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Original article

Effect of chlorophyll removal and particle size upon the nutritional and technological properties of powdered by-products from artichoke (*Cynara scolymus*, L.) industrial canning

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Summary In this study, the effect of the particle-size fractionation (Ø < 0.212 mm and 0.212 mm < Ø < 0.991 mm) and chlorophyll extraction on the nutritional and technological properties of the powdered artichoke ingredient was evaluated. The contents of minerals, protein, fat, carbohydrates and dietary fibre together with the content in bioactive compounds such as inulin and phenolics were determined. Other properties such as water- and oil-holding capacities, water activity and antioxidant capacity were measured. The ingredient with chlorophyll and the lowest particle size presented the highest phenolic content and antioxidant capacity (8.4 mg of vitamin C equivalents per 100 g of dry matter) and water- and oil-holding capacities. The removal of chlorophyll increased the oil-holding capacity (from 59.7% to 94.6%), which was much higher than in the coarse ingredient (34%), but has a deleterious effect reducing the antioxidant capacity and the inulin content. The ingredients also showed high dietary fibre (22.5–33%), inulin (9–16%) and mineral (7.5–7.8%) contents. Although the ingredient with chlorophyll and smaller particle size had the higher antioxidant capacity, the removal of chlorophyll improved the technological properties to be used as food ingredient without affecting significantly to the nutritional value.

Keywords Antioxidant capacity, artichoke, dietary fibre, inulin, total phenolics.

Introduction

Artichoke by-products are rich in bioactive compounds such as dietary fibre, inulin and phenolic compounds (Ruiz-Cano *et al.*, 2014). Fibre-rich by-products from the processing of cereals, fruits and vegetables can be recovered and used as value-added food supplements. They supply dietary fibre and bioactive compounds and may serve to improve physical and structural properties of hydration, oil-holding capacity and emulsion and oxidative stability (Elleuch *et al.*, 2011).

In food formulations, the presence of low amounts of fructans such as oligofructose and inulin is enough to be considered as functional food (Huebner *et al.*, 2007). Fructans have shown healthy effects as prebiotics, reaching the colon where they can modify the microflora (Sabater-Molina *et al.*, 2009). Different sources of fibre are increasingly being explored as an ingredient to provide desirable functional properties, including whole plant cell walls, and nonstarch oligosaccharides and polysaccharides, such as pectins and inulin (Prosky, 2000).

Previous studies have suggested that artichoke leaf extracts may have anticarcinogenic, hepatoprotective, antioxidative, antibacterial properties among others. The artichoke contains very little fat but a high content of minerals, vitamin C, fibre and polyphenolic compounds (Lattanzio *et al.*, 2009). The artichoke leaves, external bracts and stems represent a huge amount of discarded material from the industrial artichoke canning process. Artichoke flour has been used in bakery products as a functional ingredient in wheat bread, and it has even been used for technological purposes to reduce formulation costs (Frutos *et al.*, 2008). The removal of chlorophyll facilitates the application in a wider range of foods and improves some important technological properties such as the water- and oil-holding values that could be required for certain food formulations, and benefit products such as fat-free foods and low-fat foods (Vergara-Valencia *et al.*, 2007; Zhu, 2014).

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The main objective of this work was to investigate the effect of different processing steps in the powder obtained from artichoke by-products, such as the chlorophyll removal and particle-size fractionation, on its composition and technological properties as food ingredient.

Materials and methods

Chemicals and reagents

Folin–Ciocalteau reagent, inulin, gallic acid and ascorbic acid standards were purchased from Sigma-Aldrich (Buchs, Switzerland). Ethanol and HPLC-grade orthophosphoric acid were obtained from Merck.

Artichoke by-product ingredients preparation

By-products from artichoke (cultivar Blanca de Tudela) were collected from artichoke canning factories (Mediterránea de Ensaladas, S. C., El Raal, Murcia, Spain) close to the High Polytechnic School of Orihuela (Miguel Hernández University). An amount of 45 kg of artichoke by-products (87% moisture), consisting in the raw outer bracts and stems removed mechanically from artichoke heads, were divided into three independent batches that were prepared and analysed according to the experimental design. Each batch was washed and blanched at 78.4 °C for 60 s. After cooling with water to room temperature, the samples were drained and divided into five different batches and kept at −25 °C for a week before being dried in an oven at 60 °C for 24 h. The dried material from the five batches (ca. 5 kg) was milled in a grinding mill (Severin KM 3873, Germany) and passed through two sieves of 200 mm of diameter with a mesh of 0.212 mm (ASTM No. 70; CISA, Barcelona, Spain) and with a mesh of 0.991 (Tyler No. 16; Filtra, Barcelona, Spain), respectively, to obtain two fractions with different particle sizes AI-1 (Ø < 0.212 mm) and AI-2 (0.212 mm < Ø < 0.991 mm) (Fig. 1). A homogenous mixture was then formed with the artichoke

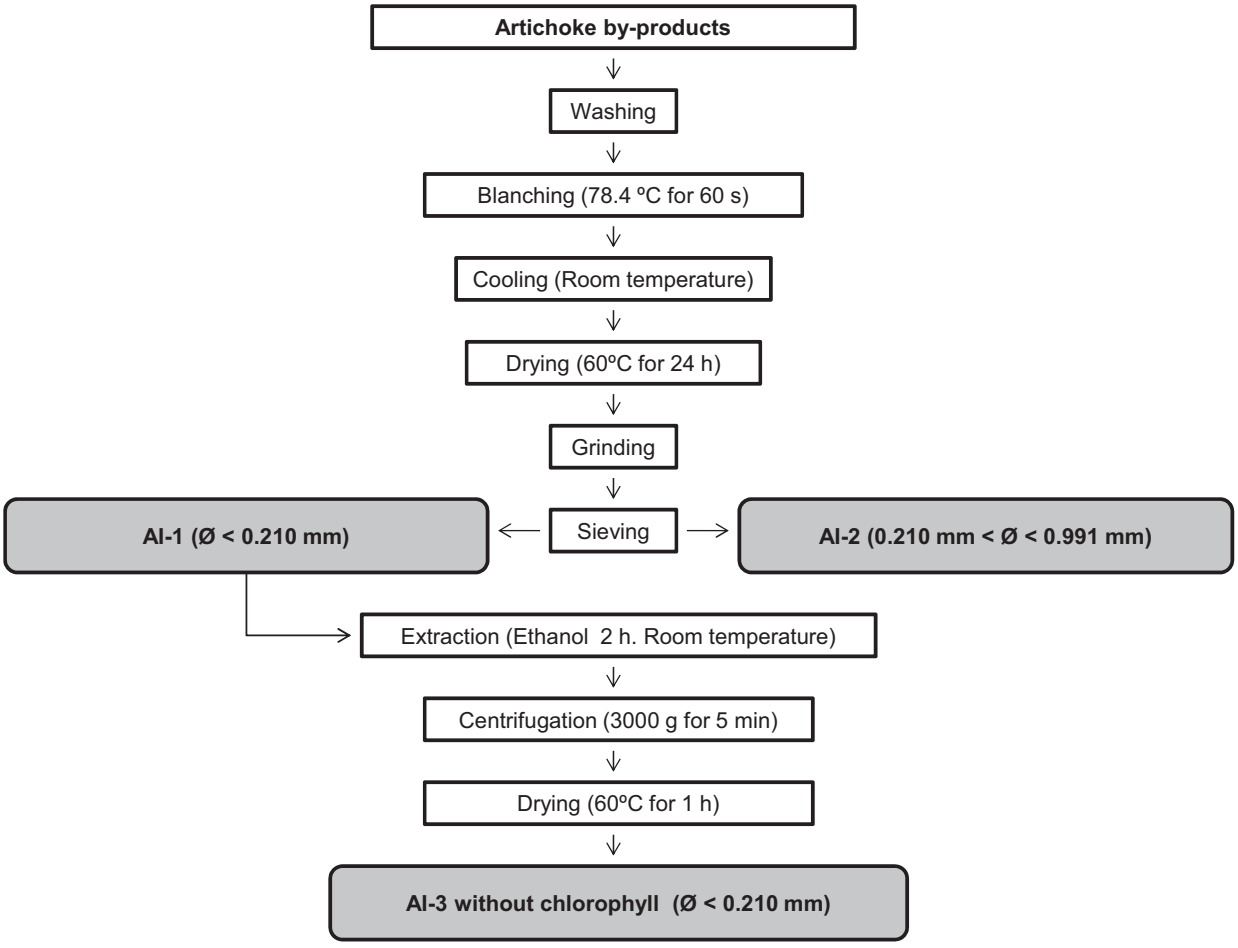

Figure 1 Flow diagram of the processing of the different artichoke powdered by-products.

ingredient (AI) obtained for each fraction. A separate particle size measurement has not been carried out.

To improve the colour characteristics of the fraction AI-1, the chlorophyll was extracted with different proportions of AI-1/ethanol 3, 4, 5, 7, 10 and 20 g AI-1 100 mL^{-1} of ethanol. Samples were kept for 2 h in flasks at 25 °C and then centrifuged at 3000 ***g*** for five min and dried in trays at 60 °C for 1 h to obtain the dried samples without chlorophyll (AI-3).

The powder of the two different fractions of each batch was stored in polyethylene bags at 3 °C until analysis in triplicate.

Chemical analysis

Ultra-pure water (Milli-Q Corp., Bedford, MA, USA) was used for all assays. The chemical parameters were analysed in the AI-1, AI-2 and AI-3 (4% w/v, AI/ethanol) ingredients using AOAC methods (2000, 2006): moisture (Method no. 945.15), ash (Method no. 942.05), protein (Method no. 920.54), fat (Method no. 920.39) and dietary fibre (Method no. 985.29).

Total carbohydrates were estimated by difference (meaning 100-the sum of moisture, protein, fat and ash). The energy value has been calculated using the conversion factors according to the European Council Nutrition Labelling Directive (EC, 1990).

Chlorophyll determination

A spectrophotometric system UV-Vis (Hewlett Packard 8453, Palo Alto, CA, USA) was used to determine the concentration of the chlorophyll *a* and chlorophyll *b* in the ethanol extracts. The measurement of absorbance was conducted in the chlorophyll extract at 663 and 645 nm, at the maximum absorbance for chlorophylls *a* and *b*. The chlorophyll concentration was calculated according to equations:

$$\text{Chlorophyll}\, a = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Chlorophyll}\, b = 22.9 \times A_{645} - 4.68 \times A_{663}$$

The concentration was expressed as milligrams total chlorophyll/100 g of AI.

Determination of inulin

Inulin content was estimated by high-performance liquid chromatography (HPLC) with a refractive index detector (RID) (Agilent 1100 Series HPLC System, Palo Alto, CA, USA), using a Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, St. Louis, MO, USA). The isocratic mobile phase used was 0.1% ortho-phosphoric acid in water, at a flow rate of 0.5 mL min^{-1}. The identification was performed by comparison with the retention time of inulin standard (Bancal & Gaudillere, 1989).

Determination of total phenolic content

Folin–Ciocalteu's reagent was used to determine the total content of the phenolic compounds of ingredients (Singleton *et al*., 1999). The ingredients were extracted in ultra-pure water (100 mg/20 mL), 0.5 mL of the extract was placed in a glass test tube with 1 mL of water, and 2.5 mL of Folin–Ciocalteu's reagent (10-fold diluted) was added. The reaction medium (3 mL) was allowed to react in darkness for 1 h, and the absorbance at 755 nm was measured with a UV-Vis spectrophotometer (Thermo Scientific Evolution 300 spectrophotometer, Loughborough, UK). The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of AI.

Determination of hydrophilic antioxidant activity

The hydrophilic antioxidant activity was measured in the samples using the method of Arnao *et al*. (1999), which is based on the ability of the antioxidants of a sample to reduce the cationic radical of 2,2′-azino-bis-3-(ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$), determined by the discoloration of $ABTS^{\cdot+}$ and measuring the quenching of the absorbance at 730 nm. This activity is calculated by comparing the values of the sample with a standard curve of ascorbic acid and expressed as milligrams of vitamin C equivalents (VCE) per 100 g of AI.

Physicochemical analysis

Water activity

The water activity (a_w) was determined in a TH-500 Sprint Novasina Thermo-constanter (Pfäffikon, Switzerland) at 25 °C.

pH

The pH was measured in a suspension resulting from blending 10 g AI with 10 mL of ultra-pure water for 2 min, using a pH meter (model pH/lon 510; Eutech Instruments Pte Ltd., Singapore).

Technological properties

The water-holding capacity (WHC) and oil-holding capacity (OHC) were measured according to Robertson *et al*. (2000). The sample (250 mg) was homogenised with 25 mL of ultra-pure water and it was left for 60 min to equilibrate and was centrifuged at 3000 ***g*** for 5 min at 4 °C, and then, the liquid was discarded and the residue was weighed. The same procedure was used for the determination of OHC using olive oil. The WHC was expressed as grams of water held per

gram of sample and the OHC as grams of oil held per gram of sample.

*Colour CIEL*a*b**

Colour was measured with a Minolta Chroma Meter CR-3200 (Minolta Camera Co., Osaka, Japan), with D_{65} as illuminant and an observer angle of 10°. In all determinations, low-reflectance glass Minolta CR-A51/1829-752 was placed on the samples. The CIELAB coordinates studied were lightness (L*), red/green (a*), yellow/blue (b*) and the psychophysical parameters, chroma (C*) and hue angle (H*). Chroma, indicating colour intensity, was calculated by the formula $(a^{*2} + b^{*2})^{1/2}$. The hue angle (H°) was calculated by the formula H° = arctan (b*/a*). The hue angle values vary from 0° (pure green colour) to 270° (pure blue colour).

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Comparisons of means were performed by one-way analysis of variance (ANOVA) followed by Duncan's test ($P \leq 0.05$). Statistical analyses were run using the computer SPSS 20.0 software (SPSS Institute Inc., Cary, NC, USA).

Results and discussion

The processing steps for the production of the artichoke ingredient fractions AI-1, AI-2 and AI-3 are represented in Fig. 1.

For the determination of the optimum proportion of ethanol for the preparation of AI-3, the amount of chlorophyll extracted and colour were measured. The higher amount of chlorophyll was extracted with 3% and 4% (w/v, AI/ethanol) (Fig. 2). The effect of the ethanol proportion on the CielL* a* b* H* and C* colour parameters is represented in Fig. 3.

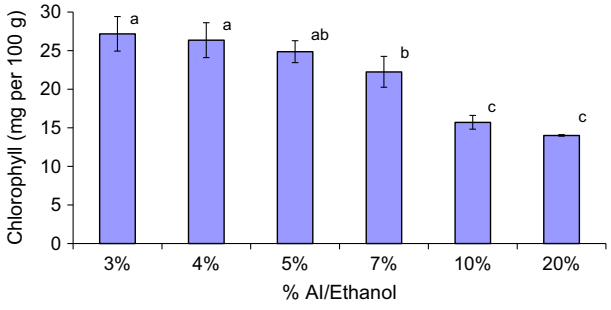

Figure 2 Chlorophyll (mg/100 g) extracted with different proportions (3%, 4%, 5%, 7%, 10% and 20% w/v) of artichoke ingredient AI-1 (Ø < 0.210 mm) and ethanol.

The L* values of the samples with the 3% and 4% (w/v, AI-1/ethanol) (61.5 and 62.0, respectively) indicate a higher lightness than the AI-1 ingredient (L* = 60.7). L* values of different flours samples varied between 59 and 62, which were lower to the 87.7, 89.5 y 94.4 found in taro, rice and pigeon pea flour, respectively (Kaushal *et al.*, 2012). All the extracted samples showed higher a* values than the AI-1, with similar values between the samples with the 3% and 10%, as a consequence of the reduction of the green colour in the chlorophyll-extracted samples. The AI samples showed similar H* values after the extractions, which were lower than those of AI-1. The ingredients after the extraction with 3% to 10% showed higher C* values than AI-1, and ranged between 19.3 and 20.7. Therefore, the parameters a*, H* and C* were similar in the extracted samples between the 3% and 10%. However, the ingredients prepared with 3% and 4% (w/v, AI/ethanol) showed the higher L* values. Those higher L* values correspond to a white flour and indicating a higher chlorophyll extraction yield (Fig. 2). The colour parameters are important for the different applications in food formulations.

The most cost-effective proportion of AI-1/ethanol (4%) was selected for the production of the AI-3 ingredient, as it was the one with less ethanol need for an optimum chlorophyll extraction.

Proximate composition

The results of proximate composition of the different artichoke ingredients are presented in Table 1. In all the ingredients, moisture ranged between 3.7% and 7.7%. The AI-3 and AI-2 ingredients showed significantly lower values ($P < 0.05$), presenting the AI-3 a 50% lower moisture contents with respect to the AI-1. The moisture content in AI-1 (7.7%) was higher than banana flour (6%), fibre-rich powder of banana flour (6.8%) (Rodríguez-Ambriz *et al.*, 2008), cowpea flour (7.4%) and horse gram flour (6.8%) (Sreerama *et al.*, 2012). Mineral content was similar in all the artichoke ingredients without significant differences ($P < 0.05$). The mineral contents (7.4–7.8%) were higher than those reported in previous studies in different commercial fibres (1–2%), fibre concentrates from citrus fruits residues (0.5–3.9%), fibre-rich powder of banana flour (2.3%), banana flour (4.4%), dried apple skin powder (ASP) of two apple cultivars; 'Northern Spy' (4.5%) and 'Idared' (4.9%), fibre-rich product of dried quince (*Cydonia oblonga* Miller) wastes (1.5%) and *Mangifera pajang* fibre (0.8%) (Wang *et al.*, 2002; Figuerola *et al.*, 2005; Rodríguez-Ambriz *et al.*, 2008; Rupasinghe *et al.*, 2008; de Escalada Pla *et al.*, 2010; Al-Sheraji *et al.*, 2011). The mineral composition of artichoke consists on relevant quantities of potassium, sodium, phosphorus, magnesium and calcium (Mataix *et al.*, 2003).

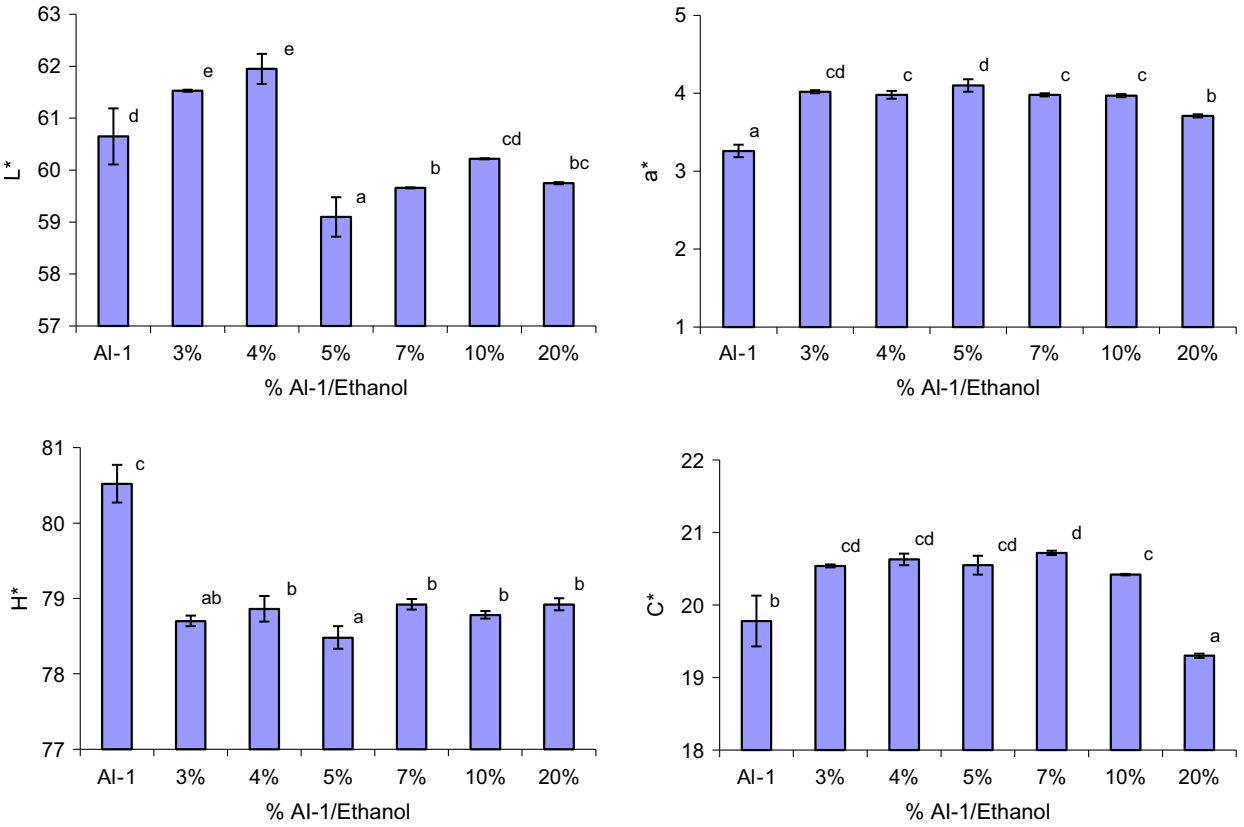

Figure 3 Colour values (L*, a*, H* and C*) of the artichoke ingredient (Ø < 0.210 mm) before (AI-1) and after (AI-3) the extraction of chlorophyll with different proportions of AI-I and ethanol (3%, 4%, 5%, 7%, 10% and 20% w/v).

The results of the protein analysis revealed a content of 13.7% in AI-3 and 12.9% in AI-2, with significantly lower contents ($P < 0.05$) with respect to AI-1 (15.2%). Proteins are important constituents of food; besides their nutritional significance, they contribute to flavour and texture of food. These protein amounts were considerably higher than those reported in fibre concentrates obtained from apple (3.7%), citrus fruit residues as lemon (7.9%) and orange (6.7%) (Figuerola *et al.*, 2005), quince by-products (6.6%) (de Escalada Pla *et al.*, 2010) and *Mangifera pajang* fibre (4%) (Al-Sheraji *et al.*, 2011), besides it was also higher than the protein content of commercial fibres with values ranging from 5% to 7% (Wang *et al.*, 2002). In comparison with other ingredients, the protein contents in the artichoke ingredients of this study were higher than those of the apple processing by-products (3%) (Rupasinghe *et al.*, 2008), banana flour (3.4%) and fibre-rich powder of banana flour (5.3%) (Rodríguez-Ambriz *et al.*, 2008), and grape seed flour obtained from wine by-products (11.5%) (Özvural & Vural, 2011).

Table 1 Proximate analysis of the artichoke ingredients expressed as % (w/w fresh weight basis)

	AI		
	AI-1	**AI-2**	**AI-3**
Moisture	7.7 ± 0.6^{b}	4.6 ± 0.5^{a}	3.7 ± 0.6^{a}
Minerals	7.5 ± 0.7^{a}	7.4 ± 0.7^{a}	7.8 ± 0.3^{a}
Proteins	15.2 ± 0.7^{b}	12.9 ± 0.1^{a}	13.7 ± 0.3^{a}
Fats	5.7 ± 0.5^{c}	4.3 ± 0.3^{b}	3.2 ± 0.7^{a}
Carbohydrates†	63.9 ± 1.5^{a}	70.8 ± 1.0^{c}	71.6 ± 1.3^{b}
Dietary fibre	22.6 ± 3.1^{a}	33.0 ± 1.6^{b}	22.5 ± 4.3^{a}
Energy value (kcal)	367.9 ± 2.2^{a}	373.3 ± 4.7^{a}	370.1 ± 0.5^{a}

AI: Artichoke ingredient. AI-1: (Ø < 0.210 mm). AI-2: (0.210 mm < Ø < 0.991 mm). AI-3: AI-1 without chlorophyll.
The values represent the mean values of fresh matter ± standard deviation (SD).
Different superscript letters within each row indicate significant differences ($P < 0.05$).
†By difference as 100-(moisture + protein + minerals + fat).

The fat content of the three artichoke ingredients ranged from 3.2% to 5.7% in the AI-3 and AI-1, respectively, with statistically significant differences ($P < 0.05$). The fat content was lower than those cited

by Rupasinghe *et al.* (2008) with 11.1% in ASP of Northern Spy and 10.2% in Idared, by de Escalada Pla *et al.* (2010) with 6.7% in fibre-rich products obtained from quince by-products and by Lecumberri *et al.* (2007) with 6.6% in dietary fibre-rich cocoa powder.

The carbohydrate content was more than 60% in all the artichoke ingredients, representing more than 70% in the AI-3 and AI-2 ingredients. The carbohydrate contents were significantly different ($P < 0.05$) in the three ingredients, with lower values for the AI-1 (63.9%).

The ingredients studied showed a high content in dietary fibre. The content was higher in the AI-2 (33%) than the AI-3 and AI-1 that presented similar values ($P < 0.05$). This could be due to the higher particle size and of the AI-2 ingredient, because the most fibrous parts from the artichoke heads present in the by-product (more external bracts and stems) are more resistant to the milling process and result in a fraction with a higher particle size. The content in AI-2 was also higher than rice bran (27%), mango dietary fibre concentrate (28%), peach dietary fibre concentrate (30.7%) and sesame coat (31.6%) (Elleuch *et al.*, 2011). Other fibre sources such as milled rye bran with a 37.7% have similar contents of dietary fibre (Rakha *et al.*, 2010). In comparison with other ingredients, the artichoke ingredients have higher dietary fibre content than banana flour (10.4%) (Rodríguez-Ambriz *et al.*, 2008) and legume flours (14.1–16.3%) (Sreerama *et al.*, 2012). The consumption of 100 g of AI-1, AI-2 and AI-3 would provide 14.4, 16.1 and 23.4 g of fibre, respectively, of the dietary intake.

Total phenolic content

The total phenolic content was higher in AI-1 with respect to the other artichoke ingredients ($P < 0.05$) (Table 2). The values were also higher than other phenolic sources as apple skin powder from Northern Spy (397.7 mg/100 g) and Idared (381.4 mg/100 g) apple varieties (Rupasinghe *et al.*, 2008). Considering the moisture content of the unprocessed artichoke by-product (86.2%), the total phenolic content in AI-1 (199 mg GAE/100 g fresh weight) would be also higher than other sources as fresh grapefruit (174 mg/100 g), freeze-dried grapefruit powder (156 mg/100 g) (Moraga *et al.*, 2012) and the food-processing by-products of other vegetables (raw and cooked potato peels: 23–45 mg/100 g fresh weight) (Mattila & Hellström, 2007). Ruiz-Cano *et al.* (2014) reported that the caffeic acid (di and caffeoylquinic acid) and polyphenols such as flavones luteolin-7-glucoside and apigenin-7-glucoside were observed in the variety *scolymus*, thus indicating the potential use of the artichoke by-product as source of phenolics acids and flavonoids.

Table 2 Technological properties, antioxidant capacity, total phenolic and inulin contents in the artichoke ingredients

	AI		
	AI-1	AI-2	AI-3
Antioxidant capacity (mg VCE/100 g)	8.4 ± 3.3[c]	4.2 ± 0.5[a]	4.8 ± 1.8[b]
Total phenolic content (mg GAE/100 g)	1330 ± 76[b]	1030 ± 53[a]	1110 ± 98[a]
pH	5.9 ± 0.2[a]	5.8 ± 0.1[a]	5.9 ± 0.1[a]
Inulin (g/100 g)	13.1 ± 0.3[b]	15.6 ± 0.2[c]	9.1 ± 0.2[a]
WHC (g/100 g)	266 ± 34.9[a]	299 ± 29.1[b]	296 ± 32.7[b]
OHC (g/100 g)	57.9 ± 5.2[b]	34.0 ± 3.1[a]	94.6 ± 8.3[c]
a_w	0.22 ± 0.01[b]	0.31 ± 0.02[a]	0.20 ± 0.02[c]

AI: Artichoke ingredient. AI-1: (Ø < 0.212 mm). AI-2: (0.212 mm < Ø < 0.991 mm). AI-3: AI-1 without chlorophyll. WHC: Water-holding capacity. OHC: Oil-holding capacity.
The values represent the mean values of fresh matter ± standard deviation (SD).
Different superscript letters within each row indicate significant differences ($P < 0.05$).

Antioxidant capacity

The antioxidant capacity was higher in AI-1 (8.4 mg VCE/100 g) with respect to the AI-3 (4.8 mg VCE/100 g) and AI-2 (4.2 mg VCE/100 g) ($P < 0.05$) (Table 2). The antioxidant capacity clearly increases with the reduction of particle size in the ingredients with lower values for AI-1. Esposito *et al.* (2005) in studies about the selected fractions of durum wheat bran by-products confirmed the antioxidant capacity increase in fractions having reduced granulometry. The presence of the phenolic compounds in these ingredients contributes to their antioxidant activity. Hence, the artichoke ingredients are a rich source of natural antioxidant phenolics and can be used as functional ingredients.

Inulin content

The inulin content (Table 2) was significantly different among the ingredients ($P < 0.05$). The AI-2 showed the higher inulin contents (15.6%) with respect to the AI-1 (13.1%) and AI-3 (9.1%). The chlorophyll extraction, although it was made with ethanol, resulted in a reduction of 30.5% of inulin in AI-3 with respect to the AI-1.

Water activity and pH

The a_w of the artichoke ingredients ranged from 0.20 to 0.31 ($P < 0.05$) (Table 2). The ingredients AI-1 and AI-3 showed a pH of 5.9. This value was higher than the pH of carrot powder 4.8 (Yousif & Alghzawi,

2000). According to Kha *et al*. (2010), a_w is a very important factor for the shelf life of powder products or dehydrated products, as a high a_w leads to biochemical degradations. Various authors have also reported that a_w values lower than 0.6 can prevent the deterioration of dried powder caused by micro-organisms and biochemical reactions being microbiologically safe (Kha *et al*., 2010). Therefore, the pH and a_w values confer a higher microbiological and a biochemical stability to the artichoke ingredients.

Water- and oil-holding capacity

The WHC presented values ranging from 266% in the AI-1, to 296% in the AI-3 and 299% in the AI-2. These high values suggest that the flour can be used in formulations of some foods such as meat, dairy and bakery products. The higher WHC in the artichoke ingredient with bigger particle size is in agreement with previous studies (Daubenmire *et al*., 1993) that reported a slightly higher water-holding capacity in coarsely ground navy bean hulls compared to finely ground hulls.

The WHC represents the amount of water retained in the fibre after centrifugation or external pressure (Raghavendra *et al*., 2004). According to this, it is strongly bound and does not affect the stool weight when consumed (de Escalada Pla *et al*., 2007) and also represents the water retained during technological operations such as kneading or during intestinal movement. The substitution of wheat flour in bakery products with these artichoke ingredients could contribute to the increase in the water retention required for optimum bread making absorption. The samples showed different OHC values being significantly higher for AI-3 (94.6%) and AI-1 (57.9%) with respect to the AI-2 (34%), ($P < 0.05$). Oil absorption depends on the microstructural characteristics of the fibre powders (de Escalada Pla *et al*., 2010). The highest OHC of the artichoke ingredients could be useful in structural interaction in food especially in flavour retention, improvement of palatability and extension of shelf life particularly in meat or bakery products where oil retention is required.

Conclusions

The powdered ingredients from processed artichoke by-products are an excellent source of dietary fibre, inulin and antioxidant phenolic compounds with beneficial effects on health. All the ingredients, regardless of the particle size, presented high dietary fibre, inulin and mineral contents. The ingredient with the larger particle size presented the highest values for dietary fibre and inulin. The artichoke ingredients represent a source of inulin for the development of functional foods and an important source of minerals due to their high ash content.

The levels of dietary fibre and inulin in the composition and the properties of the artichoke ingredients support the use of industrial artichoke by-products for the production of functional ingredients with health benefits that can be applied in the development of innovative functional foods.

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