Journal of Cereal Science 51 (2010) 313–318

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Analysis of polyphenols in cereals may be improved performing acidic hydrolysis: A study in wheat flour and wheat bran and cereals of the diet

Sara Arranz, Fulgencio Saura Calixto*

Department of Metabolism and Nutrition, ICTAN, CSIC, José Antonio Novais, 10, 28040, Madrid, Spain

ARTICLE INFO

Article history:
Received 14 August 2009
Received in revised form
18 December 2009
Accepted 12 January 2010

Keywords:
Acidic hydrolysis
Alkali hydrolysis
Cereals
Dietary polyphenols

ABSTRACT

Most analytical studies on polyphenols in cereals refer to compounds determined in aqueous-organic extracts and alkali hydrolysates, but an appreciable amount of polyphenols bound to cell wall constituents may remain insoluble in the residues of extraction and alkali hydrolysis. The main objective of this work was to determine if sulphuric acid hydrolysis may release significant amounts of polyphenols to be considered for analytical and nutritional studies. HPLC/MS analyses of polyphenols were performed in methanol–acetone extracts, alkali and sulphuric acid hydrolysates of wheat flour, bran and a pool of cereals of the diet. The amount of polyphenols found in the acidic hydrolysates (200–1600 mg/100 g) was higher than in alkali hydrolysates (0.2–372 mg/100 g). Lower amount of polyphenols were found in the methanol–acetone extracts (44–160 mg/100 g). Hydroxybenzoic, caffeic, cinammic, ferulic and protocatechuic acids were the main constituents of the hydrolysates. The contribution of cereals to the intake of dietary polyphenols in Spain was estimated around 360 mg/person/day (65 mg of extractable and 295 mg nonextractable polyphenols).

It was concluded that the acidic hydrolysis, usually omitted in analysis of polyphenols in cereals, may be allow to obtain polyphenol contents closer to the actual values.

1. Introduction

Cereals contain a wide range of phenolic compounds including benzoic and cinammic acids, anthocyanidins, quinones, flavonols, chalcones, flavanones and amino phenolics (Adom et al., 2003; Andreasen et al., 2000; Lloyd et al., 2000; Maillard and Berset, 1995). The amount of polyphenols in cereals is highly variable both in whole grain and in bran and also depends on the cereal variety and milling procedure (Adom et al., 2005).

Polyphenolic compounds may be present in free or esterified/etherified soluble form as well as in insoluble form bound to cell wall constituents such as polysaccharides, protein, lignin, cutin or suberin (Adom and Liu, 2002; Clifford, 1999; Naczk and Shahidi, 2004). Insoluble polyphenols are considered the major contributors to the total antioxidant capacity of cereals (Adom and Liu, 2002; Pérez-Jiménez and Saura-Calixto, 2005; Serpen et al., 2007, 2008).

Analytical methodology of polyphenols in cereals, including free and bound forms, generally consists of an extraction with aqueous-organic solvents to obtain soluble polyphenols followed by a hydrolysis treatment to obtain free polyphenols. Since acidic hydrolysis may degrade hydroxycinammic and benzoic acids (Krygier et al., 1982), alkali hydrolysis (saponification) is the main procedure usually performed in the aqueous-organic extracts and powdered samples for ester bond cleavage. However, an appreciable amount of polyphenols bound to cell wall constituents or trapped in cores within the food matrix may remain insoluble after aqueous-organic extraction and/or alkali treatment.

The main objective of the present work was to test strong acidic hydrolysis as a complementary assay to achieve an improved release of these insoluble polyphenols and subsequently, a more accurate quantification of total polyphenols in cereals. For this purpose HPLC–MS analysis was performed in methanol/acetone extracts and in the acidic and alkali hydrolysates of the residues of extraction. Wheat flour, wheat bran and a pool of dietary cereal products were tested.

2. Experimental

2.1. Samples and reagents

Wheat flour (NOMEN, Tarragona, Spain), wheat bran (Santiveri, S.A., Barcelona, Spain), fresh white bread (local bakery, Madrid, Spain), rice (NOMEN, Tarragona, Spain), spaghetti (El Corte Inglés, Madrid, Spain), wheat breakfast cereals (Kellog's), croissant

* Corresponding author. Tel.: +34 91 549 23 00; fax: 34 91 549 36 27.
E-mail address: fsaura@if.csic.es (F. Saura Calixto).

0733-5210/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2010.01.006

(El Corte Inglés, Madrid, Spain) and biscuits (Maria biscuit from Kraft Foods Galletas, S.A., Barcelona, Spain) were purchased in a local supermarket.

All solvents used were analytical HPLC grade from Sigma Aldrich (St. Louis, Missouri, USA) and Panreac (Castellar del Vallés, Barcelona, Spain). Water was ultrapure.

Standards of benzoic acids (gallic, vanillic, syringic, tannic, ellagic and protocatechuic acids), hydroxycinammic acids (chlorogenic, caffeic, ferulic, *p*-coumaric, sinapic and cinammic acids), flavan-3-ols (catechin, (−)-epicatechin, gallocatechin, epicatechin gallate, epigallocatechin gallate, gallocatechin gallate, procyanidin dimers B1 and B2), flavanols (naringin, hesperetin, hesperedin, phloridzin), flavonols (quercetin-3-glucoside, quercetin-galactoside, quercetin-xyloside, quercetin-rhamnoside, quercetin-arabinoside, rutin and quercetin,) and anthocyanidins (cyaniding-glucoside, malvidin-glucoside, peonidin-3-glucoside, pelargonidin-3-glucoside) were purchased from Sigma Chemical Co. (St Louis, MO, USA) and Extrasynthése (Genay, France).

2.2. Sample preparation

Wheat flour and wheat bran (sieve ring 0.5 mm) were used to compare the content and profile of polyphenols in extracts and hydrolysates. In addition, a third sample representative of the cereals daily per capita consumed in the Spanish diet was prepared on the basis of the National Dietary Survey data (Ministry of Agriculture, Fisheries and Food, 2007).

White bread (fresh, 96.8 g), rice (boiled with water, 6.7 g), spaghetti (boiled with water, 9.1 g), wheat breakfast cereals (3.8 g), croissant (fresh, 12.6 g) and biscuits (fresh, 10.7 g) were mixed (139.8 g), freeze-dried and milled (sieve ring 0.5 mm) in a centrifugal mill Retsch ZM 200 (Haan Germany). The cereals pool obtained was stored at −20 °C until analysis.

2.3. Methanol/acetone extraction and acidic and alkali hydrolysis

Extractions were performed in three replicates of each sample. Determinations were performed per triplicate in extracts and hydrolysates and results were reported as mean value ± standard deviation on a fresh matter basis.

Polyphenols in methanol/acetone extracts were obtained following conditions previously selected (Pérez-Jiménez and Saura-Calixto, 2005): 0.5 g of freeze-dried sample was placed in a capped centrifuge tube; 20 mL of acidic methanol (HCl)/water (50:50, v/v; pH 2) was added and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 3500 rpm for 10 min and the supernatant was recovered. Twenty millilitres of acetone/water (70:30, v/v) was added to the residue, and shaking and centrifugation were repeated. Methanolic and acetonic extracts were combined and used to quantify and identify polyphenols by reverse-phase HPLC–MS technique (Tomás-Barberán et al., 2001).

Residues obtained after methanol/acetone extraction were used to determine *hydrolysable polyphenols*. Two different procedures were tested:

(1) *Acidic hydrolysis*: Residues (200 mg) were treated with methanol/H_2SO_4 90:10 (v/v) at 85 °C for 20 h (Hartzfeld et al., 2002). The hydrolysates were collected and stored at −20 °C until HPLC–MS analysis.
(2) *Alkali hydrolysis*: Residues (200 mg) were treated with 2 M sodium hydroxide (in water) at room temperature for 4 h shaking under nitrogen gas. The alkali hydrolysates were neutralized with an appropriate amount of acid. The final solution was used for HPLC–MS analysis (Adom and Liu, 2002; Pellegrini et al., 2006).

2.4. Analysis of polyphenols in methanol/acetone extracts

Analysis of polyphenols in methanol/acetone extracts of samples were carried out in an Agilent HPLC 1100 series (Agilent Technologies, Waldbronn, Germany) coupled with an Agilent ChemStation software (Agilent, v. 08.03) assisted by mass spectrometry detection for separation and identification of compounds.

Briefly, 20 μL of filtered extracts (0.45 μm Teknokroma filter) were injected into a HPLC reverse-phase C18 Nucleosil column (150 × 4.6 mm i.d.; particle size 5 μm) (Agilent) with a guard column containing the same stationary phase. The method applied was based on the procedure of Tomás-Barberán et al. (2001) with some modifications. Chromatograms were acquired at 280, 320, 360 and 520 nm on the DAD.

The MS was fitted to an atmospheric pressure electrospray ionization (ESI) source, operated in negative ion mode. The electrospray capillary voltage was set to 3000 V, with a nebulizing gas flow rate of 12 L/h and a drying gas temperature of 300 °C. Mass spectrometry data were acquired in the Scan mode (mass range m/z 100–1000). Quantification was done by comparison with known standards when possible. When pure standards were not available, concentration of polyphenols in extracts were calculated using the calibration curves of the standards similar in chemical structure.

2.5. Analysis of polyphenols in acidic and alkali hydrolysates

Hydrolysable polyphenols were determined in both acidic and alkali hydrolysates following the procedure described by Bennett et al. (2004). Samples (20 μL) of filtered hydrolysates (0.45 μm Teknokroma filter) were injected into a HPLC reverse-phase C18 Gemini column (250 × 4.6 mm i.d.; particle size 5 μm) with a guard column containing the same stationary phase. This column is suitable for a wide range of pH and is appropriate for acid samples. Chromatograms were recorded at 280, 320, 360 and 520 nm on the DAD. The MS conditions were the same as those for polyphenols extracted with methanol/acetone except that the MS operated in positive mode. Quantification was achieved by comparison with known standards when possible.

3. Results and discussion

Wheat flour, the main component of most common cereal products of the diet, and wheat bran, material widely used in dietary fibre rich products, were selected as test samples due to their significant differences in polyphenol content and composition (Halvorsen et al., 2002; Pellegrini et al., 2006; Pérez-Jiménez and Saura-Calixto, 2005). In addition, a third sample representative of the cereal products daily consumed in the diet (prepared as it is described above) was also tested; this sample is made up of a mixture of cereals (139.8 g) equivalent to 80% of the amount of cereals per capita consumed in the Spanish diet (Ministry of Agriculture, Fisheries and Food, 2007).

3.1. Polyphenols in methanol–acetone extracts

Results of polyphenols analysed in methanol/acetone extracts are shown in Table 1. As expected, the total amount of polyphenols was higher in wheat bran (161.7 mg/100 g) than in wheat flour (112.2 mg/100 g). Our values for extractable polyphenols are in the same range (100 and 240 mg/100 g dry weight for wheat flour and bran respectively) than others reported in the literature (Gallardo et al., 2006; Mateo Anson et al., 2009; Vitaglione et al., 2008).

Regarding the polyphenol profile, hydroxybenzoic acid and ellagic acid were identified as major constituents in wheat flour. A peak with UV/VIS spectrum of flavanone appears in the 280 nm