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Analytical Methods

Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains

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ABSTRACT

A new microwave-assisted extraction (MAE) method has been investigated for the extraction of phenolic compounds from rice grains. The experimental conditions studied included temperature (125–175 °C), microwave power (500–1000 W), time (5–15 min), solvent (10–90% EtOAc in MeOH) and solvent-to-sample ratio (10:1 to 20:1). The extraction variables were optimised by the response surface methodology. Extraction temperature and solvent were found to have a highly significant effect on the response value ($p < 0.0005$) and the extraction time also had a significant effect ($p < 0.05$). The optimised MAE conditions were as follows: extraction temperature 185 °C, microwave power 1000 W, extraction time 20 min, solvent 100% MeOH, and solvent-to-sample ratio 10:1. The developed method had a high precision (in terms of CV: 5.3% for repeatability and 5.5% for intermediate precision). Finally, the new method was applied to real samples in order to investigate the presence of phenolic compounds in a wide variety of rice grains.

1. Introduction

Cultivated rice (*Oryza sativa L.*) is the second most widely grown cereal crop in the world and it serves as an important staple food for more than half of the world's population (OECD-FAO. Agricultural Outlook, 2011). Besides the contribution of rice to the total human calorie intake, rice contains some specific components with proven benefits for human health. Several compounds with antioxidant activity have been identified in rice and these include phenolic compounds (Zhou, Robards, Helliwell, & Blanchard, 2003). Phenolic compounds have health benefits due to their antioxidant activities, which have inhibitory effects on mutagenesis and carcinogenesis (Vattem, Ghaedian, & Shetty, 2005).

The most common forms of phenolic compounds in rice are represented by hydroxycinnamic and hydroxybenzoic acids. The predominant phenolic compounds in rice are ferulic and *p*-coumaric acids, both of which are hydroxycinnamic acids (Li, Friel, & Beta, 2010; Lin & Lai, 2011). Other compounds identified include sinapic, *p*-hydroxybenzoic and protocatechuic acids, which are benzoic acids (Goffman & Bergman, 2004; Vichapong, Sookserm, Srijesdaruk, Swatsitang, & Srijaranai, 2010; Walter & Marchesan, 2011). In addition to the aforementioned two major groups of compounds, aldehyde analogs such as vanillin are also referred to as phenolics (Qiu, Liu, & Beta, 2010). The concentrations of these compounds in rice grains have been positively correlated with antioxidant activity (Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011; Yafang, Gan, & Jinsong, 2011). Thus, phenolic compounds contribute to the antioxidant activity of rice grains.

Reversed phase-high performance liquid chromatography (RP-HPLC) has been the most commonly used analytical technique for the qualitative and quantitative analysis of phenolic compounds (Duckstein & Stintzing, 2011; Sahin, Demir, & Malyer, 2011). HPLC coupled with a photodiode-array detector (PDA) is sufficiently sensitive for the detection of eluted compounds at wavelengths across the UV and visible spectrum. This technique provides both a sensitive quantitative response and qualitative information regarding the UV–vis chromophore of phenolic compounds.

Extraction is a very important analytical step in the isolation and identification of compounds from solid samples prior to chromatographic determination. The development of an optimal procedure for the extraction of phenolic compounds from food samples can prove difficult due to the structural diversity of phenolic compounds and their potent antioxidant activity, which can lead to rapid reaction with other constituents in the matrix (Ajila, Brar, & Verma, 2011). Microwave-Assisted Extraction (MAE) appears to

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be one of the best methods to extract phenolic compounds due to the special microwave/matter interactions and the very rapid extraction time (Li, Skouroumounis, Elsey, & Taylor, 2011; Mandal, Mohan, & Hemalatha, 2007).

The MAE system rapidly generates heat and this characteristic results in a shorter extraction time and good quality extracts with better target compound recovery (Barbero, Palma, & Barroso, 2006; Liazid, Guerrero, Cantos, Palma, & Barroso, 2011). Different chemical substances absorb microwaves to different extents and this behaviour makes MAE an efficient method for extractions and, more importantly, it makes it possible to selectively extract target compounds from complex food matrices (Eskilsson & Björklund, 2000; Hemwimon, Pavasant, & Shotipruk, 2007). However, the use of MAE has not previously been reported for the extraction of phenolic compounds from rice grains.

The efficiency of the MAE process depends on extraction time, extraction temperature, solid–liquid ratio and the type and composition of solvent used (Pizarro, Pérez-del-Notario, & González-Saiz, 2007; Rostagno, Palma, & Barroso, 2007; Song, Li, Liu, & Zhang, 2011). Chemometric approaches based on the use of an experimental design have been successfully utilised to evaluate the variables that affect MAE recoveries (Zhong et al., 2010). This statistical and mathematical technique has been used to develop, improve and optimise processes (Tabaraki & Nateghi, 2011; Prasad et al., 2011).

Previously used extraction methods for phenolics in rice grains usually involve solid–liquid maceration with long extraction times (up to 1 h) (Qiu et al., 2010) and they also require some additional steps, including solvent removal by evaporation to dryness, after completion of the extraction process (Shao, Xu, Bao, & Beta, 2014).

The aim of the study reported here was to optimize a rapid MAE method for the extraction of phenolic compounds from rice grains by response surface methodology (RSM).

2. Materials and methods

2.1. Materials and chemicals

HPLC-grade methanol (MeOH), acetic acid and ethyl acetate (EtOAc) were purchased from Merck KGaA (Darmstadt, Germany). Phenolic compound standards of the highest available purity were used. Protocatechuic acid (PRO), vanillin (VAN), protocatechuic aldehyde (PARA), *p*-hydroxybenzoic acid (*p*-HBA), *p*-hydroxybenzaldehyde (*p*-HB), ferulic acid (FER) and sinapic acid (SIN) were obtained from Fluka (Buchs, Switzerland). Guaiacol (GUA), *p*-Coumaric acid (*p*-COU), caffeic acid (CAF), 5-hydroxymethyl-2-furaldehyde (HMF), furfural (FUR), 5-methylfurfural (MF) and syringic acid (SYR) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ellagic acid (ELL) was purchased from Sarsynthese (Merignac, France). Water was purified with a Milli-Q purification system (Billerica, MA, USA).

2.2. Rice samples

Rice samples were obtained from a commercial market in Spain. Each rice sample (20 g) was placed in a plastic cylinder and rice grains were milled with an Ultraturrax homogenizer (IKA® T25 Digital, Germany) for 10 min prior to extraction. The milling process was stopped every 1 min to avoid excessive heating of the sample. The fine grain was then homogenised by stirring and then stored in a closed bottle. The final extraction method was applied to 13 different rice products available in the market and these covered the varieties of short (4), long (3), aromatic (2), exotic (1), whole grain (2) and processed (1) rice grains.

2.3. Extraction of phenolic compounds

MAE experiments were performed with a Milestone Ethos 1600 system (Sorisole, Italy) equipped with vessels made from tetrafluoromethoxyl polymer with Teflon liners. Rice powder (2.5 g) was accurately weighed and placed into an extraction vessel. According to the experimental design, a set volume and type of solvent was added to the extraction vessel and the extraction was performed under different MAE conditions. After extraction, the vessels were cooled in an ice bath for 10 min and carefully opened in a fume cupboard. The solid material in the sample was filtered off and washed using fresh solvent. The combined filtrate and washings were evaporated to dryness under vacuum (rotary evaporator). The residue was re-dissolved in methanol (2 mL) and was filtered through a 0.45 μm filter prior to injection into the HPLC-FD system.

2.4. Determination of phenolic compounds

The HPLC system comprised a Dionex P680 HPLC Pump, Dionex ASI 100 Automated Sample Injector, Dionex PDA-100 Photodiode Array Detector, Dionex UCI-50 Universal Chromatography Interface, and Dionex TCC-100 Thermostatted Column Compartment. Separations were performed on a reversed phase RP 18 LiChrospher Column (LiChroCART 250 × 4 mm (5 μm), Merck KgaA, Darmstadt, Germany).

Gradient elution was carried out at a flow rate of 1.0 mL min^{-1}. A PDA-100 Photodiode Array Detector was used for UV–vis measurements and the 3D mode was set at collection rate of 1.0 Hz, 3D wavelength scan range of 250–600 nm, 3D bunch width of 1 nm and band width of 50 nm. The column compartment thermostat was set at 25 °C. The injection volume was set to 25 μL. A gradient elution was programmed using an acidified aqueous mobile phase A (2% acetic acid and 5% methanol) and mobile phase B (2% acetic acid and 88% methanol). The gradient applied was as follows: (time, solvent B): 0 min, 0%; 10 min, 25%; 25 min, 40%; 30 min, 50%; 35 min, 50%. The identification of phenolic acids in the samples was achieved by spiking and by comparison of retention times and maximum UV absorptions with those of standards.

The analytical properties of the chromatographic method for the determination of 15 phenolic compounds are listed in Table 1. Typical chromatograms at three different wavelengths (260, 280 and 320 nm) are shown in Fig. 1.

2.5. Performance of the method

The chromatographic analytical procedure used to determine phenolic compounds was carried out according to the ICH Guideline Q2 (R1) and suggestions made in ISO 17025 (ICH, 2006; ISO, 2005). The linearity, range, precision, detection and quantification limits of the method were evaluated.

Linearity was assessed in order to confirm the ability of the method to obtain test results that are directly proportional to the concentration of phenolic compounds within the range studied. Stock solutions of each phenolic compound were diluted as appropriate to give concentrations ranging from 0.15 to 30 mg L^{-1}. Gnumeric 1.10.17 was used to generate the regression analysis to obtain the calibration curves and quantify the phenolic compounds in the extracts. The standard deviation (σ) obtained for the response and the slope (m) from the regression were then used to calculate the limit of detection (LOD) and limit of quantification (LOQ) using Eq. (1) and Eq. (2), respectively.

$$\mathrm{LOD} = 3.3\,\sigma/m \quad (1)$$

$$\mathrm{LOD} = 10\,\sigma/m \quad (2)$$