Journal of Cereal Science 52 (2010) 170–176

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Identification of buckwheat phenolic compounds by reverse phase high performance liquid chromatography–electrospray ionization-time of flight-mass spectrometry (RP-HPLC–ESI-TOF-MS)

Vito Verardo [a,*], David Arráez-Román [b], Antonio Segura-Carretero [b], Emanuele Marconi [c], Alberto Fernández-Gutiérrez [b], Maria Fiorenza Caboni [a]

[a] Dipartimento di Scienze degli Alimenti, Università di Bologna, Piazza Goidanich 60, 47023 Cesena (FC), Italy
[b] Department of Analytical Chemistry, University of Granada, c/Fuentenueva s/n, 18071 Granada (GR), Spain
[c] Dipartimento di Scienze e Tecnologie Agro-Alimentari Ambientali e Microbiologiche (DISTAAM), Università del Molise, Via De Sanctis, 86100 Campobasso (CB), Italy

ARTICLE INFO

Article history:
Received 11 November 2009
Received in revised form
7 April 2010
Accepted 29 April 2010

Keywords:
Buckwheat
Phenolic compounds
RP-HPLC–ESI-TOF-MS
Flavonoids
Galloylated propelargonidins
Procyanidins

ABSTRACT

This study describes the characterization of phenolic compounds in buckwheat. Reverse phase high performance liquid chromatography (RP-HPLC) coupled to electrospray ionization-time of flight-mass spectrometry (ESI-TOF-MS) has been applied to separate and characterize thirty phenolic compounds in buckwheat flour. As far as we know, 2-hydroxy-3-O-β-D-glucopyranosil-benzoic acid, 1-O-caffeoyl-6-O-alpha-rhamnopyranosyl-β-glycopyranoside and epicatechin-3-(3″-O-methyl) gallate were tentatively identified in buckwheat for the first time. The sensitivity, mass accuracy and true isotopic pattern of the TOF-MS, legitimated the identification of phenolic compounds present in buckwheat extract.

Furthermore, other "unknown" compounds were also reported in the ethanolic extracts of buckwheat.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Buckwheat (*Fagopyrum esculentum* Möench) (Polygonaceae) is popularly recognized as an excellent selective antioxidant and hypolipidaemic nutrient food. The concentration of natural antioxidants may show strong variations depending on several factors including variety, location, and environmental conditions (Kalinová and Dadáková, 2006). Buckwheat seeds serve as a rich source of flavonoids and have been considered a good dietary source of rutin. Flavonoids isolated from buckwheat hulls showed radical scavenging activity when analyzed in the purified form. Hence interest in buckwheat is increasing because of the possible beneficial health effects of rutin (Cao et al., 2008; La Casa et al., 2000; Watanabe and Ayugase, 2008). Buckwheat is known for its high rutin (quercetin 3-rutinoside) level, but also contains other flavonoids, such as quercetin, hyperoside (quercetin 3-O-β-D-galactoside), quercitrin (quercetin 3-*O*-α-L-rhamnoside), epicatechin, orientin, vitexin, isovitexin, and isoorientin (Fabjan et al., 2003; Kalinová et al., 2006). Quercetin and isoquercitrin are precursors in the biosynthesis of rutin, which is probably formed by the 3-glycosylation of quercetin following the rhamnosylation of isoquercitrin (Kalinová and Vrchotova, 2009). Soluble condensed tannins of buckwheat are based on pelargonidin and cyanidin structures.

Reverse phase high performance liquid chromatography (RP-HPLC) hyphenated to mass spectrometry (MS) detection is one of the most important analytical techniques used for the analysis of phenolic compounds. Recently, an improvement in chromatographic performance has been achieved by using columns packed with small particles. The newest column packed with particle size less than 2 μm, operated at a pressure up to 600 bar, so that high resolution can be achieved (Ferrer et al., 2008).

The on-line coupling of RP-HPLC with MS using electrospray ionization (ESI) as an interface yields a powerful method because of its high efficient resolution and characterization of a wide range of polar compounds. ESI is one of the most versatile ionization techniques, and is the preferred one for detection of polar compounds separated by liquid chromatography.

Abbreviations: BPC, base peak chromatogram; Glc, glucose; HRF, heterocyclic ring fission; NMR, nuclear magnetic resonance; QM, quinone methide; RDA, Retro Diels–Alder reaction; RP-HPLC–ESI-TOF-MS, reverse phase high performance liquid chromatography–electrospray ionization-time of flight-mass spectrometry.

* Corresponding author. Tel.: +39 (0)547 338117; fax: +39 (0)547 382348.
E-mail address: vito.verardo@unibo.it (V. Verardo).

0733-5210/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jcs.2010.04.009

The advantages of MS detection include the ability to determine the molecular weight and to obtain structural information (Carrasco-Pancorbo et al., 2007). TOF-MS can provide excellent mass accuracy over a wide dynamic range and allow measurements of the isotopic pattern, providing important additional information for the determination of the elemental composition. Therefore, TOF-MS is a powerful detection system for identifying target compounds in a highly complex matrix.

Thus, in this study, phenolic compounds were extracted and subsequently separated and identified by RP-HPLC–ESI-TOF-MS in order to evaluate the free phenolic composition of whole meal buckwheat flour. In this sense, the use of a small particle size C_{18} column (1.8 μm) provided great resolution and made possible separation of numerous phenolic compounds.

2. Materials and methods

2.1. Sample

Whole buckwheat flour (*Fagopyrum esculentum* Moench) from organic farming was purchased in a local market.

2.2. Reagents and chemicals

HPLC-grade acetonitrile, ethanol and methanol were purchased from Labscan (Dublin, Ireland). Acetic acid analytical grade (assay$>$99.5%) was purchased from Fluka (Switzerland). Water was purified by using a Milli-Q system (Millipore, Bedford, USA). Other reagents unmarked were of an analytical grade.

2.3. Extraction of phenolic compounds

To isolate the phenolic fraction, the protocol of Van Hung and Morita (2008) was used with some modifications reported as follows. Two grams of buckwheat flour were extracted twice in an ultrasonic bath with a solution of ethanol/water (4:1 v/v). The supernatants were collected, evaporated and reconstituted with 2 mL of methanol/water (1:1 v/v). The extracts were stored at −18 °C until use.

2.4. RP-HPLC–ESI-TOF-MS analysis

RP-HPLC analysis was performed by an Agilent 1200 series rapid resolution LC system (Agilent Technologies, CA, USA) consisting of a vacuum degasser, autosampler, and a binary pump equipped with a reversed-phase C_{18} analytical column (4.6×150 mm, 1.8 μm particle size, Agilent ZORBAX Eclipse plus) was used. The mobile phase and gradient program were used as previously described by Verardo et al. (2008) with some modifications as reported below. All solvents were filtered with a 0.45 μm filter disk. A gradient elution was carried out using the following solvent system: mobile phase A, water/acetic acid (99:1, v/v); mobile phase B, mobile phase A/acetonitrile (60:40, v/v). The gradient program was as follows: from 2% B to 6% B in 16 min, from 6% to 10% in 4 min, from 10% to 17% in 4 min, from 17% to 36% in 14 min, from 36% to 38.5% in 2 min, from 38.5% to 60% in 13 min, from 60% to 100% in 5 min and from 100% to 2% in 2 min. A 10 min re-equilibration time was used after each analysis. The flow rate used was set at 0.50 mL/min throughout the gradient. The effluent from the RP-HPLC column was split using a T-type phase separator before being introduced into the mass spectrometer (split ratio = 1:3). Thus in this study the flow which arrived into the ESI-TOF-MS detector was 0.2 mL/min. The column temperature was maintained at 25 °C and the injection volume was 10 μL.

The RP-HPLC system was coupled to a microTOF™ (Bruker Daltonics, Bremen, Germany), an orthogonal-accelerated TOF mass spectrometer (oaTOF-MS), equipped with an ESI interface. Parameters for analysis were set using negative ion mode with spectra acquired over a mass range from *m*/*z* 50 to 1300. The optimum values of the ESI-MS parameters were: capillary voltage, +4.5 kV; drying gas temperature, 190 °C; drying gas flow, 7.0 L/min; and nebulizing gas pressure, 21.7 psi.

The accurate mass data of the molecular ions were processed through the newest software Data Analysis 4.0 (Bruker Daltonics, Bremen, Germany), which provided a list of possible elemental formula by using the Smart Formula Editor. The Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration, and ring-plus double bond equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (sigma value) for increased confidence in the suggested molecular formula. The widely accepted accuracy threshold for confirmation of elemental compositions has been established at 5 ppm (Cavaliere et al., 2008; Ferrer et al., 2005).

During the development of the HPLC method, external instrument calibration was performed using a Cole Palmer syringe pump (Vernon Hills, Illinois, USA) directly connected to the interface, passing a solution of sodium formate cluster containing 5 mM sodium hydroxide in the sheath liquid of 0.2% formic acid in water/isopropanol 1:1 (v/v). Using this method, an exact calibration curve based on numerous cluster masses each differing by 68 Da ($NaCHO_2$) was obtained. Due to the compensation of temperature drift in the microTOF, this external calibration provided accurate mass values (better 5 ppm) for a complete run without the need for a dual sprayer setup for internal mass calibration.

3. Results and discussion

The analysis of the aqueous ethanol extracts by RP-HPLC–ESI-TOF-MS revealed that flavonoids were the major class of phenolic compounds in buckwheat flours. Thirty phenolic compounds, twenty-eight flavonoids and two phenolic acid derivatives were characterized.

The separation method proposed by Verardo and co-workers was modified in order to obtain a high resolution and make possible the separation of several isomers.

The adapted method shows itself as a valuable tool for the analysis of phenolic compounds in buckwheat. Its sensitivity and the ease with which molecular masses may be obtained increase the possibilities of detecting and characterizing compounds present in small amounts. Besides, a good peak resolution was obtained in less than 60 min. To obtain more information a microTOF analyzer was used. Variables involved in the procedure such as capillary voltage, drying gas flow rate, drying gas temperature, and nebulizing gas pressure were optimized to improve sensitivity of TOF analysis. The optimal conditions were reported in the materials and methods section.

The obtained base peak chromatogram (BPC) is illustrated in Fig. 1. Under the proposed RP-HPLC–ESI-TOF-MS method, a large number of phenolic compounds present in buckwheat flours were detected. These are summarized in Table 1, with their formula, selected ion, experimental and calculated *m*/*z*, MS fragment, error (ppm and mDa), sigma value, tolerance and migration time.

Thus, the proposed method is able to detect thirty phenolic compounds in the same run. Furthermore, all detected compounds observed in Table 1 exhibited good sigma values smaller than 0.05, except in luteolin-glycoside, and mass accuracy (ppm and mDa) as indicated by the error values, even a low tolerance was chosen (5 ppm).

Download English Version:

https://daneshyari.com/en/article/4516347

Download Persian Version:

https://daneshyari.com/article/4516347

Daneshyari.com