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Free Water-Soluble Phenolics Profiling in Barley (*Hordeum vulgare* L.)

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The phenolic profile of barley (*Hordeum vulgare* L.) leaves, seeds, awns, and stems, collected in two different locations from Portugal, was determined by a high-performance liquid chromatography/diode array detector (HPLC/DAD). A total of 28 compounds were identified and quantified, which included 4 phenolic acids, 6 *C*-glycosylflavones, and 18 *O*-glycosyl-*C*-glycosyl flavones, with some of them acylated. Distinct profiles were noticed among the analyzed materials. The greatest diversity of compounds was found in barley leaves (26 flavonoids and 2 phenolic acid derivatives), which also exhibited the highest concentration of phenolics. Isoorientin-7-*O*-glucoside (lutonarin) was the major compound in leaves, while, in general, the pair isovitexin-7-*O*-rutinoside plus isoscoparin-7-*O*-glucoside were the main phenolics in the other materials. Thus, barley leaves may constitute an important dietary source of protective compounds, which could be used, for example, to take profit from the wastes resulting from alcoholic drink obtainment.

KEYWORDS: Barley; *Hordeum vulgare* L.; phenolic compounds; vegetal materials; HPLC/DAD

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a well-known cereal grain, cultivated throughout the world. It is used for livestock feeding, as well as for human consumption. Flat barley bread was consumed in Europe just before Roman times, and in Tibet, this cereal has been the only major staple food for centuries. In the West, it is used in soups, stews, and sauces (*1*). In Eastern countries (e.g., Japan, Korea, and China), barley is used in human food and drink, such as bread, cakes (*2*), and the Japanese "Barley Water", often flavored with lemon or orange, a well-known drink during the summer season that is said to be nutritious, for which it is given to children and invalids. Additionally, a popular drink called "Aojiru", also known as green drink or green juice, made from barley young leaves is consumed in Japan. Malt is obtained from barley grains, being used in the production of alcoholic drinks (beer, whisky, gin, and vodka), malt vinegar, in some sweets and confectionery, and as a carrier for vitamin-rich cod-liver oil (*1*).

Barley is rich in a wide range of antioxidant compounds, such as phenolic acid derivatives, proanthocyanidins, quinones, and flavonoids (*3*). Phenolic composition can vary in plants from organ to organ, but other factors, such as cultivar and geographical conditions, can also participate in their variability in plants (*4*).

The importance of these compounds is ascribed to their wide spectrum of activities. In plants, they act as antioxidants, antimicrobials, photoreceptors, visual attractors, and feeding repellents and for light screening. When present in the diet, they provide antiallergenic, antiviral, anti-inflammatory, and vasodilatation properties to the human body. However, most interest has been devoted to their antioxidant capacity (*5*). They can protect cells against the damaging effects of reactive species, and from this, they may prevent aging, cancer, atherosclerosis, ischemic injury, and neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases (*2*, *3*).

Previous studies concerned the phenolic compound determination in barley leaves and seeds and the evaluation of their antioxidant activity (*2*, *3*, *6*−*13*).

As far as we are aware, no previous work involved the comparison of the different *H. vulgare* L. plant materials. This

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study intended to define and compare the phenolics profile of barley leaves, seeds, stems, and awns collected at two different origins. For this purpose, a reversed-phase high-performance liquid chromatography/diode array detector (HPLC/DAD) methodology was applied.

MATERIALS AND METHODS

Standards and Reagents. Ferulic and chlorogenic acids, luteolin-3′,7-di-*O*-glucoside, saponarin (isovitexin-7-*O*-glucoside), isovitexin, isoorientin, and lutonarin (isoorientin-7-*O*-glucoside) were purchased from Extrasyntése (Genay, France), and *p*-coumaric acid was obtained from Sigma (St. Louis, MO). Methanol was from Merck (Darmstadt, Germany), and acetic acid was from Fisher Scientific (Leicestershire, U.K.). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Plant Material. Barley plants were collected in two different fields located in Fundão and Bragança (center and northeast of Portugal, respectively) in May 2007. The plant material was immediately transferred to the laboratory, frozen at −20 °C, and lyophilized. Leaves, seeds, awns, and stems were then separated.

Extracts Preparation. Dried barley leaves, seeds, awns, and stems were pulverized, and aqueous extracts were prepared: 0.1 g of each material was thoroughly mixed with 1 mL of water, ultrasonicated for 1 h, macerated at room temperature (18 h), and again ultrasonicated (1 h). The resulting extract was centrifuged (5000 rpm for 10 min), and the supernatant was collected and filtrated through a 0.45 μm pore-size membrane.

HPLC/DAD Analysis. HPLC analysis was carried on a Gilson unit, using a 250 × 4.6 mm i.d., 5 μm Spherisorb ODS2 column (Waters, Milford, MA). Elution was performed using 1% acetic acid (A) and methanol (B) as solvents, starting with 20% B and using a gradient to obtain 50% B at 55 min. The flow rate was 1 mL/min, and the injection volume was 20 μL. Detection was accomplished with a Gilson DAD, and chromatograms were recorded at 340 nm. Data were processed on Unipoint System software (Gilson Medical Electronic, Villiers le Bel, France).

Phenolic acids, saponarin, isovitexin, and isoorientin were identified on the basis of their chromatographic behavior, by a comparison of their retention times and UV−vis spectra, in the range of 200−400 nm, to those of authentic high-purity commercially available standards. The other compounds were identified according to our previous work

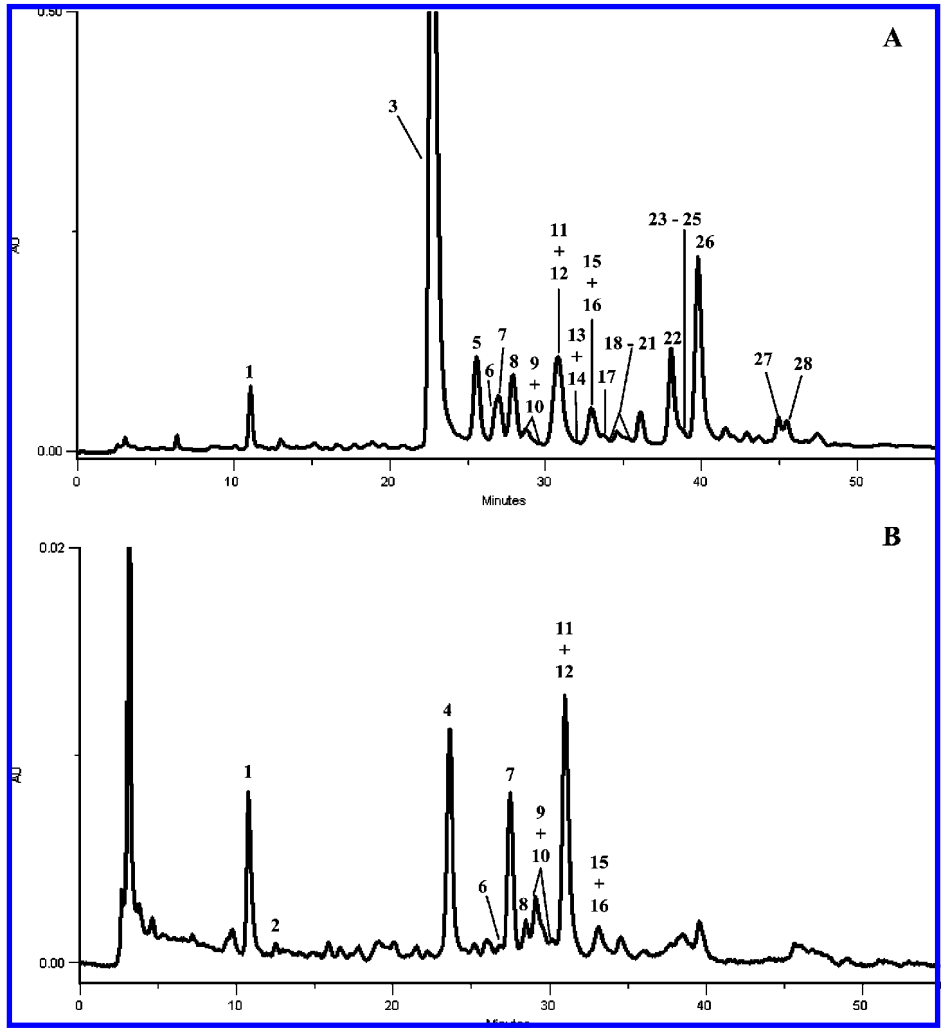

Figure 1. HPLC/DAD phenolic profile of (A) barley leaves (Fundão origin) and (B) barley stems (Bragança origin). Detection was at 340 nm. Peaks: 3-*O*-feruloylquinic acid (**1**), chlorogenic acid (**2**), isoorientin-7-*O*-glucoside (lutonarin) (**3**), *p*-coumaric acid (**4**), isoorientin-7-*O*-rutinoside (**5**), luteolin-6-*C*-arabinoside-8-*C*-glucoside (**6**), ferulic acid (**7**), isovitexin-7-*O*-glucoside (saponarin) (**8**), isoorientin-7-*O*-[6-feruloyl]-glucoside-4′-*O*-glucoside (**9**), apigenin-6-*C*-arabinoside-8-*C*-glucoside (**10**), isovitexin-7-*O*-rutinoside (**11**), isoscoparin-7-*O*-glucoside (**12**), apigenin-6-*C*-glucoside-8-*C*-arabinoside (**13**), isovitexin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**14**), isoscoparin-7-*O*-rutinoside (**15**), isoorientin (**16**), isovitexin-7-*O*-[6-feruloyl]-glucoside-4′-*O*-glucoside (**17**), isoorientin-7-*O*-glucoside-4′-*O*-[6-feruloyl]-glucoside (**18**), isoorientin-7-*O*-[6-caffeoyl]-glucoside (**19**), chrysoeriol-6-*C*-glucoside-8-*C*-arabinoside (**20**), isoscoparin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**21**), isoorientin-7-*O*-[6-sinapoyl]-glucoside (**22**), isoorientin-7-*O*-[6-feruloyl]-glucoside-2′′-*O*-glucoside (**23**), isoscoparin-2′′-*O*-glucoside (**24**), isovitexin (**25**), isoorientin-7-*O*-[6-feruloyl]-glucoside (**26**), isovitexin-7-*O*-[6-sinapoyl]-glucoside (**27**), and isoscoparin-7-*O*-[6-sinapoyl]-glucoside (**28**).

(*11*). Using the same experimental conditions, we obtained similar chromatograms in what concerns the remaining phenolics, in which the compounds had the same order of elution and the same UV spectra.

Phenolics quantification was achieved by the absorbance recorded in the chromatograms applying the external standard calibration method, using peak areas. Ferulic acid derivatives were quantified as ferulic acid; isoorientin and isoscoparin derivatives were quantified as lutonarin; and isovitexin derivatives were quantified as saponarin. The groups isoorientin-7-*O*-[6-feruloyl]-glucoside-4′-*O*-glucoside (**9**) plus apigenin-6-*C*-arabinoside-8-*C*-glucoside (**10**) and isorientin-7-*O*-glucoside-4′-*O*-[6-feruloyl]-glucoside (**18**), isoorientin-7-*O*-[6-caffeoyl]-glucoside (**19**), and chrisoeryol-6-*C*-glucoside-8-*C*-arabinoside (**20**) plus isoscoparin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**21**) were quantified as lutonarin. The pair isovitexin-7-*O*-rutinoside (**11**) plus isoscoparin-7-*O*-glucoside (**12**) as well as apigenin-6-*C*-glucoside-8-*C*-arabinoside (**13**) plus isovitexin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**14**) were quantified as saponarin. Luteolin-6-*C*-arabinoside-8-*C*-glucoside (**6**) was quantified as luteolin-3′,7-di-*O*-glucoside, and the pair isoscoparin-7-*O*-rutinoside (**15**) plus isoorientin (**16**) were quantified as isoorientin. Isoorientin-7-*O*-[6-feruloyl]-glucoside-2″-*O*-glucoside (**23**), isoscoparin-2″-*O*-glucoside (**24**), and isovitexin (**25**) were quantified together as isovitexin. The other compounds were quantified as themselves.

RESULTS AND DISCUSSION

Qualitative Analysis. For the identification of phenolic compounds in the several samples, we used the extraction conditions described before (*11*). Moreover, because we intend to focus on an alimentary approach and as a result of the use of barley in the preparation of infusions, we used water instead of organic solvents. The HPLC/DAD analysis allowed for the determination of 28 compounds, which included both phenolic acids and flavonoids (**Figure 1**). The leaves were the material presenting the highest diversity of compounds (**Figure 1A**). In both Fundão and Bragança samples, they were identified as 3-*O*-feruloylquinic acid (**1**), isoorientin-7-*O*-glucoside (lutonarin) (**3**), isoorientin-7-*O*-rutinoside (**5**), luteolin-6-*C*-arabinoside-8-*C*-glucoside (**6**), ferulic acid (**7**), isovitexin-7-*O*-glucoside (saponarin) (**8**), isoorientin-7-*O*-[6-feruloyl]-glucoside-4′-*O*-glucoside (**9**), apigenin-6-*C*-arabinoside-8-*C*-glucoside (**10**), isovitexin-7-*O*-rutinoside (**11**), isoscoparin-7-*O*-glucoside (**12**), apigenin-6-*C*-glucoside-8-*C*-arabinoside (**13**), isovitexin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**14**), isoscoparin-7-*O*-rutinoside (**15**), isoorientin (**16**), isovitexin-7-*O*-[6-feruloyl]-glucoside-4′-*O*-glucoside (**17**), isoorientin-7-*O*-glucoside-4′-*O*-[6-feruloyl]-glucoside (**18**), isoorientin-7-*O*-[6-caffeoyl]-glucoside (**19**), chrysoeriol-6-*C*-glucoside-8-*C*-arabinoside (**20**), isoscoparin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**21**), isoorientin-7-*O*-[6-sinapoyl]-glucoside (**22**), isoorientin-7-*O*-[6-feruloyl]-glucoside-2″-*O*-glucoside (**23**), isoscoparin-2″-*O*-glucoside (**24**), isovitexin (**25**), isoorientin-7-*O*-[6-feruloyl]-glucoside (**26**), isovitexin-7-*O*-[6-sinapoyl]-glucoside (**27**), and isoscoparin-7-*O*-[6-sinapoyl]- glucoside (**28**). All of these compounds have been reported before in barley leaves (*11*), with the exception of ferulic acid, identified in our work.

The seeds presented a distinct profile, composed of 13 phenolics. Besides compounds **3** and **6**−**16**, this material also exhibited *p*-coumaric acid (**4**), not detected in the leaves (**Table 1**). With the exceptions of *p*-coumaric (**4**) and ferulic (**7**) acids already described (*10*), these compounds are reported for the first time in barley seeds.

In awns, it was possible to identify 12 flavonoids and 3 phenolic acids: compounds **1**, **3**, and **5**−**16** and chlorogenic acid (**2**), not found in the previous two barley materials (**Table 1**). Stems contained some compounds of both classes of phenolics mentioned above (compounds **1**, **2**, **4**, and **6**−**16**) (**Table 1** and **Figure 1B**). As far as we know, this is the first work describing the occurrence of these compounds in barley awns and stems. Thus, the different barley materials present distinct phenolic composition.

Quantitative Analysis. The quantification of the identified compounds revealed that barley leaves were clearly the material presenting the highest phenolics contents, followed by awns (**Table 1**). However, the leaves from Fundão showed roughly 3 times the amount found in the ones from Bragança (ca. 3.7 and 1.2 g/kg, respectively), while the awns from this latter exhibited about 1.4 times the phenolic contents observed in the sample from Fundão (ca. 0.36 and 0.25 g/kg, respectively). Seeds and stems exhibited considerably lower levels of phenolic compounds (about 11−19% of the content found in awns).

Leaves and seeds revealed very particular phenolics profiles: in both plant materials, the compound in highest amounts

Table 1. Phenolics Composition of *H. vulgare* Samples (mg/kg)[a]

	samples							
	Fundão				Bragança			
compounds[b]	leaves	seeds	awns	stems	leaves	seeds	awns	stems
1	39.8 ± 0.4		61.4 ± 0.0	5.8 ± 0.1	4.3 ± 0, 1		56.4 ± 0, 9	5.1 ± 0.1
2			4.4 ± 0.1	1.1 ± 0.1			5.7 ± 0.2	0.8 ± 0.0
3	2150.8 ± 59.9	1.5 ± 0.0	37.4 ± 1.4		760.8 ± 9.0	nq[c]	76.9 ± 3.0	
4		6.2 ± 0, 2		5.3 ± 0, 8		3.4 ± 0, 0		18.6 ± 0.6
5	208.4 ± 2.6		22.8 ± 0.8		68.5 ± 5.0		38.7 ± 0.0	
6	80.5 ± 3.1	0.3 ± 0.0	14.9 ± 1.2	0.9 ± 0.0	24.6 ± 0.3	0.2 ± 0.0	nq	0.4 ± 0.0
7	33.6 ± 3.0	2.4 ± 0.1	8.6 ± 0.4	2.5 ± 0.1	25.2 ± 1.9	1.0 ± 0.1	24.9 ± 0.5	5.9 ± 0.0
8	145.3 ± 2.8	2.0 ± 0.0	4.5 ± 0.4	0.3 ± 0.0	56.4 ± 0.8	2.4 ± 0.0	6.1 ± 0.3	1.2 ± 0.1
9−**10**	30.9 ± 1.8	7.3 ± 0.4	13.2 ± 1.4	3.4 ± 0.1	14.2 ± 0.5	6.4 ± 0.0	20.2 ± 0.2	5.4 ± 0.5
11−**12**	217.5 ± 15.6	29.3 ± 0.2	63.3 ± 0.3	8.8 ± 0.1	70.5 ± 3.5	26.4 ± 0.1	82.7 ± 2.7	15.3 ± 0.3
13−**14**	14.3 ± 0.7	0.4 ± 0.0	1.5 ± 0.0	nq	7.9 ± 0.1	0.2 ± 0.0	3.6 ± 0.1	nq
15−**16**	87.6 ± 2.1	1.7 ± 0.0	21.4 ± 1.8	1.1 ± 0.0	52.3 ± 2.8	4.5 ± 0.0	47.9 ± 0.1	1.5 ± 0.0
17	3.1 ± 0.0				3.1 ± 0.3			
18−**21**	32.6 ± 1.2				23.1 ± 0.1			
22	167.3 ± 1.4				47.8 ± 0.4			
23−**25**	3.2 ± 0.3				2.0 ± 0.0			
26	494.6 ± 9.0				74.8 ± 0.9			
27	18.2 ± 0.9				2.5 ± 0.0			
28	27.7 ± 0.6				6.0 ± 0.1			
total	3740.6	50.0	252.0	28.4	1232.9	44.4	358.3	51.1

[a] Results are expressed as mean ± standard deviation of three determinations. [b] Identity of compounds as in **Figure 1**. [c] nq = not quantified.

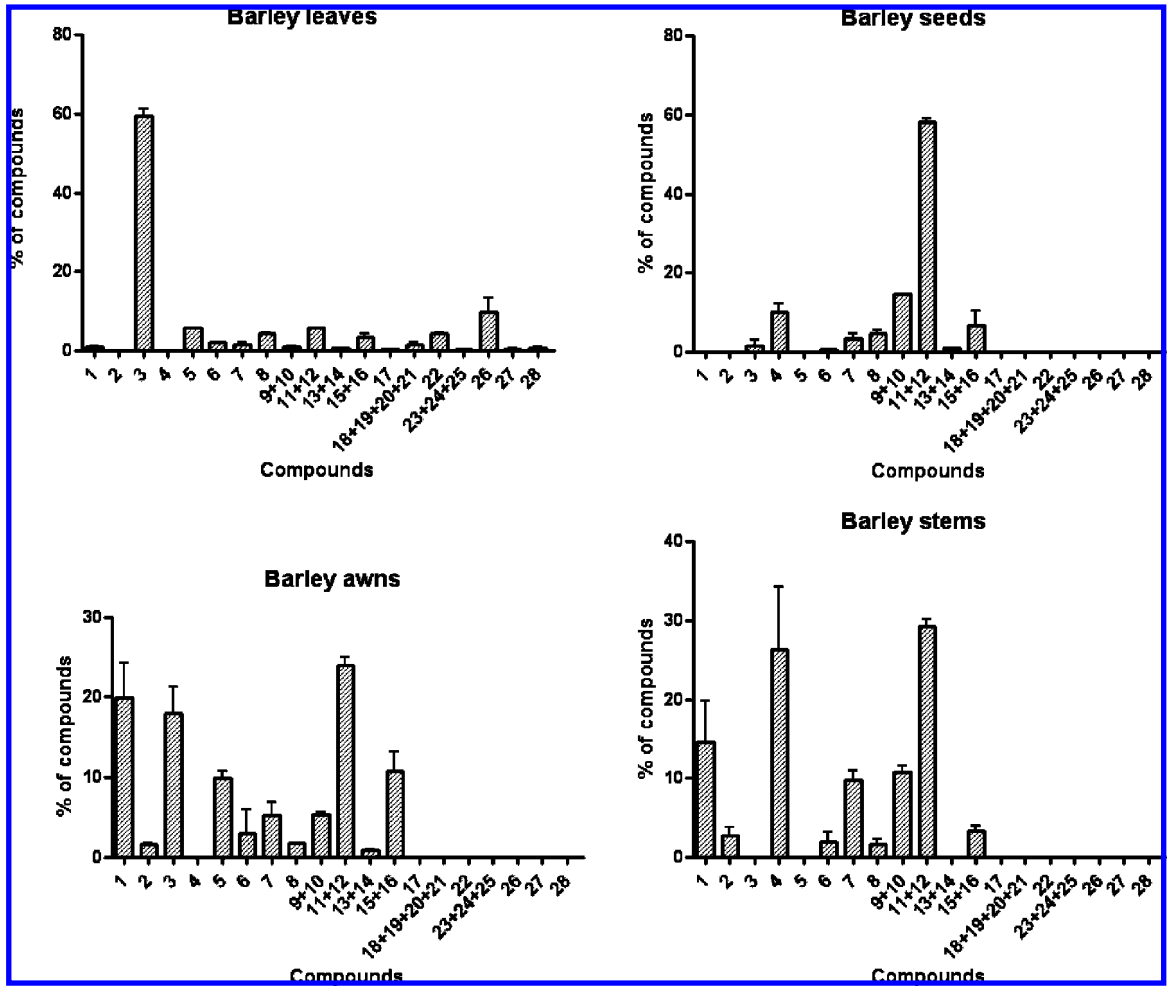

Figure 2. Phenolic compound profiles of barley leaves, seeds, awns, and stems. Values represent the mean of the different origins, and standard error bars are on the top of columns.

represented more than 50% of the total phenolic content (**Table 1** and **Figure 2**). The main compound in barley leaves and, simultaneously, the compound occurring in highest concentration in this plant was isoorientin-7-*O*-glucoside (**3**), which represented ca. 59.3% of total phenolic amount (**Figure 2**). Lutonarin is known as an antioxidant agent, with activity comparable to that of α-tocoferol and butylated hydroxytoluene (BHT). A previous report suggests that lutonarin could have a protective effect against atherosclerosis caused by lipoprotein oxidation in blood plasma (*14*). Isovitexin-7-*O*-[6-feruloyl]-glucoside-4′-*O*-glucoside (**17**), isoorientin-7-*O*-[6-feruloyl]-glucoside-2″-*O*-glucoside (**23**), isoscoparin-2″-*O*-glucoside (**24**), and isovitexin (**25**) represented ca. 0.2% of total phenolics in leaves, being the compounds in minor quantity (**Figure 2**).

Seeds exhibited a distinct profile, in which the pair isovitexin-7-*O*-rutinoside (**11**) plus isoscoparin-7-*O*-glucoside (**12**) was the most abundant, corresponding to ca. 58.2% of total phenolics. The minor compound in seeds was luteolin-6-*C*-arabinoside-8-*C*-glucoside (**6**), which represented ca. 0.55% of total compounds (**Figure 2**).

For awns and stems, the phenolic profiles were also dissimilar. However, in comparison to leaves and seeds, the difference between the levels of the major compounds and the remaining ones in those plant materials was not so prominent.

In awns, the phenolics present in highest amounts were, like in the seeds, isovitexin-7-*O*-rutinoside (**11**) plus isoscoparin-7-*O*-glucoside (**12**). 3-*O*-Feruloylquinic acid (**1**) was the second main compound, followed by lutonarin (**3**). All together, these

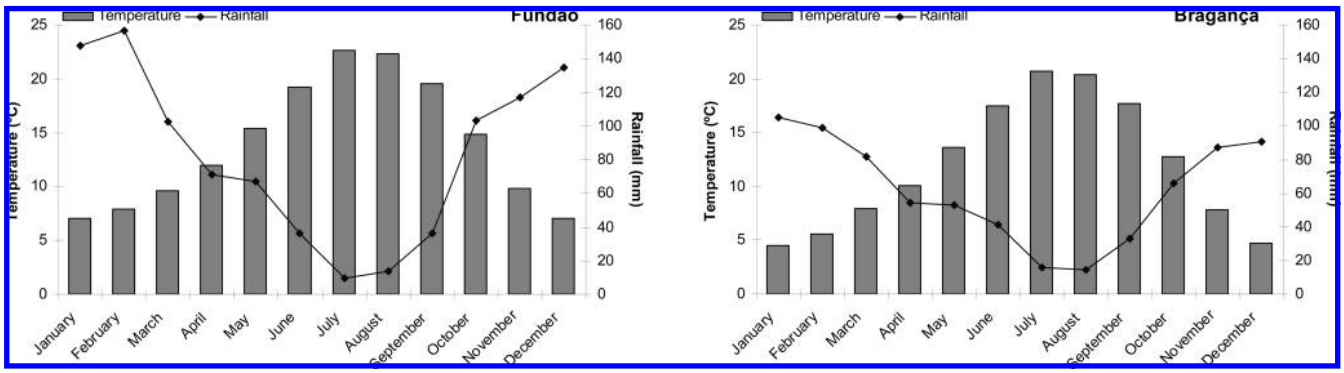

Figure 3. Average monthly temperature and accumulated rainfall, observed in Fundão and Bragança in the last 30 years.

four compounds correspond to ca. 61.8% of total phenolic content (**Figure 2**). The pair formed by isovitexin-7-*O*-rutinoside (**11**) and isoscoparin-7-*O*-glucoside (**12**) was also the most abundant in stems, followed by *p*-coumaric acid (**4**). These three compounds accounted for ca. 70.1% of the total phenolic amount in the stems. The minor compounds were apigenin-6-*C*-glucoside-8-*C*-arabinoside (**13**) and isovitexin-7-*O*-[6-sinapoyl]-glucoside-4′-*O*-glucoside (**14**) (ca. 0.8%) in awns and isovitexin-7-*O*-glucoside (**8**) (ca. 1.6%) in stems (**Figure 2**).

With the exception of stems, the relative amount of flavonoids in the different plant materials was higher than that of phenolic acids, corresponding to ca. 97.9, 73.4, and 86.8% of total phenolics in leaves, awns, and seeds, respectively. In stems, flavonoids represented ca. 46.8% of total phenolic compounds (**Figure 2**). The highest percentage of flavonoids in leaves is probably due to their well-known photoprotective effect (*15*).

Awns also exert an important role in metabolism. This part of the plant is exposed to sunlight too and, thus, also participates in the photosynthesis process (*16*). Therefore, the existence of a considerable amount of flavonoids in awns could be explained by their protective effect against photo-oxidation. The high content of flavonoids in seeds may be attributed to their role as antioxidants during germination, when the oxygen demand is high (*17*).

Besides their antioxidative properties, phenolic acids are important structural components of plant cell walls, where they serve to enhance its rigidity and strength (*18*). They have been implicated in the regulation of cellular expansion and plant defense and may reduce the digestibility of the cell wall by restricting accessibility to carbohydrates (*19*). These properties could be related with their higher contents in awns and stems.

In conclusion, for the first time, it was possible to identify and quantify 24 flavonoids and 4 phenolic acids in 4 different barley vegetable materials. Leaves presented the highest flavonoid amount, while stems presented the highest phenolic acid amount. The different plant materials of both origins, cultivated under similar climatic conditions (**Figure 3**), presented the same qualitative phenolic profile; therefore, it seems that the geographical origin does not interfere with it. However, plants growing locations may have some influence at the quantitative level, although more studies should be made to confirm this.

Our study provided evidence that this plant is an interesting source of phenolic compounds, which can improve human health. According to the data obtained, barley leaves may constitute an important dietary source of protective compounds because of their high phenolic contents, which could be used, for example, to take profit from the wastes resulting from alcoholic drink preparation.

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