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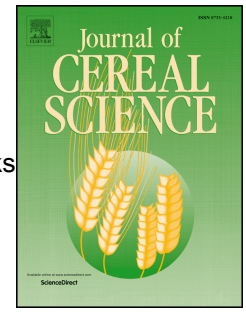

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Identification and Characterization of Phenolic Compounds Extracted from Barley Husks by LC-MS and Antioxidant Activity in Vitro

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Abstract

Phenolic compounds were extracted from Tunisian barley husks obtained through a pearling process, by using two different extraction solvents: acid treatment with sulfuric acid and alkaline delignification with sodium hydroxide. Their antioxidant properties in vitro were investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test and butylated hydroxyanisole (BHA). Antioxidants composition was evaluated with LC-MS analysis. Findings suggest that the best yields of crude extracts with high level of phenolic compounds exhibiting strong antioxidant activities were found after pre-hydrolysis and delignification step of barley husks. The lowest average total phenolic content found was 763.665 mg /100g, presenting an EC50 value of 0.93 g/L, four higher antioxidant levels than BHA (0.24 g/L). All extracted fractions showed high contents of p-coumaric acid (≥ 491.189 mg/100g), trans-ferulic acid (≥ 501.475 mg/100g) and syringic acid (≥ 192.228 mg/100g). These results contribute to enhancing the value of barley husks as a good source of natural antioxidants, which serve as new functional food ingredients and dietary supplements.

Keywords: Barley husks, pearling process, phenolic compounds, antioxidant activity in vitro

1. Introduction

Nowadays, phenolic compounds have been the issue of food and medical scientists for their remarkable antioxidant activities either in vitro or in vivo, because of their ability to scavenge free radicals and metals and to prevent radical chain reactions (Lahouar et al., 2014; Do et al., 2015; Shen et al., 2016; Gangopadhyay el al., 2016). Specifically, phenolic compounds are known as excellent dietary substances with positive antioxidant and antiradical activities. They have anti-proliferative and anti-diabetic effects (Lee et al., 2016; Idehen et al., 2017). Antioxidants are molecules at low concentration can prevent oxidation, prolong food storage and promote health by reducing risk of developing chronic diseases such as cardiovascular disease, diabetes, also cancers and oxidative stress (Lahouar et al., 2014; Zhu et al., 2015; Marecek et al., 2017). Accepted natural antioxidants include vitamin E, ascorbic acid, enzymes (catalase, glutathione peroxidase and superoxide dismutase), various phytochemicals such as phenolic acids, flavonoids, anthocyanins, etc (Barbosa-Pereira et al., 2013; Shen et al., 2016; Baba et al., 2016). These compounds are used as supplement or functional ingredients to conserve foods, for medical intentions and in cosmetics to substitute the most widely used synthetic antioxidants in food industry such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutylhydroquinone (TBHQ), in order to meet consumer preferences and health interests, for their safety issue (Barbosa-Pereira et al., 2013; Lee et al., 2016). Barley phenolic compounds exist in so-called free, soluble conjugated and insoluble bound forms, which are linked by ester or ether linkages to the cell wall materials of the grain and require acid, alkaline or enzymatic hydrolysis for their release (Gangopadhyay el al., 2015; Zhu et al., 2015; Idehen et al., 2017). In contrast, free polyphenols can be extracted by using organic solvents (methanol, ethanol, acetone, etc). The major free phenolic compounds in barley are flavanols that are habitually found in their monomeric form as catechin and

74 epicatechin, or in their polymeric chain as proanthocyanidins (Gangopadhyay el al., 2016).
75 Higher concentrations of these compounds are found in the outer layers of the kernels
76 constituting the bran. Indeed, strong antioxidant capacity has been observed in the outer layers
77 of the grain (Lahouar et al., 2014; Do et al., 2015). Thus, the pearling process which removes
78 these layers (the hull, aleurone) in covered barley significantly reduces the antioxidant
79 capacity of the whole grains (Baik and Ullrich, 2008; Blandino et al., 2015).
80 Although, over 85 % of barley production is used for animal feeds and malt production;
81 while, husks represent by-products without any useful purposes, accounting up over 15 % of
82 the grain dry weight (Lahouar et al., 2014); phenolic compounds in whole barley and
83 obviously in the husk have not received enough attention as well as phytochemicals in fruits
84 and vegetables used by industries. Research has not published on the antioxidant capacity and
85 phenolic compounds content in barley husks at the usual pearling process. Few studies (Cruz
86 et al., 2007; Garrote et al., 2008; Pereira de Abreu et al., 2012) focused on the antioxidants of
87 barley husk extracts provided from the brewing industrial wastes. In addition, the use of
88 barley husk as a feed supplement rich in carbohydrates is hindered by its low digestibility for
89 polygastric livestock. Furthermore, their high ash level makes their combustion so difficult.
90 Nevertheless, natural extracts of phenolic compounds that have remarkable antioxidant
91 properties can be recovered after pre-hydrolysis and delignification of barley husk (Cruz et
92 al., 2007; Pereira de Abreu et al., 2011). Even though, some previous researches have given
93 meaningful insights into various bioactive compounds found in barley extracts, the
94 qualification of the individual phenolic compounds that contribute to the strong antioxidant
95 activity of barley husk are still unknown. However, there's few information available about
96 the antioxidants variation in barley husks. The food processing is also keen on growing the
97 use of these new cereal ingredients in novel food products and therefore more research is
98 merited in this area. For this reason, the main objective of this work was to identify and

characterize the phenolic compounds extracted from Tunisian barley husks by using LC-MS analysis in order to develop eventual new functional ingredients and dietary supplements for use in novel food formulations.

2. Material and methods

2.1. Raw material

Plant materials used in this study were 7 six-rowed Tunisian cultivars of covered barley. Four registered official varieties (Manel, Rihane, Konouz, Lemsi) were obtained from the Experimental Research Station of the National Institute for Agricultural Research of Tunisia (INRAT), Field Crop Laboratory, located at Beja, 100 Km North-West of Tunisia. Three populations of the cultivar "Ardhaoui", grown in different areas in the South of Tunisia, were provided by the Institute of Arid Areas, Aridlands and Oases Cropping Laboratory. All the cultivars were grown from December 2013 to June 2014. After harvesting, the grains of each cultivar were cleaned and kept at 4°C for evaluation.

2.2. Chemicals

The reagents used for phenolic compounds extraction and antioxidant assays were: Sulfuric acid (H_2SO_4), Sodium Hydroxide (NaOH), ethyl acetate, methanol for HPLC, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), BHA, Folin–Ciocalteu reagent, gallic acid, sodium phosphate buffer, sodium carbonate, aluminum chloride, acid chloride, vanillin, acetonitrile, formic acid, p-coumaric acid, ferulic acid, gallic acid, quercetin and catechin. They were purchased from

Sigma–Aldrich, Inc (Sigma Chemical, Co, St-Louis, MO, USA). All other chemicals and solvents were of analytical grade.

2.3. Sample preparation

Barley grains were initially pearled to remove 20% (w/w) of the original grain weight in an abrasive-type grain testing mill (TM-05C model, Satake, Tokyo, Japan), corresponding to the external layers as described in Blandino et al., (2015). The residual 80% (w/w) of the kernels were collected; husks were stored in a dry and dark place at room temperature until utilization. The moisture content of the samples was less than 10% for all cultivars.

2.4. *Extraction of phenolic compounds*

The methods used for phenolic compounds extraction from barley husks were previously described by Cruz et al. (2007), Garrote et al. (2008) and Pereira de Abreu et al. (2012) with some minor modifications. Briefly, in a first step, samples of barley husks were subjected to acid hydrolysis with a solution of 3% H_2SO_4 for 15 min at 130 °C, at a liquid/solid ratio of 8:1 g/g to dissolve the hemicelluloses. The solid residues from treatments were separated by vacuum filtration, well washed with distilled water, air dried and then delignified with a 6.5% solution of NaOH for 60 min at 130 °C, at a liquid/solid ratio of 10:1 g/g to solubilize the lignin content, as a second step. The protocols and procedures followed for the extraction of natural antioxidants from barley husks are described in Figure 1.

Phenolic compounds were extracted from the liquid phases obtained from acid hydrolysis (noted A) and from delignification process (noted B) with ethyl acetate at a hydrolysate: ethyl acetate volume ratio (water phase/organic phase) 1:3 (v/v), 1h, 25ºC, 190 rpm in a single extraction stage. Ethyl acetate was removed by vacuum evaporation to obtain the dry material

(extracts). Extracts were re-dissolved in 10 mL methanol for HPLC that will be used in fractionation experiments after extraction yield calculation. All the crude extracts were freeze dried (lyophilized) at – 20°C until their antioxidant activity and LC–MS analysis.

2.5. Antioxidant activity measurement

The antioxidant activity (AA) of the extract solutions was determined using the DPPH radical scavenging test according to the method as described by Von Gadow et al. (1997) with some minor modifications. Exactly, 50 μL of a methanolic solution of the extract were added to 2 milliliters of a 6×10^{-5} mol / L methanolic solution of DPPH, and mixed vigorously on a vortex mixer. The decreases in DPPH absorbance were registered in a UV-VIS Spectrophotometer (Jasco–V–650, Japan) at 515 nm during 16 min. The inhibition percentage (IP) of the DPPH radical was calculated by using the formula:

$$\mathbf{IP = (A0 - A16) / A0}$$

Where A0 is the absorbance of the extract at 0 min and A16 is the absorbance at 16 min.

All measurements were done in triplicate and the mean values are recorded. BHA was used as reference antioxidant. The AA of the barley husk extracts was determined as the equivalent concentration of the antioxidant causing a 50% inhibition of the initial DPPH radical, as EC50. The EC50 value is expressed as g/L and allows comparison of the AA of all samples analyzed within the same conditions. The parameter EC50 was calculated from the IP data as the amount of ethyl acetate soluble extracts, dissolved in methanol required to inhibit 50% of the hydroxyl radical formation (Cruz et al., 2007; Lee et al., 2016; Baba et al., 2016).

2.6. LC-MS analysis

The LC–MS – 2020 Liquid Chromatography – Mass Spectrometry UFLC * R system (Shimadzu – Japan) comprised a Thermo Accela liquid chromatography coupled to a TSQ Quantum access MAX mass detector controlled by Xcalibur software. Chromatographic separation was performed with an AQUASIL C18-HL column (150 mm × 3 mm, 3 µm particle size) at 60 °C, all from Thermo Fisher Scientific Inc. (Supelco, USA). An aliquot (10 µL) was injected into the column and eluted at 60 °C with a constant flow rate of 0.5 mL/min at the following gradient conditions for the mobile phase composed by acetonitrile /0.25 % formic acid (F) and water (W): F:W (10:90) for 5 min, changed to F:W (50:50) for 30 min and held for 5 min, changed to F: W (10:90) for 5 min. MS/MS detector settings: negative electro-spray ionization mode, spray voltage: 2500 V, vaporizer temperature: 250 °C, sheath gas pressure: 12L N2/min, argon gas pressure: 25 psi, probable temperature: 400 °C. Antioxidants were identified via LC-MS system analysis by comparison with standard phytochemicals. The limit of detection (LOD) and the limit of quantification (LOQ) were 5 µg/kg and 16 µg/kg, respectively.

2.7. Statistical analyses

All analyses were carried out in triplicate with the exception of the extraction yield and concentrations of crude extracts and LC-MS quantification, performed as one replicate. The results are reported as the mean of the three replicates. The entire variations coefficients were less than 10. The data were reported as means ± standard error. Statistical analysis was carried out using SAS (V.9.1). Proc ANOVA (Analysis of Variance) with the option of $LSD_{0.05}$ to compare means was used for each trait. Statistical significance was set at $p < 0.05$.

3. Results and discussion

197 *3.1. Extraction yield and antioxidant activity of crude extracts*

198

199 The extraction yields and antioxidant activity (AA) measurements of crude extracts obtained
200 after acid treatment (pre-hydrolysis) and alkaline extraction (delignification) of barley husks
201 were reported in table 1. The fractionation process used allowed to obtain fractions with
202 different concentrations in raw antioxidants. Crude extracts showed high level of phenolic
203 compounds. In fact, the percentages varied from 1.16% in acid hydrolysis for Ardhaoui
204 Medenine cultivar to 5.16% in alkaline hydrolysis for Manel variety. Results indicated that
205 the best yields of natural extracts were obtained after pre-hydrolysis and delignification of the
206 solid residues (3.09% – 5.16%). It demonstrated that the extraction solvent properties
207 significantly affected the total phenolic compounds (TPC) of barley husk extracts (Cruz et al.,
208 2007; Garrote et al., 2008; Pereira de-Abreu et al., 2012). Phenolic compounds which are
209 susceptible to have strong antioxidant capacity and to be used as food preservatives and
210 natural antioxidants, were extracted and recovered with ethyl acetate from the liquids obtained
211 after the alkaline hydrolysis. These results are comparable to those of Cruz et al. (2004) and
212 Gonzalez et al. (2004).

213 Konouz variety had the highest extraction yield after acid hydrolysis (1.77%, sample 5A) and
214 Manel variety had the highest extraction yield after the basic hydrolysis (5.16%, sample 4B).
215 The raw phenolic compounds accounted for 1.16 – 5.6 g/100 g oven-dry barley husk. This
216 yield is comparable to other conventional aqueous or organic solvent extraction yield
217 processed from different materials (Garrote et al., 2008). The yields obtained revealed a great
218 variation in the raw antioxidants level between the different barley husk samples analyzed.
219 These differences can be explained by genetic make-up and environmental conditions
220 (Barbosa-Pereira et al., 2013; Lahouar et al., 2014). Thus, the greater variability noted in this
221 study may be important for the optimum utilization of these barley husks for production of

natural antioxidants which could be used for development of functional foods and industrial uses (Cruz et al., 2007; Pereira de Abreu et al., 2012).

Scavenging of DPPH radicals is a widely used model to evaluate the free radical scavenging activity of mixed and pure antioxidants level in crops, fruits, vegetables and natural plants (Lee et al., 2016). Among the radical scavenging assays, the utilization of DPPH was chosen for its simplicity and worldwide acceptance for comparative purposes. It is very popular and frequently used in the food processing owing to its cost effectiveness, easy control and direct free radicals inhibition (Cruz et al., 2007; Pereira de Abreu et al., 2012). The parameter EC50 values of the crude extracts were reported in table 1. All the raw extracts had higher DPPH radical scavenging activities as compared to the BHA. As presented in Table 1, antioxidant properties through DPPH assays showed significant differences ($p < 0.001$) in each cultivar fractions compound, and their positive controls exhibited high effects with the EC50 values. Furthermore, it is noted that the scavenging activity of barley husk extracts was two higher antioxidant levels than BHA, commonly used in food industry ($p < 0.001$). All the phenolic compounds extracts present an EC50 values showing more than twice higher AA than BHA in terms of EC50. The parameter EC50 values of the crude extracts ranged from 0.43g/L to 1.46 g/L; in contrast, the EC50 of BHA was 0.24 g/L. Ardhaoui Tataouine cultivar, treatment A (2A), showed the most potent antioxidant compound with an EC50 value of 1.46g/L ± 0.47, and Lemsi forage variety, treatment B (7B) exhibited the second highest scavenging activity (EC50 = 1.45g/L ± 0.3). In addition, fractions 3A, 4A, 4B, 5A, 5B, 6A, 6B and 7A also revealed significant degrees of AA (EC50 more than 4 times the BHA). These crude extracts displayed high radical scavenging abilities, which may be attributed to their high natural content in phytochemicals; since such activity of the sample is greatly influenced by the phenolic composition (Cruz et al., 2007; Barbosa-Pereira et al., 2013). The contents of other antioxidants in the samples may also be responsible for the major contribution to antioxidant

capacities against the DPPH radical. In other words, significant differences of crude phenolic compounds at different environments showed deeply variations in the scavenging activities on DPPH radical. Consequently, the genetic, environment and extraction process exhibited remarkable differences in barley cultivars AA regarding the DPPH radical (Lee et al., 2016; Zhu et al., 2015). Therefore, the results indicated that barley husk had strong DPPH radical scavenging activity. This antioxidant capacity is related to the molecular structure or configuration of the phenolic compounds. The fractionation process employed allowed to obtain fractions with very different AA ($p < 0.001$). The average scavenging activities against the DPPH method were similar to the results obtained by Cruz et al. (2007), Pereira de Abreu et al. (2012) and Barbosa-Pereira et al. (2013). Therefore, these cultivars may be considered as excellent natural sources of potent free radical scavengers, nutraceuticals and healthy foods. These cultivars may also be recommended as potential cultivars to develop better barley owing to its high phenolic contents. Moreover, the pearling process is an important technique to keep high phenolic contents and potent antioxidant effects of barley husk. The results of EC50 have demonstrated the efficacy of natural extracts antioxidants obtained from barley husk which can be used as antioxidant agents (Cruz et al., 2007; Garrote et al., 2008). Thus, barley husk can be considered as a rich source of natural antioxidants comparing to other cereals (Lahouar et al., 2014; Lee et al., 2016). These antioxidant extracts may be optimized to be used in a vast type of functional foods. As industrial relevance, the use of barley husk, which is usually a residue of the brewing process or livestock production, can be optimized to produce natural extracts with high AA and potential health benefits, and it may work as a cancer preventative and brain booster (Pereira de-Abreu et al., 2012).

3.2. Mass spectrometric identification of the major antioxidants

Phenolic compounds in barley husks raw extracts were successfully identified and quantified by LC–MS method based on analysis of their molecular structure. Individual antioxidants revealed in our extracts are summarized in Tables 2 and 3. Referring to some previous studies (Cruz et al., 2007; Pereira de Abreu et al., 2011; Barbosa-Pereira et al., 2013; Do et al., 2014; Lee et al., 2016), twenty one (21) isolated antioxidants were confirmed in the samples investigated. As illustrated in Tables 2 and 3, a major part of our extracts presented more than thirteen compounds (Fractions A and B). All the extracted fractions showed high contents of p-coumaric acid and syringic acid in the acid treatment (fractions A, Table 2), and p-coumaric acid followed by trans-ferulic acid in the basic treatment (fractions B, Table 3), as illustrated also by chromatograms in figure 2. Thus, the most abundant phenolic acids shown in barley husk extracts were p-coumaric acid, trans-ferulic acid and syringic acid, respectively. Each phenolic acid was present in the crude extracts with a large amount according to the extraction solvent. Total concentrations of p-coumaric acid ranged from 491.189 mg/100g for Ardhaoui Sfax cultivar (1A sample) to 1954.002 mg/100g for Ardhaoui Tataouine cultivar (2B sample) at a retention time of 16.939 min. Similarly, the concentrations of trans-ferulic acid were very important and varied between 501.475 mg/100g for 5B sample (barley Rihane) and 849.146 mg/100g for 3B (Ardhaoui Medenine cultivar) at a retention time of 18.392 min, which indicated that trans-ferulic acid is the second important phenolic acid in barley husks raw extracts. Syringic acid also shows great amounts in all fractions A; its concentrations varied from 192.228 mg/100g for 2A sample to 786.351mg/100g for the last sample (7A) at a retention time of 13.838 min (chromatograms in figure 2). These results are very interesting and partially consistent with some previous researches which found that p-coumaric acid and ferulic acid were the major phenolic acids present in barley grains (Hernanz et al., 2001; Andersson et al., 2008; Li et al., 2008; Lahouar et al., 2014; Zhu et al., 2015).

As expected, barley husks extracts are a complex of phenolic substances mixed with other antioxidants such as beta-carotene difficult to solve. The LC-MS system analysis revealed eighteen individual phenolic compounds and three other natural antioxidants in total in our extracts. Thirteen phenolic compounds were identified in fractions A, and eleven phenolic compounds were identified in fractions B obtained after the delignification process of barley husks with NaOH, at different concentrations. Some compounds such as p-coumaric acid, protocatechuic acid, naringin, hyperoside (quercetin-3-o-galactoside), naringenin and cirsiliol were present in all fractions provide from the two extraction processes employed, but at very different concentrations (0.312 – 1954.002 mg/100g). These differences might be due to genetic makeup, cultivar variations and extraction solvent employed. Phenolic compounds present in these extracts include different phenolic acids and flavonoids. Therefore, the high AA of our barley husks materials has been related to the phenolic acids such as p-coumaric acid, syringic acid, trans-frulic acid, gallic acid and caffeic acid. In addition, Cirsiliol, Naringin and Protocatechuic acid are also recognized as natural antioxidants that contribute to scavenging free radicals and prevent chain reactions (Barbosa-Pereira et al., 2013; Zhu et al., 2015). As well avowed, this corresponds to phenolic compounds being the main responsible for the strong antioxidant activities. The phenolic acids identified in these barley husks extracts were divided into two groups: hydroxybenzoic acid derivatives and hydroxycinnamic acids. The first group contains syringic acid, gallic acid, protocatechuic acid and salviolinic acid. The cinnamic acids identified were p-coumaric acid, trans-ferulic acid and caffeic acid. Other compounds were detected by the LC-MS analysis and correspond to cirsiliol, catechin, epicatechin, rutin, sylimarin and 4,5-di-O-caffeoyquinic acid but at small concentrations, ranged between 0.098 and 8.183 mg/100g of extract. Some classes of flavanones such as naringin and naringenin were also showed in our barley husks extracts. Apegenin was equally detected as a flavone (Fractions A). Acacetin which belongs to the O-methylated flavone was

identified as another compound. Mass spectrometry identification revealed that flavonols present in these extracts as epicatechin, catechin (+), naringin, naringenin and acacetin were homogeneously distributed in all extracts at near amounts, between 0.098 and 5.731 mg/100g of natural extract for every compound. Beta-carotene was also present as antioxidant but only in the last sample corresponding to 'Lemsi forage variety' at a concentration of 0.394mg/100g for fraction A and 0.276 mg/100g for fraction B. Finally, other group of bioactive compounds like stigmasterol and sitosterol were equally detected at minor concentrations especially into fractions B. Consequently, major phenolic compounds present in these natural extracts include phenolic acids. P-coumaric acid was present at greater concentrations, corresponding to the most dominant phenolic acid in all extracted fractions provided from barley husks. It accounts about 50% of the identified phytochemicals, known as the deepest radical scavenger protecting efficiently from oxidation. In addition, the natural derived antioxidants showed great amounts of flavonoids, cirsiliol and other phenolic acids responsible for remarkable AA, comparable to the synthetic antioxidants: BHA and BHT extremely used in food processing. The major flavonoids revealed in this study were flavan-3-ols and flavonols such as epicatechin and catechin (+), equally acacetin but at very low concentrations (Tables 2 and 3). These results were expected because of the originality of the barley pearls, and are partially consistent with some researches (Piazzon et al., 2010) which identified phenolic acids in beers at similar contents, showing that ferulic acid is the most abundant compound, followed by caffeic acid and p-coumaric acid. The flavan-3-ol such as catechin (+) was characterized as flavonoids class responsible for the high free radicals scavenging activities of barley grain and malt; it is shown in all fractions A. Other studies (Gökmen et al., 2009; Gangopadhyay et al., 2015; Shen et al., 2016) have also reported that flavan-3-ols such as gallocatechin and epigallocatechin are the main cause of the high AA of brewing materials. Therefore, commercialization of barley husks raw extracts as powerful and natural antioxidants should

be encouraged for increasing the prospect to be used as functional food additives and preservatives, preventing lipid pre-oxidation and protecting from oxidative spoilage during storage time, which enhance the shelf life of food products. André et al. (2010), Barbosa-Pereira et al. (2013) and Zhu et al. (2015) have previously suggested that generally, the composition of phenolic compounds in natural extracts depend widely on the genetic of plant species, agricultural technique, soil production, and on the technological processes used to precede the raw materials in the case of agro-food industries, cosmetics and pharmaceuticals. Other important variability factors, environmental conditions such as cultivation areas, seasonal climate and maturity stage also influence greatly the content of phenolic compounds present in natural extracts. Currently, researches give so much attention into the antioxidant properties and phenolic compounds associated with potential health benefits; human estimated daily intake of phenolic acids varied between 25 mg and 1 g, coming from fruits, vegetables, whole grains, green tea, coffee, spices and cereals (Leitao et al., 2011; Zhu et al., 2015; Gangopadhyay et al., 2015).

4. Conclusion

In summary, barley husks raw extracts demonstrated high levels of phenolic compounds exhibiting strong antioxidant activity. LC-MS analysis and statistical evaluation of the DPPH results reflected more the impact of the variety, location and of the extraction solvent on the TPC. A greater variability among the individual cultivars and between extraction solvents noted in this study may be important for the optimum utilization of these barley pearls to introduce several natural antioxidants. Ardhaoui cultivars can be selected as the best genotypes enriched of these natural compounds followed by ‘Lemsi’ forage variety. It can be concluded that application of the pearling process is an effective tool to produce barley pearls

as a good source of natural antioxidants that might be employed for functional foods and therapeutics. Thus, there is a need to explore the possibility of increasing consumption of barley husk ingredients and derived-end products in food processing. Incorporation of these materials in human foods would enhance their nutritional and physiological properties. However, functionality and acceptability should be given a particular attention when manufacturing fiber-rich products. Future researches are needed to better understand the nutraceutical values of barley husk considering the consumers sensory acceptability.

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Table 1

Extraction rate (%) and antioxidant activity (EC50) of the barley husks crude extracts

Cultivars	Fraction	Concentration in crude extracts (g/L)	% Extract from 100 g barley husk	EC50 (g/L)
Ardhaoui Sfax	1 A	121.4	1.33	0.93^{bc} ±0.48
	1 B	211.2	4.63	0.43^{d} ±0.06
Ardhaoui Tataouine	2 A	66.5	1.46	1.46^{a} ±0.47
	2 B	70.6	3.09	0.93^{bc}±0.05
Ardhaoui Medenine	3 A	52.8	1.16	1.12^{abc} ±0.02
	3 B	156.2	3.42	0.88^{c} ±0.04
Manel	4 A	64.8	1.42	1.08^{abc}±0.01
	4 B	235.2	5.16	1.32^{ab}±0.01
Rihane	5 A	79.6	1.74	1.15^{abc}±0.02
	5 B	233.7	5.12	1.27^{abc}±0.08
Konouz	6 A	80.7	1.77	1.2^{abc}±0.03
	6 B	228.7	5.01	1.43^{a} ±0.03
Lemsi	7 A	77.5	1.70	1.18^{abc}±0.029
	7 B	228.8	5.01	1.45^{a}±0.028

A = Liquid from acid hydrolysis process; B = Liquid from delignification process; BHA: EC50= 0.24±0.05 g/L; Mean in the same column followed by different superscript letters differ significantly ($p < 0.05$).

Table 2

Individual antioxidants identified by LC-MS (mg/100g) into fractions A (acid treatment) of the barley husks crude extracts

Antioxidants	Cultivars						
	Ardhaoui Sfax	Ardhaoui Tataouine	Ardhaoui Medenine	Manel	Rihane	Konouz	Lemsi
Gallic acid	3.531	5.232	5.402	4.379	4.051	5.295	5.428
Protocatechuic acid	11.425	17.438	15.430	15.443	16.534	13.233	15.639
Catechin -(+)	1.408	2.055	2.902	3.832	3.200	4.47	6.229
Syringic acid	201.478	192.228	237.423	280.715	451.510	426.984	786.351
p-coumaric acid	491.189	666.037	849.872	733.894	602.784	678.915	633.279
Naringin	5.731	4.607	6.983	6.417	5.556	5.66	6.854
Hyperoside (quercetin-3-o-galactoside)	1.343	0.990	0.957	0.845	1.126	0.456	1.032
Salviolinic acid	-	-	-	-	3.877	-	4.375
Rutin	2.189	2.051	3.810	2.059	1.884	1.280	1.087
4,5-di-O-caffeoyquinic acid	5.759	4.495	8.183	5.945	4.964	5.260	7.166
Naringenin	1.927	1.082	2.202	1.574	1.514	0.906	2.255
Cirsiliol	35.376	21.421	28.602	42.402	52.114	28.382	60.164
Apegenin	0.732	1.948	1.272	1.548	2.778	2.689	1.663
Acacetin	1.577	-	-	-	-	-	-
Beta carotene	-	-	-	-	-	-	0.394
Sitosterol	-	67.895	-	10.493	5.914	3.458	2.047
Total	763.665	987.479	1163.038	1109.546	1157.806	1176.988	1533.963

- N.D: No defined peak of the antioxidant

Table 3

Individual antioxidants identified by LC-MS (mg/100g) into fractions B (delignification) of barley husks crude extracts

Antioxidants	Cultivars						
	Ardhaoui Sfax	Ardhaoui Tataouine	Ardhaoui Medenine	Manel	Rihane	Konouz	Lemsi
Protocatechuic acid	11.425	28.897	11.856	7.200	8.154	11.056	10.899
Epicatechin	0.098	0.309	0.158	0.156	0.182	0.136	0.158
Caffeic acid	5.427	8.587	4.675	4.267	5.873	7.423	7.113
p-coumaric acid	1339.646	1954.002	1867.568	1075.800	1338.168	1292.520	1740.248
Trans-ferulic acid	696.499	801.185	849.146	515.171	501.475	703.267	545.539
Naringin	4.603	3.593	3.242	2.379	3.469	3.628	2.554
Hyperoside (quercetin-3-o-galactoside	0.806	0.312	0.539	0.519	0.680	0.692	0.298
Salviolinic acid	2.837	4.704	2.536	1.721	3.183	2.426	2.638
Naringenin	0.538	1.161	1.626	0.697	0.749	0.887	0.712
Silymarin	0.319	0.099	0.385	0.305	0.537	0.276	0.609
Cirsiliol	43.546	43.256	22.976	11.564	20.674	14.670	18.831
Beta-carotene	-	-	-	-	-	-	0.276
Stigmasterol	0.371	29.254	7.283	9,476	2,222	2.942	2.152
Sitosterol	4.674	139.636	98.870	58.934	30.814	35.045	42.782
Total	2110.789	3014.995	2870.86	1688.189	1916.18	2074.968	2374.809

- N.D: No defined peak of the antioxidant

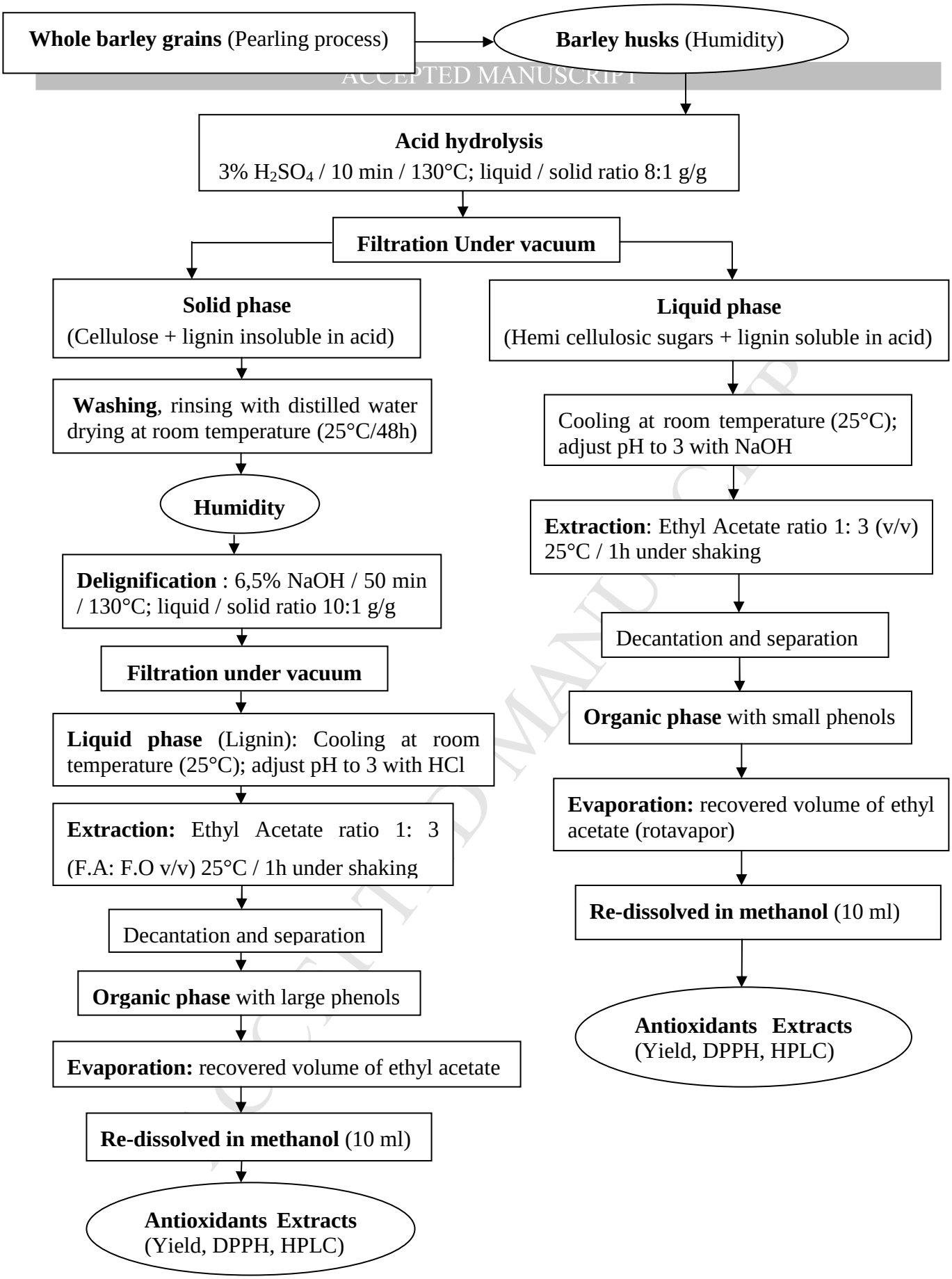
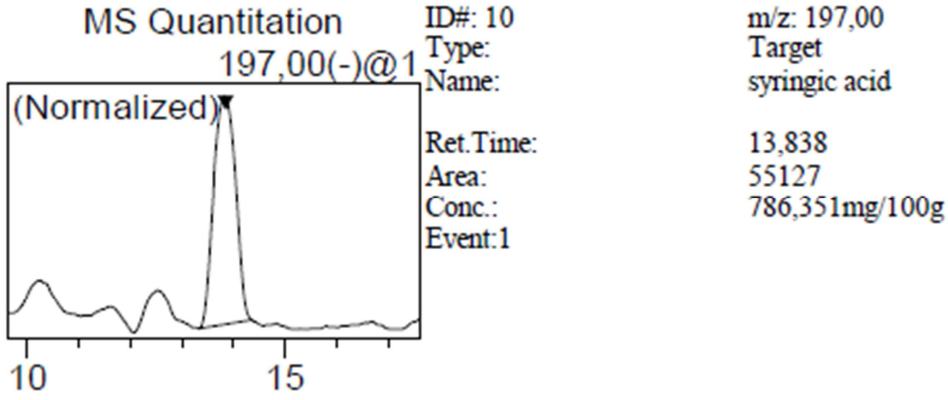


Figure 1. Extraction process of antioxidants from barley husks (Cruz et al., 2007; Garrote et al., 2008; Pereira de Abreu et al., 2012; Barbosa-Pereira et al., 2013).

a



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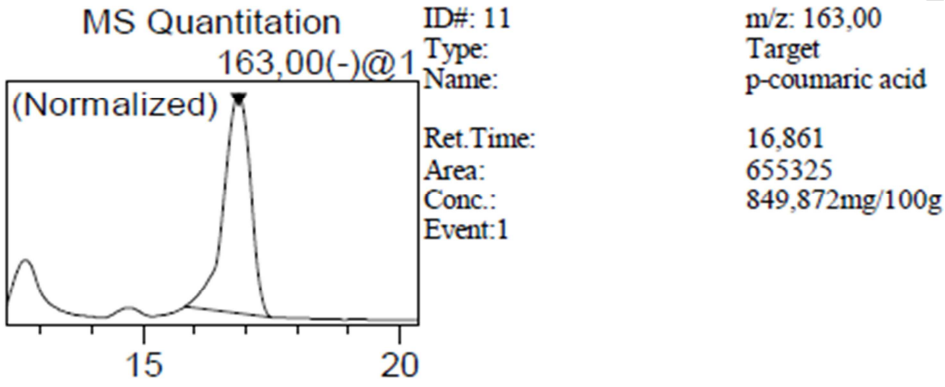

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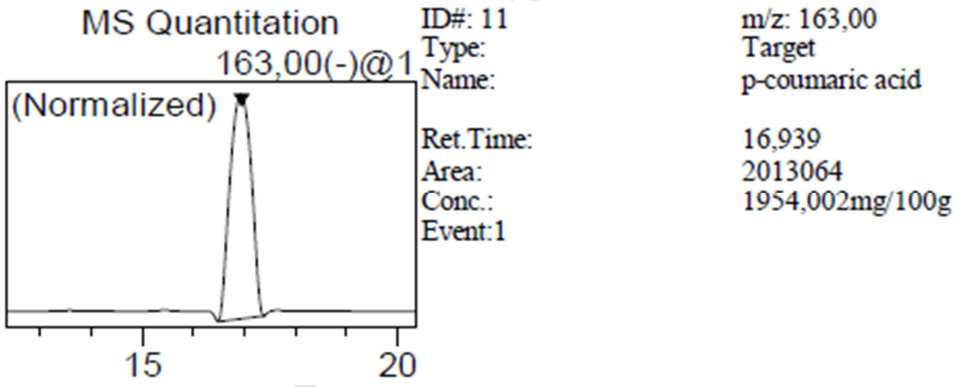

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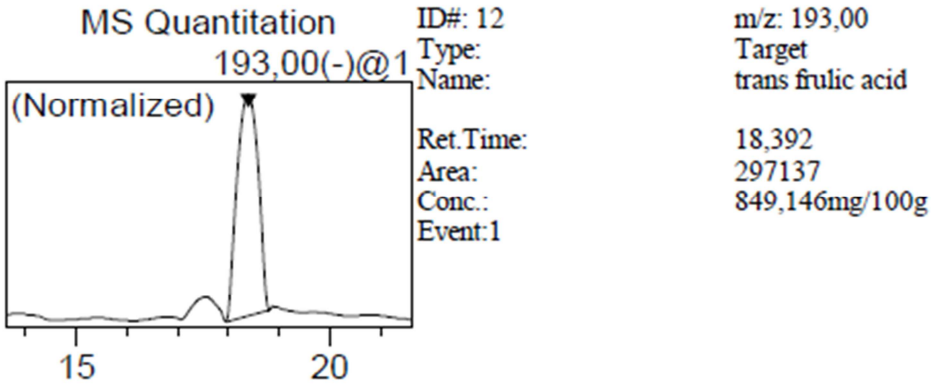

Figure 2. Best antioxidants quantified by LC-MS/MS analysis: (a and b) syringic acid and p-coumaric acid identified in fractions A (samples 7A and 3A); (c and d) p-coumaric acid and trans-ferulic acid identified in fractions B (samples 2B and 3B)

Highlights

- High level of phytochemicals exhibiting potent antioxidant activity was found;
- Extraction solvent affected so much the level of phenolic compounds;
- Wide composition of natural antioxidants was identified by LC-MS analysis;
- New functional ingredients can be developed from barley husks crude extracts.