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Evaluación nutricional y de la actividad biológica de los bulbos silvestres de *Allium ampeloprasum* L.

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Palabras clave: *Allium ampeloprasum* L.; compuestos bioactivos; composición nutricional; capacidad antioxidante

Resumen:

Allium ampeloprasum L. posee un gran potencial nutricional con presencia de compuestos antioxidantes de interés para la salud. Diversos estudios epidemiológicos han demostrado los efectos beneficiosos del consumo de diversas especies pertenecientes al género *Allium*, destacando sus efectos anticancerígenos.
En el presente trabajo se estudia el contenido de nutrientes (hidratos de carbono, proteínas, grasa y minerales), compuestos bioactivos (ácidos orgánicos, vitamina C, tocoferoles, perfil de ácidos grasos) y actividad antioxidante (DPPH, poder reductor, inhibición de la decoloración del β-caroteno y TBARS) la parte comestible del ajo porro (*A. ampeloprasum*). Para ello, se recolectaron muestras de los puerros (bulbos y parte basal de las hojas) de dicha especie silvestre en dos localidades diferentes del centro peninsular durante dos años consecutivos (2007-2008), sobre los cuales se llevaron a cabo los análisis mencionados.
Los resultados del análisis de los compuestos anteriormente mencionado indican que, a pesar de la amplia variabilidad natural detectada, esta planta destacó por ser una buena fuente de fibra (3,5 – 4,7 g/100 g), hierro (0,20 – 0,92 mg/100 g) y cinc (0,03 – 1,67 mg/100 g) en comparación con otras especies del mismo género, así como por su bajo valor calórico (59-97 Kcal/100 g). Asimismo, presentó un alto porcentaje de ácidos grasos poliinsaturados, siendo el ácido linoleico el principal ácido graso (53% del total de ácidos grasos). Se obtuvieron valores equilibrados en cuanto a la presencia de compuestos bioactivos hidrófilos y lipófilos en esta planta. La bioactividad de este bulbo se caracterizó por su actividad antioxidante medida como poder reductor (correlacionado con los tocoferoles) y TBARS (correlacionado con los compuestos fenólicos totales).

Assessment of nutritional and biological activity of wild bulbs of *Allium ampeloprasum* L.

Palabras clave: *Allium ampeloprasum* L.; nutritional composition; bioactive compounds; antioxidant capacity

Allium ampeloprasum L. has a great nutritional value as it presents antioxidant compounds with potentially beneficial effects on human. Different epidemiological studies have shown the healthy effects of the consumption of various species of the genus *Allium*, highlighting its anticancer effects.
In the present work, the nutritional composition (carbohydrates, proteins, fat and minerals), bioactive compounds (organic acids, vitamin C, tocopherols and fatty acids) and antioxidant activity (DPPH, reducing power, inhibition of β-carotene bleaching and TBARS methods) of the edible part of the wild leek (*A. Ampeloprasum*) were studied. Samples of wild leek were collected from two different wild populations located in the center of the Iberian Peninsula for three consecutive years (2007-2008), on which the aforementioned analysis were conducted. Despite the large natural variability detected, this plant stood out as a good source of fiber (3.5 to 4.7

g/100 g), iron (0.20 to 0.92 mg/100 g) and zinc (0.03 to 1.67 mg/100 g), compared to other species of the same genus. Wild leeks also showed a high proportion of polyunsaturated fatty acids, being linoleic acid the major one (53% of total fatty acids). Values were balanced in terms of the presence of hydrophilic and lipophilic bioactive compounds in this plant. The bioactivity of this species was better characterized by antioxidant activity measured as reducing power assay (correlated with tocopherols) and TBARS (correlated with total phenolic compounds).

Introduction

Wild leek *Allium ampeloprasum* L. is distributed in the Mediterranean region, and its bulbs and basal stems are traditionally consumed in the Iberian Peninsula, raw in salads, cooked, or salted in omelettes (Tardío et al., 2006). It has a very long folk medicinal history of use in a wide range of diseases due to their fungicidal, antiseptic and anthelmintic properties, but also as aperitive, hipocolesterolemic, antiasthmatic, antispasmodic, cholagogue, diuretic digestive and in the prevention of atherosclerosis. One of the mechanisms involved in *Allium* species bioactivity may be radical scavenging. The main nutrients in *Allium* species are polysaccharides, mainly resistant and digestible starch, and proteins. Dietary fiber show great beneficial functional effects, not only promoting bowel health, but also lowering cholesterol, balancing blood glucose, and promoting healthy physiology and well-being (EFSA, 2010). Other important components are minerals with an important role as cofactors of antioxidant enzymes or in the prevention of chronic diseases and in the immune function (Badui, 2006).

Material and Methods

In order to have representative samples, the edible parts of the wild leek, i.e the bulb and the pseudostem formed by the overlapping leaf bases, were collected in two different locations in Central Spain, during at least two years from 2007 to 2008. Fresh samples were immediately homogenized in a laboratory blender. Aliquots were taken to analyze dry matter, pH, titratable acidity, organic acids and vitamin C (ascorbic acid- AA and dehydroascorbic acid- DHAA). The other determinations were performed on freeze-dried materials. Dry matter (DM), pH and titratable acidity (TA) were determined following AOAC official methods (2006). Determinations were made for total available carbohydrates (TAC) (Osborne and Voogt, 1986), total dietary fiber (method 985.29, AOAC, 2006), total protein (AOAC, 2006), lipids (continuous extraction with ethyl-ether at 120ºC for 6 h), ash content (method 930.05; AOAC, 2006) and mineral elements by Atomic Absorption Spectrocopy (Fe, Cu, Mn, Zn, Ca, Mg, Na and K) according to AOAC (2006). Enregy value was calculated from lipids, available carbohydrates, proteins and fibre contents, according to the conversion factors recommended by European Regualtion 1169/2011.

Vitamin C (AA and DHAA) and individual organic acids (oxalic, glutamic, malic, citric and fumaric acids) was determined by HPLC-UV (Sánchez-Mata et al., 2012). Total phenolics and flavonoids were estimated based on procedures described by Wolfe et al. (2003) and Jia et al. (1999), respectively, with modifications. Tocopherols content was determined by HPLC-FL (Morales et al., 2012a), and fatty acids analysis were determined by GC-FID/capillary column, using a standard that allows to quantify each fatty acids as % of total (Morales et al. (2012a). Antioxidant activity was determined on methanolic extracts by DPPH, Reducing power, Inhibition of β-carotene bleaching and (TBARS) assays, following a procedure previously described by Morales et al., (2012b).

Results and discussion

Nutritional evaluation

Table 1 includes the results of physicochemical parameters analysis that describe samples characterization, including macronutrients composition and energy values. *Allium amperoprasum* showed a moisture content around 78.32%, between the values described for *Allium sativum* and *Allium porrum* (64% and 86%, respectively; Souci et al. (2008). Wild leek did not show high acidity (14.35 mL N/10 NaOH per 100 g) and pH values were relatively low and stable (5.49-6.09). Carbohydrates composition was characterized by TAC (16.60 g/100 g) and dietary fiber (4.23 g/100 g

as an average value). Both parameters showed variability between years and locations ($p<0.05$), especially for TAC concentration.

Table 1. Physico-chemical and nutritional characterization (g/100 g fw; mean ± SD, n=3) and energy value (kcal/100 g fw) of *Allium ampeloprasum* L. bulbs.

Proximate composition	2007		2008		Global average
	Site 1	Site 2	Site 1	Site 2	
Moisture	81.50 ± 0.02 ^{d}	76.02 ±0.10 ^{a}	78.10 ± 0.19 ^{c}	77.68 ± 0.17 ^{b}	78.32 ± 2.0
pH	6.09 ± 0.05 ^{b}	5.43 ± 0.04 ^{a}	5.91 ± 0.03 ^{b}	5,61 ± 0.17 ^{a}	5.76 ± 0.29
Tritable acidity (mL NaOH/100g fw)	10.04 ± 1.12 ^{a}	13.22 ± 0.21 ^{a}	16.87 ± 2.23 ^{b}	17.27 ± 2.27 ^{b}	14.35 ± 3.40
Total available carbohydrates	12.04 ± 0.48 ^{a}	20.92 ± 0.27 ^{c}	16.76 ± 1.40 ^{b}	16.63 ± 0.93 ^{b}	16.60 ± 3.08
Proteins	1.20 ± 0.10 ^{a}	2.02 ± 0.44 ^{a}	1.77 ± 0.25 ^{a}	1.64 ± 0.22 ^{a}	1.67 ± 0.36
Fat	0.23 ± 0.01 ^{b}	0.12 ± 0.00 ^{a}	0.14 ± 0.01 ^{a}	0.21 ± 0.03 ^{b}	0.18 ± 0.05
Fiber	3.56 ± 0.10 ^{a}	4.72 ± 0.43 ^{c}	4.08 ± 0.13 ^{b}	4.53 ± 0.14 ^{c}	4.23 ± 0.51
Energy (Kcal/100g)	59.40 ± 3.02 ^{a}	97.87 ± 1.89 ^{c}	80.03 ± 6.06 ^{b}	77.08 ± 3.06 ^{b}	78.92 ± 13.92
Ashes	0.97 ± 0.09 ^{c}	0.48 ± 0.01 ^{a}	0.91 ± 0.01 ^{c}	0.79 ± 0.02 ^{b}	0.79 ± 0.20

In each row, different letters mean significant differences between individual samples ($P<0.05$).

Compared with other species, *Allium ampeloprasum* showed higher carbohydrate content than those reported by Carnovale et al. (1989) for *A. sativum* and *A. porrum* (11.2 g/100 g and 6.75 g/100 g, respectively). Wild leek fiber content was higher than the levels found in cultivated species, *Allium porrum* (2.9%) (Souci et al., 2008). Fiber requirements are around 25 g per day (EFSA, 2010). As can be seen in Table 4, wild leek can be considered as an interesting source of dietary fiber, as a 100 g portion can provide 17 % of the daily amount required for adults, and can be considered as a "source of fiber", according the European Regulation 1924/2006 (more than 3 g/100g).

Table 2. Macro and microelements (mg/100 g fw; mean ± SD, n=3) of *Allium ampeloprasum* L. bulbs.

Macroelements (mg/100 g)	2007		2008		Global average
	Site 1	Site 2	Site 1	Site 2	
K	533.19 ± 19.68 ^{d}	146.62 ± 2.75 ^{a}	294.28 ± 9.20 ^{c}	232.87 ± 3.30 ^{b}	309.37 ± 164.25
Na	53.08 ± 10.40 ^{a}	48.30 ± 8.45 ^{a}	67.14 ± 10.83 ^{a}	43.64 ± 9.50 ^{a}	54.60 ± 12.75
Ca	30.24 ± 5.23 ^{a}	78.04 ± 7.84 ^{b}	81.74 ± 3.98 ^{b}	80.13 ± 11.98 ^{b}	70.16 ± 21.07
Mg	8.88 ± 1.08 ^{a}	15.44 ± 0.89 ^{c}	16.41 ± 1.64 ^{c}	13.50 ± 1.80 ^{b}	14.03 ± 2.95
Microelements (mg/100 g)					
Mn	0.14 ± 0.01 ^{b}	0.15 ± 0.02 ^{b}	0.08 ± 0.01 ^{a}	0.06 ± 0.00 ^{a}	0.11 ± 0.04
Fe	0.92 ± 0.17 ^{c}	0.20 ± 0.02 ^{a}	0.69 ± 0.18 ^{bc}	0.62 ± 0.10 ^{b}	0.60 ± 0.32
Zn	0.68 ± 0.02 ^{b}	0.03 ± 0.00 ^{a}	1.67 ± 0.02 ^{c}	0.61 ± 0.09 ^{b}	0.75 ± 0.63
Cu	0.22 ± 0.03 ^{c}	0.05 ± 0.01 ^{a}	0.11 ± 0.02 ^{b}	0.06 ± 0.01	0.11 ± 0.07

In each row, different letters mean significant differences between individual samples ($P<0.05$).

Other components of wild leek were proteins, with a content around 1.67 g/100 g, similar to proteins content in *A. sativum* and *A. porrum* (0.9 g/100 g and 2.1 g/100 g, respectively). Wild leek presented a total lipid content around 0.18 g/100 g, close to *A. porrum* and *A. sativum* (0.1-0.6 g/100 g). Regarding energy content (Table 1) wild leek presented global average of 78.92 kcal/100 g; this energetic value seems to be influenced mainly by TAC (positive correlation, $p<0.05$), as the main macronutrient of the bulb. The studied samples presented TAC and energy values in a range similar or higher than *A. porrum*, and lower than *A. sativum* (Souci et al., 2008; USDA, 2013).

Considering mineral content, *Allium ampeloprasum* stands out by K content, around 309.37 mg/100 g (Table 2), levels of Na and K in *A. porrum* are 4 and 279 mg/100g of edible portion, respectively; it can be seen that Na content is lower than the values observed in wild leek, and has a low Na content according to the requirements of European Regulation 1924/2006 (no more 0.12 g/100 g) *A. porrum* (leek) presented Ca content of 63 mg/100 g, and Fe levels of 0.81 mg/100 g. Both values are in the same range of the wild leek (30.24 to 81.7 mg/100 g and 0.20 to 0.92 mg/100 g, respectively) as it is showed in the literature (Souci et al., 2008).

Bioactive molecules and antioxidant activity

The contents of antioxidant compounds of hydrophilic nature, including vitamin C (AA and DHA) and other organic acid, in wild leek bulbs, are included in Table 3. Total vitamin C of samples presented annual fluctuations, ranging between 2.37 - 11.54 mg/100 g fw, depending on the location or year of gathering; AA was the major vitamin C form (4.30 mg/100 g fw), being around the double content of DHAA (2.14 mg/100 g fw). Comparing with its relatives, wild *Allium ampeloprasum* showed similar values to those found in cultivated *Allium Porrum* (5.15 mg/100 g; Tsouvaltzis et al., 2007) and *Allium sativum* (14 mg/100 g fw, Souci et al., 2008). Citric acid (CA, Table 1) was the main organic acid in the wild leek (0.12 g/100 g fw) follow by oxalic acid (0.8 g/100g). Several authors (e.g. Guil et al., 1996) recommended an oxalic acid/Ca ratio not higher than 2.5 in foods to avoid a decrease of Ca availability. In the present study, oxalic acid/Ca ratio was 1.18, so oxalic acid concentration in this bulb is not considered as to decrease Ca availability.

Table 3. Vitamin C (mg/100g fw, ascorbic and dehydroascorbic acid) and organic acids (g/100g fw) (mean ± SD, n=3) in *Allium ampeloprasum* L. bulbs.

	2007		2008		Global average
	Site 1	Site 2	Site 1	Site 2	
Ascorbic acid	4.23 ± 0.23 ^{b}	3.49 ± 0.26 ^{b}	7.89 ± 1.17 ^{c}	1.58 ± 0.27 ^{a}	4.30 ± 2.64
Dehydroascorbic acid	3.83 ± 0.21 ^{a}	1.15 ± 0.16 ^{a}	3.16 ± 2.36 ^{a}	0.43 ± 0.17 ^{a}	2.14 ± 1.61
Total Vitamin C	8.06 ± 0.41 ^{a}	4.77 ± 0.01 ^{a}	11.54 ± 1.23 ^{b}	2.39 ± 0.61 ^{a}	6.69 ± 3.98
Oxalic acid	0.01 ± 0.00 ^{a}	Tr.	0.24 ± 0.00 ^{b}	0.08 ± 0.02 ^{ab}	0.08 ± 0.07
Malic acid	0.01 ± 0.00 ^{a}	Tr.	0.16 ± 0.01 ^{b}	0.03 ± 0.01 ^{a}	0.07 ± 0.06
Citric acid	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.27 ± 0.05 ^{b}	0.21 ± 0.07 ^{b}	0.12 ± 0.02
Fumaric acid	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.06 ± 0.00 ^{b}	0.04 ± 0.02 ^{a}	0.04 ± 0.01

In each row, different letters mean significant differences between individual samples ($P<0.05$).

In the bulb studied the total phenolics and flavonoids content were shown (Table 5), with 5.77 mg GAE/g extract and 0.86 mg CE/ g extract, higher values comparing with the results of soluble phenolics content (0.369 mg GAE/g) obtained by Tsouvaltzis et al. (2007) in cultivated *Allium ampeloprasum* var. porrum.

As far as we know, this is the first report on tocopherols composition in wild leek (Table 4), with values of 0.05 mg/100 g of total tocopherols, being α-tocopherol the major form (0.3 mg/100 g). There

is information about cultivated species, *A. sativum* and *A. porrum*, which presented a higher total tocopherol content (100 and 547 mg/100 g, respectively) (Souci et al., 2008). At least twenty individual fatty acids were identified in the wild leek GC profile (Table 4). Saturated fatty acids (SFA) provide 38.23% of total fatty acids, being palmitic acid (PA, C16:0) the major one with 26.42% followed by C18:0 and C22:0, with 3.30 and 2.75%, respectively. MUFA provided only 7.61%, being oleic acid (C18:1n9) the major fatty acid (7.39%). PUFA content in wild leek was around 54.16%, and the major PUFA, LA (C18:2n6), with 53.45%. While α and γ-linolenic acids (ALA and GLA, 18:3n3 and 18:3n6) were not found in the analysed sample. PUFA/SFA ratio was also calculated; wild leek presented a good PUFA/SFA ratio, higher than 0.45 (Table 3). From these values, and following the requirements of European Regulation 1924/2006, this product could be labelled as "low fat" and even "fat-free" (content not higher than 0.5 g/100 g), as well as "saturated fat-free" (not exceeding 0.1 g/100 g).

Table 4. Lypophilic compounds in *Allium ampeloprasum* L. bulb (mean ± SD, n=3): Individual fatty acids (%) and tocopherols (mg/100g fw)**.**

Total SFA (%)	**38.23 ±0.63**	**Total MUFA (%)**	**7.61 ± 0.44**	**Total tocopherols (mg/100g fw)**	**0.05 ± 0.01** [b]
C8:0	0.33 ± 0.07	C16:1	0.22 ± 0.02	α-tocopherol	0.03 ± 0.01 [a]
C10:0	0.21 ± 0.02	C18:1n9	7.39 ± 0.42	α-tocopherol	nd
C11:0	0.05 ± 0.01	C20:1	nd	β-tocopherol	nd
C12:0	0.18 ± 0.03	**Total PUFA (%)**	**54.16 ± 0.29**	γ-tocopherol	0.02 ± 0.00 [a]
C13:0	0.04 ± 0.02	C18:2n6	53.45 ± 0.27	δ-tocopherol	
C14:0	0.64 ± 0.03	C18:3n6	nd		
C15:0	0.55 ± 0.03	C18:3n3	nd		
C16:0	26.42 ± 0.30	C20:2	0.17 ± 0.02		
C17:0	0.89 ± 0.13	C20:3n6	nd		
C18:0	3.30 ± 0.35	C20:3n3+C21:0	0.44 ± 0.04		
C20:0	0.80 ± 0.22	C20:5n3	0.10 ± 0.01		
C22:0	2.75 ± 0.05				
C23:0	0.34 ± 0.07				
C24:0	1.73 ± 0.49				

Different letters mean significant differences (P<0.05).

The wild leek antioxidant capacity was evaluated by four different *in vitro* assays (Table 5), DPPH and ferric reducing power assays were aplied to evaluate total antioxidant capacity, obtaining EC_{50} values ranging between 15.12 and 0.70 mg/mL of methanolic extract, respectively. β-carotene bleaching inhibition and TBARS assays were used for lipid peroxidation inhibition assay, with values around 1.66 and 0.11 mg/mL of extract, respectively.

Table 5. Antioxidant properties of methanolic extracts of *Allium ampeloprasum* L. bulb (mean ± SD, n=3).

Phenolics		**EC_{50} values (mg/mL methanolic extract)**	
Total phenolics GAE/g extract	5.70 ± 0.62	DPPH	15.12 ± 1.21 [d]
Total flavonoids CE/g extract	0.86 ± 0.05	Reducing power	0.70 ± 0.12 [b]
		β-carotene bleaching inhibition	1.66 ± 0.24 [c]
		TBARS assay	0.11 ± 0.01 [a]

Different letters mean significant differences (P<0.05).

Conclusions

Allium ampeloprasum L. bulb stood out as a good source of fiber (3.5 to 4.7 g/100 g), iron (0.20 to 0.92 mg/100 g) and zinc (0.03 to 1.67 mg/100 g), compared to other species of the same genus, as well as due to its low energy value (59-97 Kcal/100 g). Substantial levels of hydrophylic antioxidant compounds, including vitamin C (AA and DHA) and organic acids, total phenolics and flavonoid were found and also a good oxalic acid/Ca ratio. LA was the main fatty acid (53% of total fatty acids). The bioactivity of this bulb was better characterized by reducing power assay and TBARS method; the first one was better correlated with tocopherols, while TBARS assay was more correlated with total phenolics. However, the EC_{50} values were not as higher as estimated. Concerning nutritional molecules, wild leek can be considered as a good source of fiber, low Na content, fat-free and saturated fat-free (according to nutrition claims, regulated by Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods). Furthermore, this non-conventional wild bulb provides very low amount of energy, so it is a good alternative for low caloric diets.

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