Food Chemistry 116 (2009) 484–490

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of the extraction method on phytochemical composition and antioxidant activity of high dietary fibre powders obtained from asparagus by-products

J.M. Fuentes-Alventosa [a], S. Jaramillo-Carmona [a], G. Rodríguez-Gutiérrez [a], R. Rodríguez-Arcos [a], J. Fernández-Bolaños [a], R. Guillén-Bejarano [a], J.A. Espejo-Calvo [b], A. Jiménez-Araujo [a,*]

[a] Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), Avda. Padre García Tejero no 4, Sevilla 41012, Spain
[b] Consejo Regulador de la D.O.E. "Espárrago de Huétor Tájar", Ctra. de la Estación s/n, Huétor-Tájar-18360, Granada, Spain

ARTICLE INFO

Article history:
Received 20 October 2008
Received in revised form 12 January 2009
Accepted 25 February 2009

Keywords:
Asparagus by-product
Dietary fibre
Phytochemicals
Bioactive compounds
Flavonoids
Saponins
Ferulic acid
Fructooligosaccharides
Phytosterols
Antioxidant activity
Fibre-enriched foods

ABSTRACT

Asparagus (*Asparagus officinalis L.*) spears are highly appreciated for their composition of bioactive compounds. The method by which their by-products are treated affects the phytochemical composition and antioxidant activity of the fibre-rich powders. Factors such as the treatment intensity, the solvent used, and the drying system were studied. Among the asparagus phytochemicals, hydroxycinnamic acids (HCA), saponins, flavonoids, sterols, and fructans were quantified. HCA varied from 2.31 and 4.91 mg/g of fibre, the content being affected by the drying system and, in some cases, the solvent. Fibres from intense treatments had significantly higher amounts of saponin than samples isolated by gentle treatments. Saponin content ranged from 2.14 to 3.64 mg/g of fibre. Flavonoids were the most affected by processing conditions, being present (0.6–1.8 mg/g of fibre) only in three of the samples analysed. Continuous stirring during processing could be the main reason for this result. Sterols and fructans were present in minor amounts, 0.63–1.03 mg/g of fibre and 0.2–1.4 mg/g of fibre, respectively. Soluble and total antioxidant activities were also measured. Fibres with the highest activities corresponded to those with the highest levels of flavonoids and HCA.

1. Introduction

Besides their culinary quality, green asparagus spears are appreciated for their composition of bioactive compounds. Eastern civilisations have been using asparagus extracts as stimulants, laxatives, antitussives, diuretics, etc. for hundreds of years. In recent pharmacological studies (Kamat, Boloor, Devasagayam, & Venkatachalam, 2000; Wiboonpun, Phuwapraisirisan, & Tip-pyang, 2004; Yu et al., 1996), these extracts have been shown to have several biological activities, including antitumoral and antioxidant activities. Among all the bioactive compounds present in asparagus spears, saponins, flavonoids, and hydroxycinnamates are the main compounds responsible for the characteristics cited above. Asparagus saponins are steroidal glycosides, with protodioscin being the most abundant. Several activities of this compound have been described, with cytotoxicity against several lines of human cancerous cells (Chin, 2006; Hibasami et al., 2003; Wang et al., 2003) being of special interest. Flavonoids are phenolic compounds with high antioxidant activity. Additionally, they have antitumoral and antimicrobial activities, and participate in the prevention of cardiovascular diseases (Cushine & Lamb, 2005; Nijveldt et al., 2001). Rutin is the most abundant flavonoid in asparagus spears, in addition to others that have been recently described (Fuentes-Alventosa et al., 2007, 2008). Hydroxycinnamic acids, especially ferulic acid, are strong antioxidants. Thus, ferulic acid may be beneficial in the prevention of disorders linked to oxidative stress, including Alzheimer's disease, diabetes, cancers, hypertension, atherosclerosis, inflammatory diseases, and others (Zhao & Moghadasian, 2008). If linked to dietary fibre, ferulic acid would be desesterified in the intestinal lumen, which could offer a way to provide a slow-release form of ferulic acid that might provide a prolonged physiological effect (Plate & Gallaher, 2005).

Plant sterols (phytosterols) and fructans, mainly fructooligosaccharides, are also present in asparagus spears, but in lower amounts. The nutrition role of phytosterols is based on their cholesterol-lowering effect in human blood, based on their ability to competitively inhibit intestinal cholesterol uptake (Jiménez-Escrig, Santos-Hidalgo, & Saura-Calixto, 2006). β-Sitosterol is the most abundant compound within this group of phytochemicals. Fructooligosaccharides (FOS) have a beneficial effect on human health because they are prebiotics. FOS are not hydrolysed by digestive

* Corresponding author.
E-mail address: araujo@cica.es (A. Jiménez-Araujo).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.02.074

enzymes, but gut microbiota are able to ferment them. Several studies have found that FOS and inulin promote calcium absorption in both the animal and human gut (Heuvel, Muys, Dokkum, & Schaasfma, 1999; Zafar, Weaver, Zhao, Martin, & Wasttney, 2004). The intestinal microflora in the lower gut can ferment FOS, which results in a reduced pH. Calcium is more soluble in acid and, therefore, more is released from the food and is available to move from the gut into the bloodstream.

Of the asparagus-producing countries, Spain ranks fifth after China, Peru, the USA, and Germany. During industrial processing, around half of the total length of each spear is discarded, which creates significant waste for producers. Assuming that the by-products have a similar composition to the edible part of the spears, their fibre-rich products could contain significant amounts of all the phytochemicals mentioned above. In addition to changes in chemical composition and functional characteristics of the fibres (Fuentes-Alventosa et al., 2009), by-product processing conditions could modify the composition of bioactive compounds and, in doing so, the intrinsic antioxidant activity of these fibre-rich products. The aim of this work is to characterise phytochemicals from asparagus fibres and to study the effects that processing conditions have on their composition and antioxidant activity.

2. Materials and methods

2.1. Asparagus by-products

Asparagus by-products were obtained from Centro Sur S.C.A. (Huétor-Tajar, Granada, Spain). Prior to canning, freshly harvested asparagus spears were cut to obtain the 15 cm long upper portion (edible part) and the rest of spear (15–18 cm) was considered a by-product. This by-product was sent to our lab within the next 24 h and held at 4 °C until processing.

2.2. Asparagus by-product treatment

Three variables were studied (Table 1): extraction treatment (intense – 90 min at 60 °C, or gentle – 1 min at room temperature), extraction solvent (water or 96% ethanol), and drying system (freeze-drying or oven treatment at 60 °C for 16 h). Two kilograms of the by-product portions were cut, homogenised, and mixed using a professional homogeniser (Sirman Orione, Marsango, Italy). Homogenisation was performed at top speed with each of the extraction solvents in a 1:1 ratio (solid:liquid) (w/v), and at the programmed temperature (60 °C or room temperature) for 1 min. Afterwards, gently-extracted samples (G) were directly processed using a industrial juicer (Tecno-Chufa, Valencia, Spain) to separate the asparagus liquid extract from the wet fibrous residue. Intensely-extracted samples (I) were treated in an open reactor designed in our laboratory, with time and temperature controls and continuous stirring. After 90 min of extraction, samples were centrifuged as described above. Each treatment was performed in duplicate. One batch of wet fibrous residue was freeze-dried (F-D) and the other dried in an oven (O-D). Dried fibres were ground in a hammer mill to a particle size lower than 1 mm and stored at 4 °C until analysis.

2.3. Determination of bioactive compounds

2.3.1. Hydroxycinnamic acids

Total hydroxycinnamic acids (HCA) present in fibre samples were extracted and quantified as previously described (Jaramillo et al., 2007). Briefly, samples (in duplicate) were treated with 2 N NaOH for 24 h, at room temperature, under nitrogen and darkness. After filtration, *trans*-cinnamic acid was added as an internal standard. Solutions were acidified and extracted three times with ethyl acetate. Ethyl acetate extracts were evaporated under nitrogen, re-dissolved in 50% methanol, and analysed by HPLC. Phenolic compounds were quantified using a Synergy 4μ Hydro-RP80A reverse-phase column (25 cm × 4.6 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, UK). The gradient profile was formed using solvent **A** (10% aqueous acetonitrile plus 2 ml/l acetic acid) and solvent **B** (40% methanol, 40% acetonitrile, and 20% water plus 2 ml/l acetic acid) in the following program; the proportion of **B** increased from 10% to 42.5% for the first 17 min, held isocratically at 42.5% for a further 6 min, increased to 100% over the next 17 min, and finally returned to the initial conditions. The flow rate was 1 ml/min. HCA were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μl loop). Quantification was performed by integration of peak areas at 280 nm, with reference to calibrations done using known amounts of pure compounds.

2.3.2. Extraction of saponins and flavonoids

Two and a half grams of each fibre (in duplicate) were extracted with 100 ml of 80% ethanol. The samples were blended in a Sorvall Omnimixer, Model 17106 (Du Pont Co., Newtown, CT), at maximum speed for 1 min, and then passed through filter paper. Ethanolic extracts were stored at −20 °C until analysis.

2.3.3. Quantification of saponins

Five millilitre aliquots (in duplicate) of each ethanolic extract were dried under air flow and re-dissolved in 2 ml of distilled water by sonication. Water solutions were extracted twice with 2 ml of hexane, ethyl acetate, and butanol, sequentially. Butanol extracts were collected and dried under air flow, the residue was re-dissolved in 2 ml of distilled water, and then loaded onto a 1 ml Sep-Pak C_{18} cartridge (Waters Corporation, Milford, MA) preconditioned with 96% ethanol. Cartridges were washed with 5 ml of water and then with 5 ml of 96% ethanol. Ethanol fractions were assayed for saponin content.

A colorimetric method for saponin quantification was developed in our laboratory based on reactive anisaldehyde–sulphuric acid–acetic acid for TLC staining (Wang, Lii, Chang, Kuo, & Chen, 2007). Two hundred microlitres of purified ethanol fractions were dispensed in quadruplicate and 400 μl of a reactive acid (sulphuric

Table 1
Different conditions for obtaining a fibre-rich powder from asparagus by-product.

	Description
I/W O-D	Intense treatment (90 min at 60 °C) with water and oven-drying
I/W F-D	Intense treatment (90 min at 60 °C) with water and freeze-drying
I/E O-D	Intense treatment (90 min at 60 °C) with ethanol and oven-drying
I/E F-D	Intense treatment (90 min at 60 °C) with ethanol and freeze-drying
G/W O-D	Gentle treatment (1 min at room temperature) with water and oven-drying
G/W F-D	Gentle treatment (1 min at room temperature) with water and freeze-drying
G/E O-D	Gentle treatment (1 min at room temperature) with ethanol and oven-drying
G/E F-D	Gentle treatment (1 min at room temperature) with ethanol and freeze-drying

acid:acetic acid, 1:1) were added. After mixing, 20 μl of *p*-anisaldehyde were added to three of the replicates, with the same volume of water being added to the fourth to be used as a sample blank. All tubes were heated at 95–100 °C in a water bath for 2 min and then cooled with tap water. The absorbance at 630 nm was determined. In each determination, a calibration curve was done, using diosgenin as a reference standard.

2.3.4. Quantification of flavonoids

Flavonoids were detected and quantified by HPLC (Fuentes-Alventosa et al., 2007) using a Synergy 4μ Hydro-RP80A reverse-phase column as before. The gradient profile was formed using solvent **A** (10% aqueous acetonitrile plus 2 ml/l acetic acid) and solvent **B** (40% methanol, 40% acetonitrile, and 20% water plus 2 ml/l acetic acid) in the following program; the proportion of **B** increased from 10% to 42.5% **B** for the first 15 min, increased to 70% over the next 6 min, remained at 70% for 3.5 min, increased again to 100% over the next 5 min, and finally returned to the initial conditions. The flow rate was 1 ml/min, and the column temperature was 30 °C. Flavonoids were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μl loop). Spectra from all peaks were recorded in the 200–600 nm range, and the chromatograms were acquired at 360 nm. Quantification was performed by integration of peak areas at 360 nm, with reference to calibrations done while using known amounts of pure compounds.

2.3.5. Sterols

The methods applied for hydrolysis, saponification, silylation and quantification were adapted from Jiménez-Escrig et al. (2006). Fibre samples (0.5 g in duplicate) were weighed in 50 ml screw-capped Erlenmeyer flasks, 4 ml of internal standard solution (20 μg cholesterol/ml ethanol) and 10 ml of 6 M HCl were added to each sample, and the flask were heated at 80 °C for 1 h in a shaking water bath. The flasks were cooled to room temperature and 20 ml of hexane:diethyl ether (1:1) mixture were added. The samples were shaken for 10 min and allowed to stand for phase separation. The upper phase was evaporated to dryness under vacuum at 50 °C. Saponification was performed as follows: 8 ml of 2 M ethanolic KOH were added to the dry extracts, the mixtures were transferred to a 50 ml screw-capped Erlenmeyer flask, and heated at 80 °C for 30 min in a shaking water bath. Then, 20 ml of cyclohexane and 12 ml of distilled water were added to each sample. The samples were shaken for 10 min and allowed to stand for phase separation. The unsaponifiable upper phase was evaporated to dryness under vacuum at 50 °C. The residue was re-dissolved in 1 ml of dichloromethane and loaded onto a 1 ml Sep-Pack C_{18} cartridge, preconditioned with methanol. Sterol fractions were eluted with 15 ml of dichloromethane:methanol (95:5) and evaporated to dryness under vacuum at 50 °C. The residue was re-dissolved in 0.5 ml of dichloromethane. Aliquots of 100 μl of the sterol fractions were placed in a pre-silanised screw-capped vial. The solvents were evaporated under nitrogen and the TMS ether derivatives of the sterols were prepared by adding 100 μl of the silylation reagent (BSTFA:TMCS, 99:1) and 100 μl of anhydrous pyridine. The samples were then heated at 60 °C for 30 min or left overnight at room temperature for silylation. The excess silylating reagent was removed under nitrogen at 50 °C, and the residue was dissolved in 600 μl of hexane. Sterol quantification was by GC. A Hewlett–Packard 5890 Series II chromatograph, fitted with a 30 m × 0.25 mm (film thickness = 0.25 μm) cross-linked methyl siloxane capillary column (HP-1 from Agilent, Santa Clara, CA), was employed. The oven temperature program used was as follows: initial, 50 °C, 2 min; raised at 30 °C/min to 245 °C, 1 min; raised at 3 °C/min to 275 °C, and held for 28.5 min. The carrier gas was helium at a flow rate of 1 ml/min. The injector temperature was 250 °C and the FID temperature was 280 °C.

2.3.6. Fructans

Fructan amounts were determined using a Megazyme kit (K-FRUC), whose procedure is described in detail at http://www.megazyme.com/downloads/en/data/K-FRUC.pdf. This assay is based on AOAC method 999.03 and AACC method 32.32. Briefly, fibre samples (0.5 g in duplicate) were extracted twice with 20 ml each of hot distilled water for 15 min at 80 °C with continuous stirring. After filtering through glass filter paper, both extracts were collected and the volume made up to 50 ml. Two aliquots of 50 μl of the extracts were placed in test tubes and 50 μl of sucrase/amylase solution were added. After incubation (30 min, 40 °C), 50 μl of a 10 mg/ml sodium borohydride solution in 50 mM sodium hydroxide were added and the tubes were incubated under the same conditions. To remove excess borohydride and adjust the pH to approximately 4.5, 125 μl of 0.2 M acetic acid were added. Carefully, 50 μl of this final solution were transferred to test tubes (×3), and 25 μl of fructanase solution were added to two of the tubes and 25 μl of 0.1 M sodium acetate buffer were added to the third (sample blank). After incubation (40 °C for 20 min) to produce complete hydrolysis of fructans, 1.25 ml of *p*-hydroxybenzoic acid hydrazide (PAHBAH), reactive for reducing sugars, were dispensed into each tube. Tubes were placed in a boiling water bath for 6 min and allowed to cool in cold water. Absorbance at 410 nm was measured against a reagent blank. During each determination, a calibration curve was prepared using fructose as reference standard.

2.4. Antioxidant capacity

Soluble antioxidant activity was determined after fibre extraction (in duplicate) with methanol:water (50:50) and acetone:water (70:30) (Larrauri, Rupérez, & Saura-Calixto, 1997) by the DPPH· method (Rodríguez, Jaramillo, Rodríguez, et al., 2005). Total antioxidant activity was evaluated as described by Serpen, Capuano, Fogliano, and Gokmen (2007). Between 3 and 20 mg of fibre was transferred to an Eppendorf tube (for weights lower than 3 mg, fibre had to be diluted with cellulose as inert material), and the reaction was started by adding 1 ml of the DPPH· reagent (3.8 mg/50 ml methanol). After 30 min of continuous stirring, samples were centrifuged and the absorbance of the cleared supernatants was measured (in triplicate) at 480 nm. Both antioxidant activities were expressed as millimoles of Trolox equivalent antioxidant capacity (TEAC) per kilogram of sample by means of a dose–response curve for Trolox.

2.5. Statistical analysis

Results were expressed as mean value ± standard deviation. To assess for differences in the composition and physicochemical characteristics between the different treatments, multiple sample comparison was performed using the Statgraphics Plus program Version 2.1. (StatPoint Inc., Warrenton, VA). Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to contrast the groups. The level of significance was $p < 0.05$.

3. Results and discussion

Asparagus by-products could be considered an interesting source of dietary fibre. By-product processing involves several factors that affect fibre composition and functionality (Fuentes-Alventosa et al., 2009). In this work, we report the composition of bioactive compounds and the antioxidant activity of a fibre-rich

product obtained from asparagus by-product, which may be used as an ingredient in the preparation of additional food products. Three factors have been taken into account (severity of treatment – temperature and time, solvent, and drying system) and their effects on fibre characteristics will be discussed.

3.1. Bioactive compound composition

Table 2 shows the composition of several bioactive compounds analysed in fibre-rich powders obtained from asparagus by-products.

3.1.1. Hydroxycinnamic acids

The hydroxycinnamic acids present in the green spears are mainly coumaric acid, ferulic acid, and its dimers. Middle and basal portions of the spears are richer in these compounds than the upper portion, especially after a storage period (Rodríguez, Jaramillo, Guillén, et al., 2005). In the fibre-rich powders assayed, the amount of HCA ranged from 2.31 to 4.91 mg/g, with ferulic acid derivatives (FAD) of 0.8 to 1.8 mg/g. In both intense and gentle treatments, significant differences were caused by the choice of drying system, with higher HCA content in the freeze-dried samples than in those dried in an oven. In the intense treatment process, the solvent did not cause any differences, but in the gentle process, fibres obtained with water had the highest HCA content.

The amounts of HCA quantified in the fibre-rich powders were higher than previously reported for green asparagus (0.6 mg HCA/g cell wall material in fresh asparagus and 1.6 mg HCA/g of cell wall material in stored spears; Rodríguez, Jaramillo, Guillén, et al., 2005), but lower than that found in the white spears, (0.7 mg FAD/g of cell wall material in fresh asparagus and 2.9 mg FAD/g of cell wall material in stored spears; Jaramillo et al., 2007). It is important to take into account that these authors, when working with green asparagus, analysed only the edible portion of the spears. HCA and FAD (monomers, dimers and trimers) especially are related to asparagus hardening during storage, *via* cross-linking of plant cell wall polymers (Rodríguez-Arcos, Smith, & Waldron, 2004). Asparagus by-products are harder and more fibrous than the edible portions, therefore, their HCA and FAD content must be higher than that of edible portions. One gram of these fibre-rich powders brings to the diet as much FAD as a serving of some fruits (berries, plums, and apples), vegetables (tomato, carrot, and lettuce), and beer (Zhao & Moghadasian, 2008). From a nutritional point of view, FADs have been considered to impede the degradation of polysaccharides by intestinal bacteria. Recent studies (Funk, Braune, Grabber, Steinhart, & Bunzel, 2007) indicate that low to moderate amounts (1.5–15.8 mg FAD/g of cell wall material) do not interfere with hydrolysis of cell walls by human gut microbiota. Additionally, FADs have high antioxidant activity (Chen & Ho, 1997) and many other potential health benefits (Plate & Gallaher, 2005). When linked to arabinoxylan oligosaccharides, they stimulate the growth of *Bifidobacterium bifidum* (Yuan, Wang, & Yao, 2005).

3.1.2. Saponins

Saponins are also present in the fibre-rich powders from asparagus by-products. These bioactive compounds are present in amounts between 2.14 and 3.64 mg/g. During intense treatments, the samples treated with ethanol had higher saponin content than those treated with water, but during gentle treatments, all the samples had almost the same saponin content. In general, fibres from gentle treatments had significantly lower saponin content than samples from intense treatments, probably due to a higher solubilisation of material in the latter. In this work, we quantified saponin amounts by spectrometry, so the results expressed here could be overestimated. However, previously reported data on the basal zone of asparagus shoots (Wang et al., 2003) by LC/MS (25 mg protodioscin/100 g of fresh sample) are in agreement with our results. Garlic and onion are two species of the *Allium* genus (*A. sativum* and *A. cepa*, respectively) widely used in folk medicine as hypotensive, hypoglycaemic, antimicrobial, antithrombotic, anticarcinogenic, and diuretic agents. These pharmacological activities are partially due to steroidal saponins. The content of this compound in Allium species is 2–3 mg/g of dry matter (Smoczkiewicz, Nitschke, & Wieladek, 1982), similar to the quantified amounts in the asparagus fibre-rich powders.

3.1.3. Flavonoids

Other bioactive compounds quantified in the asparagus by-product, fibre-rich powders are flavonoids. Asparagus flavonoids (mainly rutin) are partly responsible for the antioxidant characteristics of this vegetable (Guillén et al., 2008; Makris & Rossiter, 2001; Rodríguez, Jaramillo, Rodríguez, et al., 2005). Only three of the analysed samples (Table 2) had significant amounts of flavonoids. Makris and Rossiter (2001), working with green asparagus, concluded that simply chopping and macerating caused a decrease of as much as 18.5% in rutin content without the liberation of quercetin. Therefore, rutin must be oxidatively cleaved rather than hydrolysed. However, hydrolysis might occur to some extent, but the quercetin was oxidised as soon as it was liberated from the sugar, and, thus, did not accumulate in detectable amounts. The authors in the same work quantified a 43.9% decrease in total flavonols when asparagus was boiled for 60 min. Asparagus had high levels of peroxidase activity (Rodríguez, Guillén, Heredia, Fernandez-Bolaños, & Jiménez, 1999), with these enzymes having some specificity for flavonols (Hirota, Shimoda, & Takahama, 1998). As observed, several factors could affect the stability of flavonoids during vegetable processing. Additionally, in our processing experiences, another factor must be taken into account, since continuous stirring was applied during 90 min. of processing in an open reactor. Therefore, flavonol oxidation caused by air inclusion within the asparagus by-product homogenate may occur

Table 2
Bioactive components of different fibrous residues from asparagus by-products (mg/g dry matter).[a]

		Hydroxycinnamic acids[b]	Saponins	Flavonoids	Sterols	Fructans
I/W	O-D	2.81 ± 0.29a	2.50 ± 0.12a	–	0.82 ± 0.04a	–
	F-D	3.89 ± 0.31b	3.18 ± 0.33b	–	0.81 ± 0.01a	0.21 ± 0.03a
I/E	O-D	3.01 ± 0.04a	3.37 ± 0.01bc	0.64 ± 0.03a	1.03 ± 0.06b	1.24 ± .09b
	F-D	3.83 ± 0.10b	3.64 ± 0.33c	1.82 ± 0.31b	1.05 ± 0.01b	1.43 ± 0.25c
G/W	O-D	3.07 ± 0.23a	2.44 ± 0.07a	1.08 ± 0.09a	0.64 ± ± 0.09c	0.33 ± 0.04a
	F-D	4.91 ± 0.30c	2.56 ± 0.24a	–	0.63 ± ± 0.07c	0.26 ± 0.03a
G/E	O-D	2.31 ± 0.06d	2.14 ± 0.02d	–	0.83 ± 0.06a	0.20 ± 0.04a
	F-D	2.85 ± 0.01a	2.74 ± 0.01a	–	0.92 ± 0.05a	0.77 ± 0.19d

[a] All analyses are done in quadruplicate at least.
[b] Means within a column bearing the same letter are not significantly different at 5% level, as determined by the Duncan multiple range test.

to some extent. Presently, an optimised process (patent pending) is being applied to obtain fibre-rich powders from asparagus, in which most of the factors affecting flavonoid stability have been controlled. The relatively low content of flavonoids in these samples led to low levels of antiradical activity, which will be commented on below.

3.1.4. Sterols

Plant sterols are bioactive compounds present in asparagus spears and, therefore, in their by-products. In the fibre-rich powder, the amount of analysed phytosterol varied between 0.63 and 1.03 mg/g. The amount of phytosterol was affected by the solvent and intensity of treatment, but not by the drying system. Samples treated with ethanol had higher amounts of sterols than those treated with water. Samples obtained by gentle processing had lower amounts of sterols than those obtained from intense treatment. As seen in Fig. 1, β-sitosterol was the most abundant sterol (more than 50% of total quantified sterols), as is the case in most fruits and vegetables (Jiménez-Escrig et al., 2006).

3.1.5. Fructans

Fructans were also present in asparagus by-products and their amounts in the fibres varied with processing method, from 0.2 to 1.4 mg/g of fibre. The highest content was found in fibres obtained after intense treatment with ethanol (I/E), with the rest of the fibres having almost the same fructan content. Fructans present in asparagus were mainly fructooligosaccharides (FOS – with low polymerisation degree) instead of inulin (high molecular weight) (Shiomi et al., 2007). FOS content of asparagus by-product was around 4 mg/g of dry weight (data not shown) and during the fibre obtaining process, it decreased between 25% and 95%, a reduction that was explained by the high solubility of these low molecular weight carbohydrates (3–4 polymerisation degree) in water/ethanol mixtures, even at room temperature.

3.2. Antioxidant activity

To measure antioxidant activity, the antiradical capacity (ARC) against DPPH· was assayed in both the soluble fraction and total fibre. In Fig. 2, the dose–response lines are presented. The correlation between dose and decrease in DPPH· concentration was very high in all cases ($r > 0.9$). There are two fibres that have almost the same ARC; these were I/E, F-D and G/W, O-D fibres, which also had the highest content of flavonoids (Table 2). The I/E, O-D fibre also contained flavonoids and the ARC of its soluble extract (Subfigure (a)) was near those commented on above. Soluble extract from G/W, F-D fibre, in which flavonoids were not detected, had a lower ARC than extracts with these antioxidant compounds. However, looking at the total ARC of the fibres (Subfigure (b)) I/E, O-D and G/W, F-D fibres had the same ARC. This fact could be explained by the fact that although the latter fibre did not contain flavonoids, it had the highest amount of hydroxycinnamates (Table 2). These compounds, especially the ferulates, have a high antioxidant capacity and are mainly found esterified to fibre polymers in asparagus spears, therefore the ARC caused by ferulates could be measured only in the total antioxidant assay. The rest of the fibres (I/W and G/E) had similar ARC, which was much lower than those described above were.

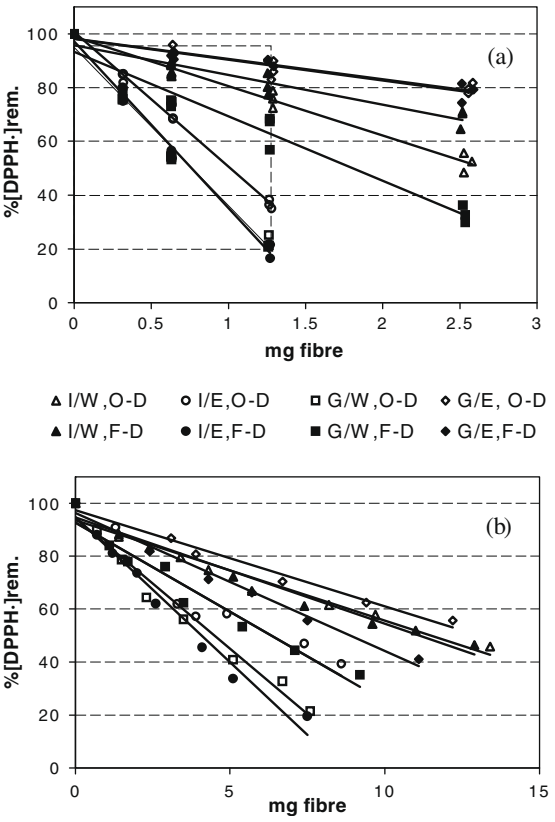

Fig. 2. Dose–response lines of antiradical capacity of asparagus by-product fibres. Antiradical capacity is expressed as percent DPPH· remaining in solution (%DPPH· rem.) after 30 min of reaction. (a) Antiradical capacity of soluble fractions. (b) Total antiradical capacity of fibres. Each individual point in the graphs is the average value of three replicates.

In Table 3, the results for antioxidant capacity expressed as Trolox equivalents/g of fibre (TEAC) are presented. The strongest antioxidant fibres (I/E, F-D, and G/W, O-D) had an equivalent of about 13 μmol Trolox/g of total fibre or 11 μmol Trolox/g of fibre (soluble extract). The second strongest ARC group (I/E, O-D and G/W, F-D) had about half of the activity listed above, and the rest at about one-third. Looking at the soluble activity only, there were differences within the last group, with G/E fibres having the lowest activity, nearly one tenth of that determined for the I/E, F-D and G/

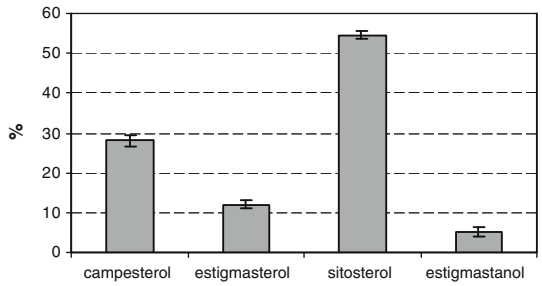

Fig. 1. Percentage contribution of each sterol to the total sterol quantified. Error bars correspond to the average value of the eight analysed samples.

Table 3
Antioxidant activity (total and soluble) of the fibre-rich asparagus powders, expressed as Trolox equivalents (μmol TE/g fibre).

		TEAC (soluble)	TEAC (total)
I/W	O-D	3.30	3.80
	F-D	2.05	3.96
I/E	O-D	5.16	7.29
	F-D	11.43	13.11
G/W	O-D	11.41	12.17
	F-D	5.33	7.50
G/E	O-D	1.33	3.12
	F-D	1.40	4.55

W, O-D fibres. The ARC of soluble extracts had a high correlation with the flavonoid content of fibres ($r = 0.8056$), but the total activity of fibres correlated better with ferulic acid derivatives plus flavonoids ($r = 0.8291$). The strongest antioxidant fibres from asparagus by-products are in the same range of activity as a fibre-rich product from cocoa bean husks (Lecumberri et al., 2007), and about one tenth of the ARC of fibres from different citrus by-products (Marín, Soler-Rivas, Benavente-García, Castillo, & Pérez-Álvarez, 2007), but they are much lower than fibre from guava fruit by-product (Jiménez-Escrig, Rincón, Pulido, & Saura-Calixto, 2001) and from red grape pomace and stem (Llobera & Cañellas, 2007). The difference must be due to a higher amount of polyphenols, in addition to the presence of other antioxidant compounds (condensed tannins and ascorbic acid).

4. Conclusions

Besides the chemical composition and functional characteristics (Fuentes-Alventosa et al., 2009), the bioactive profile of asparagus by-product fibres was affected by processing to obtain the powders. In general, intense treatments led to fibres with the highest content of bioactive compounds, especially those treated with ethanol, probably due to a concentration effect. The drying system had a clear effect only in the case of hydroxycinnamic acids, freeze-dried fibres having higher amounts than oven-dried ones. The most affected components were flavonoids, which are mainly responsible for the antioxidant capacity of the fibres. These compounds are oxidised very easily and were lost under most processing conditions of the fibres, decreasing the overall antioxidant capacity. Presently, we are working on an optimised process (patent pending) that maintains the maximum flavonoid content.

Although the content of bioactive compounds and the antioxidant capacity of these fibres were relatively low, it is important to remark that they are interesting sources of dietary fibres, which can be added as functional ingredients in fibre-enriched food. In comparison, other fibre sources such as cellulose, wheat bran, glucomannans, and others lack this intrinsic antioxidant activity and other bioactive compounds. These facts, together with the functional properties (water and oil holding capacities, and glucose retardation index); (Fuentes-Alventosa et al., 2009) and technical compatibility (Sanz, Salvador, Jiménez, & Fiszman, 2008) possessed by fibre from asparagus by-products, increases its attractiveness as an alternative source of dietary fibre. *In vivo* studies on the antioxidant status of asparagus fibre-fed rats and other physiological parameters are under way. These results could be decisive for the use of this added-value agricultural by-product.

Acknowledgements

This research was supported by the Ministerio de Ciencia y Tecnología (Spain), Proyect PETRI PTR1995-0968-OP, and Centro Sur S.C.A. (Huétor-Tajar, Granada, Spain). J.M.F.-A. is the holder of a grant FPI from IFAPA (Instituto de Investigación y Formación Agraria y Pesquera), Junta de Andalucía (Spain).

References

Chen, J. H., & Ho, C.-T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry, 45*, 2374–2378.

Chin, C. K. (2006). Functional elements from asparagus for human health. In *Proceedings of the XI international asparagus symposium in acta horticulturae*.

Cushine, T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*, 343–356.

Fuentes-Alventosa, J. M., Jaramillo, S., Rodríguez-Gutiérrez, G., Cermeño, P., Espejo, J. A., Jiménez-Araujo, A., et al. (2008). Flavonoid profile of green asparagus genotypes. *Journal of Agricultural and Food Chemistry, 56*, 6977–6984.

Fuentes-Alventosa, J. M., Rodríguez, G., Cermeño, P., Jiménez, A., Guillén, R., Fernandez-Bolaños, J., et al. (2007). Identification of flavonoid diglycosides in several genotypes of asparagus from the Huétor-Tájar population variety. *Journal of Agricultural and Food Chemistry, 55*, 10028–10035.

Fuentes-Alventosa, J. M., Rodríguez-Gutiérrez, G., Jaramillo-Carmona, S., Espejo-Calvo, J. A., Rodríguez-Arcos, R., Fernández-Bolaños, J., et al. (2009). Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. *Food Chemistry, 113*, 665–671.

Funk, C., Braune, A., Grabber, J. H., Steinhart, H., & Bunzel, M. (2007). Moderate ferulate and diferulate levels do not impede maize cell wall degradation by human intestinal microbiota. *Journal of Agricultural and Food Chemistry, 55*, 2418–2423.

Guillén, R., Rodríguez, R., Jaramillo, S., Rodríguez, G., Espejo, J. A., Fernandez-Bolaños, J., et al. (2008). Antioxidant from asparagus spear: Phenolics. *Acta Horticulturae, 776*, 247–254.

Hibasami, H., Moteki, H., Ishikawa, K., Katsuzaki, H., Imai, K., Yoshioka, K., et al. (2003). Protodioscin isolated from fenugreek (*Trigonella foenumgraecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *International Journal of Molecular Medicine, 11*, 23–26.

Hirota, S., Shimoda, T., & Takahama, U. (1998). Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *Journal of Agricultural and Food Chemistry, 46*, 3497–3502.

Jaramillo, S., Rodríguez, R., Jiménez, A., Guillén, R., Fernandez-Bolaños, J., & Heredia, A. (2007). Effects of storage conditions on the accumulation of ferulic acid derivatives in white asparagus cell wall. *Journal of the Science of Food and Agriculture, 87*, 286–296.

Jiménez-Escrig, A., Rincón, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *Journal of Agricultural and Food Chemistry, 49*, 5489–5493.

Jiménez-Escrig, A., Santos-Hidalgo, A. B., & Saura-Calixto, F. (2006). Common sources and estimated intake of plant sterols in the Spanish diet. *Journal of Agricultural and Food Chemistry, 54*, 3462–3471.

Kamat, J., Boloor, K., Devasagayam, P. A., & Venkatachalam, S. R. (2000). Antioxidant properties of *Asparagus racemosus* against damage induced by irradiation in rat liver mitochondria. *Journal of Ethnopharmacology, 71*, 425–435.

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Mango peel fibres with antioxidant activity. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung, 205*, 39–42.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L., & Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). *Food Chemistry, 104*, 948–954.

Llobera, A., & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chemistry, 101*, 659–666.

Makris, D. P., & Rossiter, J. T. (2001). Domestic processing of onion bulbs (Allium cepa) and asparagus spears (*Asparagus officinalis*): Effect on flavonol content and antioxidant status. *Journal of Agricultural and Food Chemistry, 49*, 3216–3222.

Marín, F. R., Soler-Rivas, C., Benavente-García, O., Castillo, J., & Pérez-Álvarez, J. A. (2007). By-products from different citrus processes as a source of customized functional fibres. *Food Chemistry, 100*, 736–741.

Nijveldt, R. J., VanNood, E., VanHoorn, D. E., Boelens, P., VanNorren, K., & VanLeeuwen, P. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition, 74*, 418–425.

Plate, A. Y. A., & Gallaher, D. D. (2005). The potential health benefits of corn components and products. *Cereal Foods World, 50*, 305–314.

Rodríguez, R., Guillén, R., Heredia, A., Fernandez-Bolaños, J., & Jiménez, A. (1999). Postharvest changes on cell wall and peroxidases of white asparagus (*Asparagus officinalis* L.). *Acta Horticulturae, 479*, 477–482.

Rodríguez, R., Jaramillo, S., Guillén, R., Jiménez, A., Fernandez-Bolaños, J., & Heredia, A. (2005a). Cell wall phenolics of white and green asparagus. *Journal of the Science of Food and Agriculture, 85*, 971–978.

Rodríguez, R., Jaramillo, S., Rodríguez, G., Espejo, J. A., Guillén, R., Fernandez-Bolaños, J., et al. (2005b). Antioxidant activity of ethanolic extracts from several asparagus cultivars. *Journal of Agricultural and Food Chemistry, 53*, 5212–5217.

Rodríguez-Arcos, R., Smith, A. C., & Waldron, K. W. (2004). Ferulic acid cross-links in asparagus cell wall in relation to texture. *Journal of Agricultural and Food Chemistry, 52*, 4740–4750.

Sanz, T., Salvador, A., Jiménez, A., & Fiszman, S. M. (2008). Yogurt enrichment with functional asparagus fibre. Effect of extraction method on rheological properties, colour, and sensory acceptance. *European Food Research and Technology, 227*, 1515–1521.

Serpen, A., Capuano, E., Fogliano, V., & Gokmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural and Food Chemistry, 55*, 7676–7681.

Shiomi, N., Benkeblia, N., Onodera, S., Omori, T., Takahashi, N., Fukushima, M., et al. (2007). Saccharide and fructooligosaccharide contents, and invertase, 1-KHE, 1-SST, 1-FFT and 6G-FFT activities in green asparagus spears during storage: Effects of temperature and spear portion. *Journal of Applied Glycoscience, 54*, 187–194.

Smoczkiewicz, M. A., Nitschke, D., & Wieladek, H. (1982). Microdetermination of steroid and triterpene saponin glycosides in various plant materials. I. Allium species. *Mikrochimica Acta, II*, 43–53.

van den Heuvel, E. G. H. M., Muys, T., Dokkum, W. v., & Schaasfma, G. (1999). Oligofructose stimulates calcium absorption in adolescents. *American Journal of Clinical Nutrition, 69*, 544–548.

Wang, T.-S., Lii, C.-K., Chang, J.-Y., Kuo, C.-I., & Chen, H.-P. (2007). Anti-Fenton reaction activity of three taxa of water yam (*Dioscorea alata* L.). *International Journal of Food Science and Technology, 42*, 1107–1113.

Wang, M., Tadmor, Y., Wu, Q.-L., Chin, C.-K., Garrison, S. A., & Simon, J. E. (2003). Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. *Journal of Agricultural and Food Chemistry, 51*, 6132–6136.

Wiboonpun, N., Phuwapraisirisan, P., & Tip-pyang, S. (2004). Identification of antioxidant compounds from *Asparagus racemosus*. *Phytotherapy Research, 18*, 771–773.

Yu, S., Chee-Kok, C., Chi-Tang, H., Wei, M., Garrison, S. A., Ho, C., et al. (1996). Antitumor activity of the crude saponins obtained from asparagus. *Cancer Letters, 104*, 31–36.

Yuan, X., Wang, J., & Yao, H. (2005). Feruloyl oligosaccharides stimulate the growth of *Bifidobacterium bifidum*. *Anaerobe, 11*, 225–229.

Zafar, T. A., Weaver, C. M., Zhao, Y., Martin, B. R., & Wasttney, M. E. (2004). Nondigestible oligosaccharides increase calcium absorption and supress bone resorption in ovariectomized rats. *Journal of Nutrition, 134*, 399–402.

Zhao, Z., & Moghadasian, M. H. (2008). Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chemistry, 109*, 691–702.