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

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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

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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