.01a	1.68±0.01 c	0.85±0.1a	9±0.02b	11±0.03b
Fe	0.36±0.01b	1±1c	5.29±0.01 d	0.25±0.01 a	2.55±0.01a	8.25±0.02a
Na	120±0.03 a	360±0.1d	220±0.1b	260±0.04c	580±0.1a	610±0.03e
K	500±0.01d	320±0.01 a	380±0.01b	410±0.01c	480±0.01 e	380±0.01b
Ca	440±0.2b	390±0.1 a	550±0.38ca	430±0.32b	460±0.01f	450±0.3d

Values with the same letter within the same column are not significantly different (p ≤ 0.05). Values are means±Standard Deviations (SD) of three determinations.

3.2. Chemical composition

The nutritional quality of cereals products from wheat, barley and oat is related to the chemical composition of the cereals grains. Whole durum wheat, barley and oat grains provide many essential nutrients and health-protective components. Table 1 shows the chemical compositions of these studied cereals. In all studied varieties, the highest moisture content was found in the durum wheat Manel variety (12.93%) however the lowest was in oat Meliane (11.5%). A significant difference ($p \leq 0.05$) was observed in moisture content between all species. Comparing the tested cereals, on average, the highest protein content was observed in durum wheat (12.09%) followed by oat (11.81%) and barley (10.98%). Among durum wheat varieties, Maali variety had a slightly higher protein content (12.43%) than Karim (11.76%) while in barley varieties Rihane had the highest one (11.53%).

Concerning carbohydrates contents, on average, whole grain barley had the highest carbohydrates contents (70.95%) followed by whole grain durum wheat (69.66%), while the lowest carbohydrates content was found in whole grain oat (64.78%). Significant differences ($p \leq 0.05$) were observed in carbohydrate content of all studied cereals. For the tested varieties, barley Manel (71.33%), durum wheat Maali (70.58%) and oat Ghzella (65.90%) showed the highest carbohydrate contents. Examining Table 1, oat whole grains had the highest ash content on average, followed by barley and durum wheat (3.93%, 2.97 % and 2.76%, respectively). A significant difference in ash contents was observed for barley and durum wheat whole grains but no significant difference was observed for oat whole grains. The high ash content was detected in Meliane oat variety (3.93%) whereas in durum wheat the range of ash content is around 2.04%. These results confirm the high fiber content in oat and barley species compared to durum wheat species. Significant differences ($p \leq 0.05$) were observed in the lipid content of all studied cereals. On average, barley and durum wheat whole grains contained relatively lower lipid content (2.6% and 2.4%, respectively) compared to oat whole grains (6.75%). Among all studied cereals, the highest lipid content in oat, barley and durum wheat whole grains was observed in Meliane (7%), Rihane (2.7%) and Karim (4.8%) varieties.

3.3. Mineral contents

Minerals are divided into macro- and micro-elements based on their concentration in foods (22). The macro-elements include calcium, phosphorus, potassium, magnesium and sodium. The rest are iron, manganese, zinc, selenium and cobalt which are the nutritionally important micro-elements in the barley kernel (23). Table 2 shows that the most abundant mineral in whole cereal grains was calcium with values ranging from 390 mg/100 g DM (in durum wheat Maali variety) to 550 mg/100 g DM (in barley Rihane variety). Results revealed that, in average, the oat whole grain had the highest levels of potassium and sodium compared to others varieties.

3.4. Dietary fiber content

Whole grain cereals are excellent sources of insoluble dietary fiber. The content of cellulose, hemicellulose, NDF and lignin in barley, durum wheat and oat whole grain varieties are presented in Table 3. On average, results reveal that hemicellulose was the mainly abundant dietary fibres in all studied cereal species. The highest hemicellulose content was observed in durum wheat (59.59%) followed by oat (52.68%) and barley (39.42%) whole grains. In durum wheat, the highest hemicellulose content was found in Karim (60.4%) followed by Maali (58.76%) varieties. However, the highest NDF content, on average, was observed in oat (67.39%) followed by durum wheat (62.86%) and barley (47.67%) whole grains.

Concerning lignin content, in average, durum wheat showed the lowest content (2.54%) followed by barley (6.37%) and oat (12.89%) species. The β-glucan contents of different Tunisian barley and oat varieties are given in Table 3. A statistically significant difference ($p \leq 0.05$) was found between the different barley and oat varieties. The β-glucan contents ranged between 4.99% (Manel variety) and 5.9% (Meliane variety). The obtained results revealed that, on average, the oat varieties (11.1%) have higher β-glucan contents compared to the barley varieties (10.39%).

3.5. Phytochemical composition of cereal grains

Phenolic compounds are naturally concentrated in the outer layers of the cereal kernel. In general, Folin–Ciocalteu is used principally as an antioxidant method to determine total phenolics content.

For that reason, whole grains were used in experiments. TPC, TFC and CTC of different cereal grains extracts are summarized in Fig 1 (A). In most cases, significant differences ($p \leq 0.05$) in TPC and TFC values were obtained from the studied cereal samples. However, results revealed no significant difference in the CTC values between oat and durum wheat. The highest contents of TPC, TFC and CTC were observed in barley and oat whole grains varieties with values of 212.91 mg GAE/100 g DM and 110.92 mg GAE/100 g DM, 108.46 mg CE/100 g DM and 65.6 mg CE/100 g DM, 72.66 mg CE/100 g DM and 33.49 mg CE/100 g DM, respectively. Among the studied species, the highest TPC was found in Maali (87.16 mg GAE/100 g DM), followed by Ghzella (118.16 mg GAE/100 g DM) and Rihane (231.5 mg GAE/100 g DM). The obtained values are in accordance with previous studies (43). Meliane, Maali and Rihane have the highest TFC compared to other studied cereal varieties. The Rihane variety showed the highest amount of CTC (76.83 mg CE/100 g DM), followed by Maali (37.76 mg CE/100 g DM) and Meliane (36 mg CE/100 g DM).

Table 3. Fibers composition of whole small grain cereals grown in Tunisia

Species		β- glucans (%)	NDF (%)	Falling number (s)	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Durum Wheats	**Karim**	n.d	64.56±8.8 c	418±1	2.93±0.92a	60.4±8.41b	1.22±0.83a
	Maali	n.d	61.17±11.54 bc	327±1	2.14±0.21a	58.76±11.4b	0.36±0.07a
Barley	**Rihane**	5.4±0.02a	49.36±3.4ab	444.66±0.01	6.79±0.25b	40.94±4.3a	1.62±0.9a
	Manel	4.99±0.01c	45.9±10.3a	445±1	5.94±0.65b	37.9±9.71a	2.13±1.44a
Oat	**Meliane**	5.9±0.03d	64.41 ±1.9c	65.33±0.01	14.41±0.61 d	48.73±1.95ab	1.26±0.95a
	Ghzella	5.2±0.01b	70.36±0.6c	60.12±0.01	11.37±1.24 c	56.63±1.1b	2.35±1.6a

Values with the same letter within the same column are not significantly different ($p \leq 0.05$). Values are means±Standard Deviations (SD) of three determinations. NDF: neutral detergent fiber. n.d: not determined.

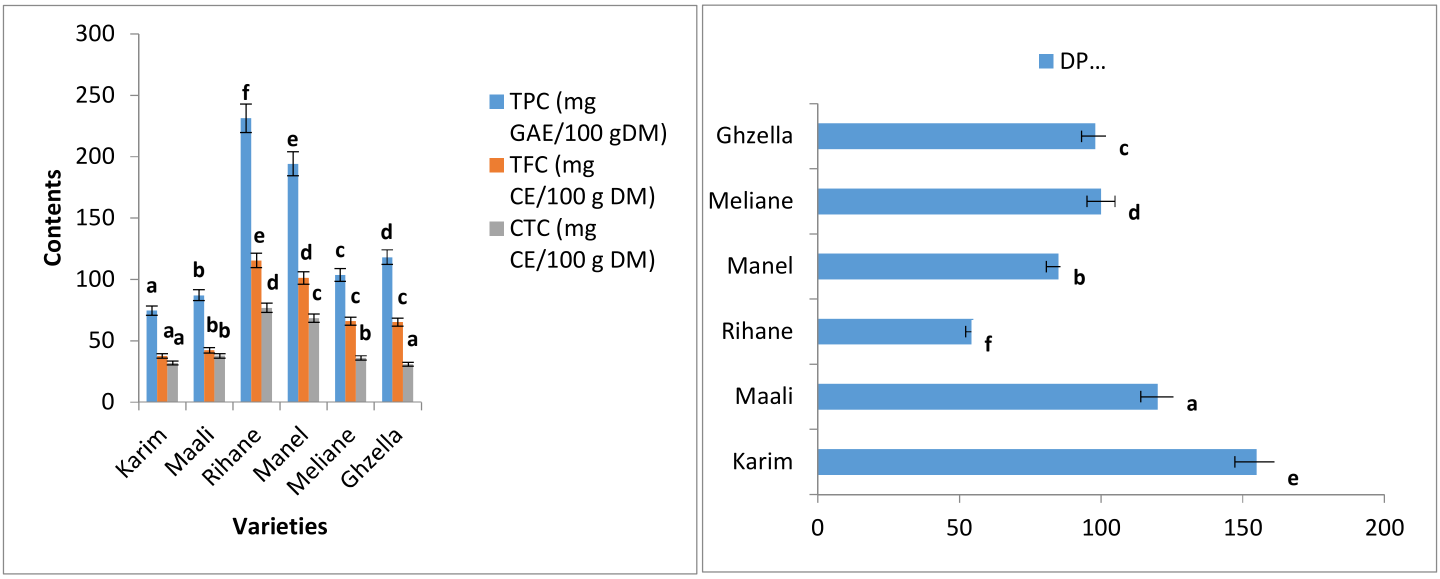


Figure 1. (A) TPC. TFC and CTC of different grain extracts, and (B) antioxidant activities of cereal grains. TPC: total polyphenols content (mg GAE/100 g DM); TFC: total flavonoids content (mg CE/100 g DM); CTC: condensed tannins content (mg CE/100 g DM); DPPH (IC_{50}. µg/mL); GAE:gallic acid equivalents; CE: catechin equivalents.

3.6. Antioxidant activity

In the present study, the antioxidant capacity of different cereal grain extracts was determined using the DPPH method, in which the antiradical activity was evaluated by the capacity of the antioxidant compound to reduce the DPPH radical. The antioxidant activity is a very important parameter to evaluate the health benefits of whole grain cereals consumption. Results are presented in Fig. 1 (B) and significant differences ($p \le 0.05$) were obtained between the different varieties. Rihane variety showed the best radical scavenging activity with IC_{50} of 55 µg /mL and was two times stronger than Meliane oat variety (100 µg /mL). Also, barley Rihane has a good antioxidant activity compared to durum wheat Karim variety (155 µg /mL).

3.7. RP-HPLC analysis

The phenolic acid composition in small grain cereals was determined using RP-HPLC method (Table 4). The obtained chromatograms for Maali, Rihane and Meliane varieties are presented in Fig. 2. In examining the results, significant differences ($p \le 0.05$) were observed among varieties within each species. Results showed that the highest content, in average, of the total phenolic compound was found in barley whole grain (1.43 mg/g DM) followed by oat (1.18 mg/g DM) and durum wheat (0.62 mg/g DM). In each variety, the order of total phenolic compound based on RP-HPLC method was: Manel<Rihane for barley whole grain, Ghzella<Meliane for oat whole grain and Karim<Maali for durum wheat whole grain. This order is similar to that obtained for TPC analysis.

In the present study, sinapic acid (0.42 mg/g DM) was reported as the most dominant phenolic acid in barley whole grain varieties followed by chlorogenic acid (0.3 mg/g DM), protocatechuic acid (0.25 mg/g DM), ferulic acid (0.24 mg/g DM), 3.4-dihydroxybenzoic acid (0.21 mg/g DM), gentisic acid (0.19 mg/g DM), gallic acid (0.16 mg/g DM) and caffeic acid (0.12 mg/g DM). Whereas, vanillic, p-coumaric and syringic acids were found in moderate levels.

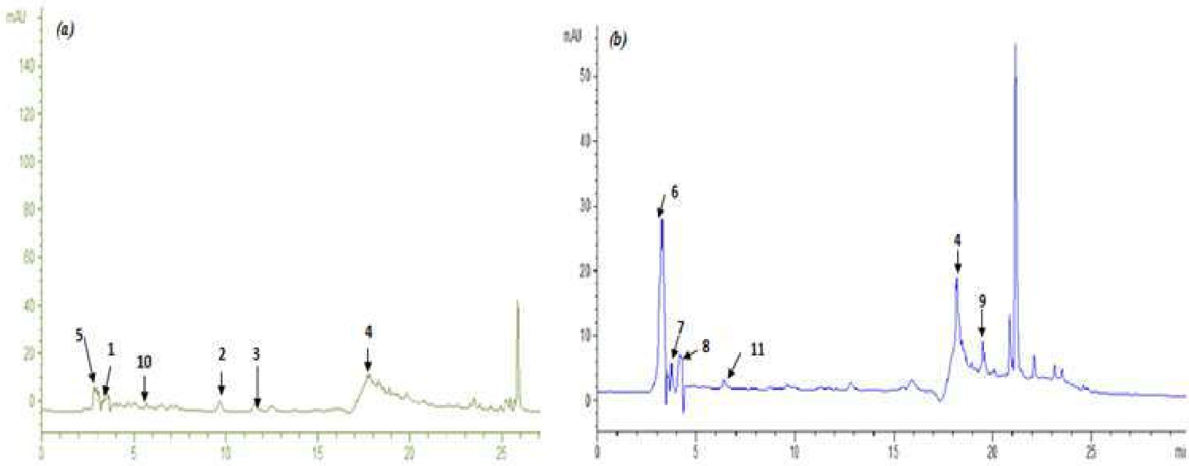
In our studied oat varieties, gallic acid (0.31 mg/g DM) was reported as the most dominant phenolic acid followed by ferulic (0.26 mg/g DM), gentisic (0.23 mg/g DM), caffeic (0.21 mg/g DM), chlorogenic (0.14 mg/g DM) and syringic acids (0.12 mg/g DM).

Concerning durum wheat grains, sinapic acid was the major phenolic acid (0.18 mg/g DM) followed by (+)-catechin hydrated acid (0.11 mg/g DM) and gallic acid (0.1 mg/g DM).

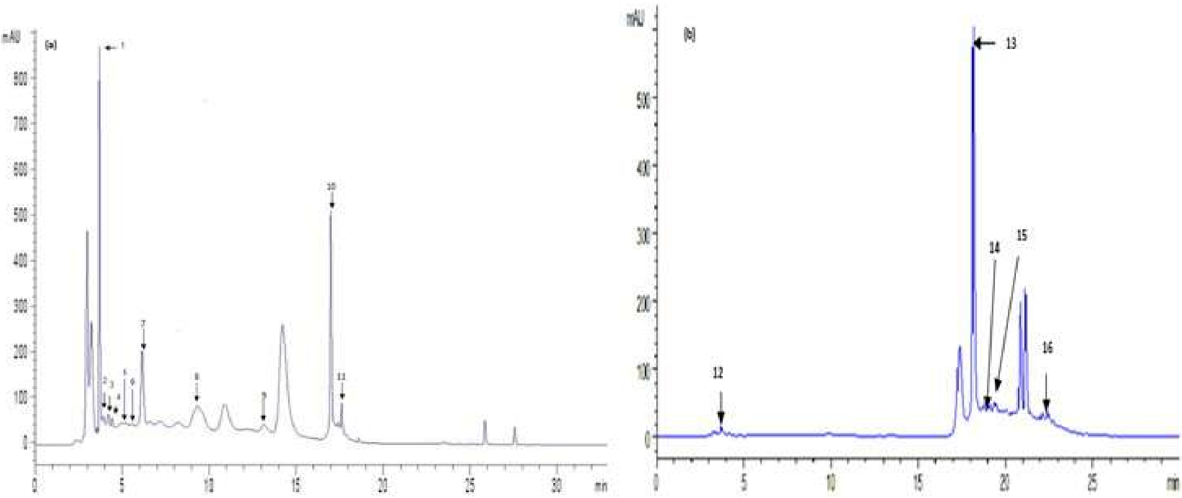
Table 4. Phenolic acid contents (mg /g DM) of grain cereals grown in Tunisia.

	Phenolic compounds	Content (mg/g DM)					
		Durum wheat		*Barley*		*Oat*	
		Maali	Karim	Rihane	Manel	Meliane	Ghzella
1/	Gallic acid	0.1±0.001d	0.07±0.001c	0.04±0.001b	0.16±0.001e	0.31±0.001f	0.01±0.001a
2/	Protocatechuic acid	0.03±0.001 b	0.08±0.01 c	0.25±0.001 d	n.d	0.03±0.01 b	0.09±0.001 c
3/	3.4-dihydroxybenzoic acid	0.01±0.001 b	0.003±0.001 a	0.21±0.01 e	0.24±0.001 f	0.08±0.02 d	0.05±0.001 c
4/	Gentisic acid	n.d	n.d	0.19±0.001 d	0.18±0.001 c	0.02±0.001 b	0.23±0.01 e
5/	Resorcinol	n.d	n.d	n.d	n.d	n.d	n.d
6/	Chlorogenic acid	0.01±0.001 a	0.08±0.001 d	0.30±0.001 f	0.01±0.001 b	0.14±0.001 e	0.07±0.001 c
7/	Syringic acid	0.07±0.001 e	0.06±0.001 d	0.05±0.001 c	n.d	0.12±0.001 f	0.03±0.001 b
8/	p-Coumaric acid	0.05±0.001 e	n.d	0.004±0.0001 b	0.01±0.001 c	0.036±0.001 d	0.09±0.001 f
9/	Naringin	n.d	n.d	n.d	n.d	n.d	n.d
10/	Quercetin	0.02±0.001 b	0.002±0.0001 a	0.05±0.001 d	0.04±0.001 c	0.04±0.001 e	0.02±0.001 b
11/	Apigenin	n.d	n.d	n.d	n.d	0.04±0.01 c	0.020±0.01 b
12/	Sinapic acid	0.18±0.001 e	0.11±0.001 d	0.42±0.001 f	0.06±0.001 c	0.02±0.001 a	0.04±0.001 b
13/	(+)-Catechin hydrated	0.11±0.03 d	0.10±0.001 c	0.03±0.001 b	n.d	n.d	n.d
14/	Caffeic acid	0.08±0.01 d	0.02±0.001 a	0.04±0.001 c	0.12±0.001 e	0.03±0.001 b	0.21±0.001 f
15/	Vanillic acid	n.d	n.d	0.02±0.001 c	0.01±0.001 b	0.04±0.001 d	0.07±0.001 e
16/	Epicatechin	n.d	n.d	n.d	n.d	n.d	n.d
17/	Ferulic acid	n.d	n.d	0.24±0.01 d	0.12 c	0.26±0.001 e	0.09±0.001 b
18/	Luteolin	n.d	n.d	0.05±0.001 e	0.04±0.001 d	0.026±0.001 b	0.035±0.001 c
19/	Trans-cinnamic acid	0.01±0.001 c	0.04±0.001 d	0.01±0.001 b	0.008±0.001 a	0.007±0.001 a	0.01±0.001 b
20/	Kaempferol	n.d	n.d	n.d	n.d	0.008±0.001 b	0.023±0.001 c
	TPAC	0.62±0.001 a	0.61±0.001 a	1..96±0.001 e	0.91±0.001 b	1.20±0.001 d	1.16±0.001 c
	Mean TPAC	0.62±0.002 a		1.43±0.44 c		1.18±0.22 b	

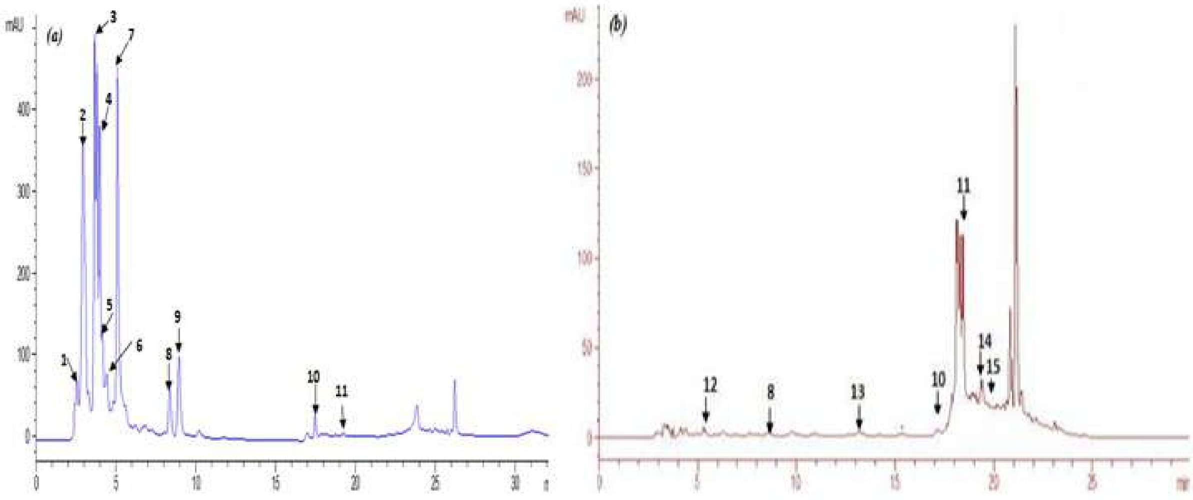
Values with the same letter within the same column are not significantly different (p ≤ 0.05). Values are means±Standard Deviations (SD) of three determinations.



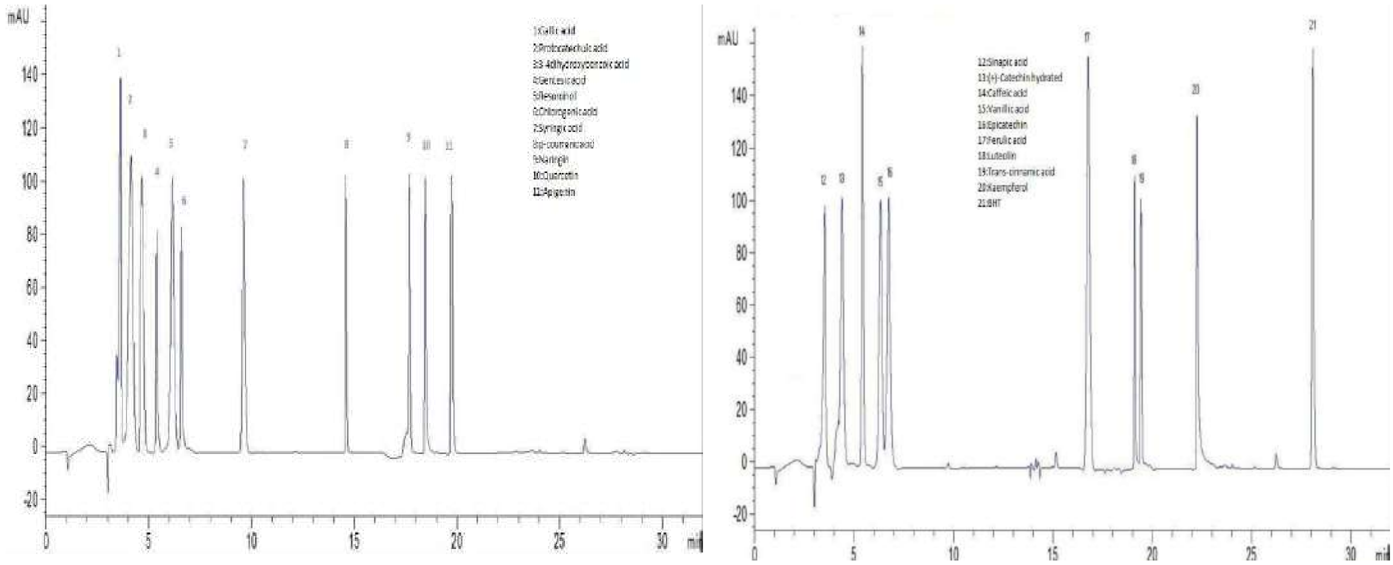
Durum Wheat



Oat



Barley



(A) (B)

Figure 2. Phenolic compounds in durum wheat "Maali" (2), barley "Rihane" (3) and oat "Meliane" (4) varieties analyzed by RP-HPLC at 280 nm (a) and 330 nm (b). (a) and (b): RP-HPLC chromatograms of phenolic acid standards.

1: Gallic acid; 2: Protocatechuic acid; 3: 3,4-dihydroxybenzoic acid ; 4: Gentisic acid; 6: Chlorogenic acid; 7: Syringic acid; 8: p-caumaric acid; 10: Quercetin; 11: Apigenin; 12 : Sinapic acid; 13: (+)-Catechin hydrated; 14: Caffeic acid; 15: Vanillic acid; 17 : Feurelic acid ; 18: Luteolin; 19: Trans cinamic acid; 20: Kaempferol.

4.Discussion

According to the results, the TGW values of Tunisian durum wheat varieties are higher than those of spring durum cultivars from Turkey (values ranging fom 42 to 50 g) (24). However, SW values are comparable with those of Indian durum wheat varieties (83.3 kg/hl) (25). Based on these results, it can be predicted that all studied durum varieties have a good semolina yield after milling.

Tunisian durum wheat genotypes have a TGW similar to those reported for the American durum wheat which showed a TGW of 47.3 g and 52.5 g (26).

Variations in physical properties between all cereal varieties could be attributed both to their genotypes and agronomic factors. These results provide evidence that all studied cereal grains were sound, unbroken and appropriate for high milling yields. Since the ratio of endosperm to bran is greater in larger kernels, a higher milling yield can be expected from these kernels.

In this study, the protein contents of Tunisian barley varieties are not similar to those found for Brazilian (12.55% to 15.92% DM) (27) hull-less barley. The observed variation in protein content of all studied cereals was due to the genotype variations (cereal, species, variety) and the growing conditions (soil, climate, fertilization).

For the carbohydrate contents, our results are lower than those reported for Canadian barley (77.90 %) and oat (83.1%) varieties (28). However, carbohydrate contents of Tunisian durum wheat varieties are higher than those reported by Koehler and Wieser (29) (59.4%).

Ash contents of the studied Tunisian cereal species were lower than those found in Italian durum wheat (30) and Australian barley (31) varieties, respectively.

Sterna *et al*. (32) showed that the wild oat species are rich in lipids (max 12.40%) in contrast to wheat species (1.7%) (29). Results found for lipid content in barley are lower than those stated by Moreau *et al*. (33). The high of lipid content in oat must be taken into consideration during grain conservation.

The obtained mineral results are similar to those recorded by Jeantet *et al*. (34) who reported that calcium, potassium and sodium values are ranging from 0.35% to 0.7%. The presence of potassium and calcium minerals in these cereal grains improves the beneficial role in maintaining the electrolyte balance of body fluids. In addition, whole cereal grains could provide reasonable amounts of minerals needed for adequate human nutrition.

Concerning fibers, Ragaee *et al*. (35) reported that whole grain cereals contain a higher content of fiber compared to wheat flour and would enhance dietary fiber intake. Barley and oat are preferred not only for their nutritional importance but also for its nutraceuticals properties. Several studies are interested in the active component in barley and oat having nutraceutical properties which is the soluble dietary fiber β-Glucan (36).

β-glucan is found predominantly in the cell walls of the endosperm and aleurone layer of oat and barley grains and in smaller amounts in rye and wheat (37). It has been reported that one of the main health effects of cereal β-glucan, shared with other soluble dietary fibres, is its ability to lower serum cholesterol, leading to a decreased risk of cardiovascular diseases (22).

The obtained results of β-glucans are in accordance with a previous study on native oat and barley extracts (38). Moreover, our values are similar to those reported by Lahouar *et al*. (22) and Zarroug *et al*. (39) on the Tunisian barley varieties. However, Messia *et al*. (40) reported lower β-glucan contents (values ranging from 1.5% to 3.5%) for the Italian barley genotypes.

Concerning phytochemicals, Gordana et *al.* (41) revealed that the TPC in barley and oat whole grains was higher than that found in wheat and rye grains. Chen *et al*. (42) found lower TPC in whole oat varieties as compared to Tunisian oat grain. These variations in phenolic compounds are due to several factors such as genetic differences, analytical methods, maturity stage, varieties used and environmental conditions.

Phenolic compounds are the most bioactive compounds that promote cellular protection and play an important role against oxidative damage in plants. The presence of these phenolic compounds in the studied cereal grains promotes their use in food products as a natural source of antioxidants that have potential health benefits. These results are important for Tunisian durum wheat and oat varieties since small information have been undertaken to study their phytochemical and antioxidant compositions.

Our findings are in agreement with those found for the total phenolic and flavonoid contents of each cereal grain extracts. The difference in IC_{50} values is strongly related to the amount and the type of phenolic compounds present in each cereal grain variety. According to Ragaee *et al*. (35), Lahouar *et al.* (43) and Zarroug *et al*. (39), the studied cereal grain varieties are a rich source of natural antioxidant compounds that can serve for food and nutraceutical formulations.

In barley genotypes, many studies have shown the presence of ferulic acid, hydroxybenzoic acid, vanillic acid, sinapic acid and syringic acid (44). It was reported that ferulic acid was the most abundant phenolic acid in barley flour (6, 44). The ferulic acid contents of the Spanish and the Czech barley varieties (ranged from 0.81 to 1.20 mg/g and 0.68 to 1.37 mg/g, respectively) (45) were higher than those found in our Tunisian barley varieties (0.24 and 0.12 mg/g).

The contents of ferulic and caffeic acids, which are hydroxycinnamic acids, were much higher than those previously studied in Chinese oat varieties (42). In fact, having a high content of hydroxycinnamic acids may be a special characteristic in Tunisian oats.

In this study, ferulic acid was not found in durum wheat varieties which is not consistent with the results reported by Călinoiu and Vodnar. (46) who showed that ferulic acid is the predominant phenolic acid found in whole wheat grains.

The descending order of total phenolic acid content (TPAC): barley > oat > wheat was reported by Zielinski and Kozlowska. (41) was partly confirmed by our data.

5. Conclusions

Extensive studies of the physicochemical properties, antioxidant activities and phenolic acids profile of Tunisian durum wheat, barley and oat whole grains have not been reported previously.

This study has revealed that the six studied varieties contained appreciable amounts of fat, protein, fiber, mineral and carbohydrates. These good nutrients have a beneficial effect on human health. Results showed also that barley Rihane and oat Meliane have significant β-glucan contents compared to other Tunisian barley and oat varieties. In addition, barley, oat and durum wheat whole grain extracts demonstrated high phenolic content and potential antioxidant activity determined by DPPH method. Barley Rihane, oat Meliane and durum wheat Maali varieties have the highest antioxidant activity and the highest total phenolic, total flavonoid and condensed tannin contents. Moreover, the highest content of total phenolic compound was found in barley, followed by oat and durum wheat whole grains. The genetic diversity observed for all tested cereal grains on physicochemical composition, antioxidant activity and phenolic acids suggest that it's possible to select breeding for lines with high nutritional qualities, in order to improve diet requirements for the consumer's health. Indeed, the incorporation of whole grain into bakery products would enhance their nutritional qualities, but their functionality and acceptability will be negatively affected.

Conflict of ineterst

The authors have no conflict of interest.

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