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LC-ESI-MS/MS Analysis and Extraction Method of Phenolic Acids from Gluten-Free Precooked Buckwheat Pasta

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Abstract In many countries, common buckwheat (*Fagopyrum esculentum* Möench) has been cultivated for its nutritive value as food ingredient. This plant is a rich source of vitamins and exogenic amino acids. Many of the health-promoting effects of *F. esculentum* have been attributed to a large amount of phenolic compounds. Presented in this paper, precooked buckwheat pasta, produced by extrusion cooking, is a gluten-free product without any technological additives. Moreover, it contains natural polyphenolic antioxidants and therefore could be classified as convenience food. The phenolic acid compositions of precooked buckwheat pasta were as follows: gallic, protocatechuic, gentisic, 4-OH-benzoic, vanillic, *trans*-caffeic, *cis*-caffeic, *trans*-p-coumaric, *cis*-p-coumaric, *trans*-ferulic, *cis*-ferulic, and salicylic. A very important step of sample pretreatment before quantitative analysis is the choice of extraction conditions. Therefore, in this study, before quantitative analysis (liquid chromatography-mass spectrometry, LC-ESI-MS/MS), optimization of ultrasound-assisted extraction (UAE) of phenolic acids from precooked buckwheat pasta was performed. The most effective conditions for the isolation of phenolic acids from precooked buckwheat pasta with the use of UAE were as follows: 80 % aqueous ethanol, 60 °C, ultrasound frequency 20 kHz, power 100 W, and time 40 min.

Keywords *Fagopyrum esculentum* Möench · Precooked buckwheat pasta · Ultrasound-assisted extraction · Phenolic acids · High-performance liquid chromatography-mass spectrometry

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Introduction

The genus *Fagopyrum* includes several species (Fu-hua et al. 2013; Morishita et al. 2007). Common buckwheat (*Fagopyrum esculentum* Möench) has been cultivated in many countries in Europe, Asia, South Africa, and USA for its nutritive value as food ingredient (Choi and Ma 2006; Kiprovski et al. 2015; Wu et al. 2015). Studies revealed that the consumption of buckwheat can reduce the blood glucose level (Kawa et al. 2003) cholesterol level (Inglett et al. 2010), provided protection from oxidative stresses (Mukoda et al. 2001; Przybylski et al. 1998), and showed antimutagenic activity (Yokozawa et al. 2002). This plant is a rich source of vitamins and exogenic amino acids (Kiprovski et al. 2015).

Many of the health-promoting effects of *F. esculentum* have been attributed to a large amount of phenolic compounds. Polyphenols are secondary plant metabolites, which show antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (Campillo et al. 2015; Oniszczuk and Podgórski 2015; Sharma et al. 2012; Tarola et al. 2013). They are applied in the prevention of cancer and cardiovascular diseases (Luthria and Mukhopadhyay 2006; Ness and Powles 1997).

Extrusion cooking seems to be one of the best methods for obtaining the maximum nutritive value of several plant materials. It is a high-temperature, short-time process, which combines the respective sequences of mixing, heating, shearing, forming, and shaping (Wójtowicz 2012). The first obtained precooked buckwheat pasta was produced by extrusion cooking in the Department of Food Process Engineering,

University of Life Sciences in Lublin, as a gluten-free product without any technological additives.

An extremely significant step of sample pretreatment is the choice of extraction conditions. Therefore, in this study, before quantitative analysis (liquid chromatography-mass spectrometry, LC-ESI-MS/MS), optimization of ultrasound-assisted extraction of phenolic acids from precooked buckwheat pasta was performed.

Materials and Methods

Chemicals and Plant Material

Analytical grade standards of phenolic acids were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). Liquid chromatography grade and analytical grade solvents were obtained from J.T. Baker (Phillipsburg, USA). Roasted buckwheat seeds (*F. esculentum*) were purchased from a local market (distributor Awiko, Lublin, Poland). Seeds were ground to flour (particle size below 500 μm).

Pasta Processing

Raw materials were moistened and mixed to obtain a moisture content of the dough at 32 %. After mixing, the raw materials were processed using a modified single-screw extrusion-cooker TS-45 (Metalchem, Gliwice, Poland) with L/D = 18:1 and compression ratio 3:1, equipped with an additional cooling section with glycol (chiller SW 8P MINI's Cool, Chotomów, Poland). Pasta products were shaped using a forming die with 12 openings with a diameter of 0.8 mm and processed at 100 rpm screw speeds.

Ultrasound-assisted extraction

Extraction was performed in an ultrasonic bath (20 kHz, 100 W; BANDELIN electronic GmbH & Co. KG, Germany). Each 2-g portions of the powdered sample was extracted with 40 mL of 80 % aqueous methanol (80 % MeOH) or 80 % aqueous ethanol (80 % EtOH) for 40 min (two cycles for 20 min) at 60 °C (Oniszczuk and Olech 2016). Extraction temperature was adjusted to 55, 60, or 65 °C. Extracts were filtered, combined, and evaporated to dryness. The residues were dissolved in 5 mL of methanol. The procedure was repeated three times for every condition.

LC-ESI-MS/MS Analysis of Phenolic Acids

The samples were analyzed by high-performance liquid chromatography and electrospray ionization mass spectrometry (HPLC-ESI-MS/MS) according to the method described previously (Oniszczuk and Olech 2016). Analysis was performed

Table 1 Content of phenolic acids in gluten-free precooked buckwheat pasta extracts ($n = 3$)

Yield of phenolic acids (μg g^{-1} of dry weight)											
UAE method	Gallic acid	Protocatechuic acid	Gentisic acid	4-OH-benzoic acid	Vanillic acid	*trans*-Caffeic acid	*cis*-Caffeic acid	*trans*-p-Coumaric acid	*cis*-p-Coumaric acid	*Cis*-Ferulic acid	Salicylic acid
80 % MeOH 60 °C	2.566	2.725	0.355	3.052	0.438	0.152	0.059	0.414	0.203	0.116	0.942
SD	0.0061	0.2023	0.0021	0.0231	0.0052	0.0006	0.0001	0.0021	0.0013	0.0013	0.0048
80 % EtOH 55 °C	2.553	2.677	0.348	2.804	0.395	0.109	0.053	0.442	0.294	0.127	0.920
SD	0.0069	0.0015	0.0020	0	0.0087	0.0013	0.0001	0.0014	0.0024	0.0006	0.0034
80 % EtOH 60 °C	3.073	3.063	0.408	3.108	0.451	0.155	0.120	0.647	0.354	0.348	1.124
SD	0.0348	0.0069	0.0041	0.139	0.0022	0.0001	0.0003	0.0084	0.0014	0.0005	0.0139
80 % EtOH 65 °C	2.736	2.741	0.380	3.039	0.384	0.148	0.116	0.623	0.322	0.328	1.096
SD	0.0561	0.0072	0.0021	0.0071	0.0035	0.0007	0.0005	0.0004	0.0042	0.0013	0.0011

SD standard deviation

using Agilent 1200 Series HPLC (Agilent Technologies, USA) equipped with a binary gradient solvent pump, a degasser, an autosampler, and a column oven. Phenolic acids were separated at 25 °C, on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8-μm particle size; Agilent Technologies, USA), using 3-μL injections. The solvents used were as follows: water containing 0.1 % HCOOH (solvent A) and methanol containing 0.1 % HCOOH (solvent B). The following gradient elution program at a flow rate of 400 μL min^{-1} was applied: 0–1 min, 5 % B; 2–4 min, 20 % B; 8–9.5 min, 70 % B; 11.5–15 min, 5 % B. MS detection was performed in a 3200 QTRAP mass spectrometer (AB Sciex, USA) equipped with an electrospray ionization source (ESI) and a triple quadrupole-ion trap mass analyzer that was controlled by the Analyst 1.5 software. The ESI interface was operated in the negative-ion mode.

The correlation coefficient of all calibration curves was $R^2 > 0.9992$. Analytical results and parameters of LC-MS/MS quantitative method—data for calibration curves, limit of detection (LOD) and the limit of quantification (LOQ) values for each analyzed phenolic acids—are presented in the paper described previously (Oniszczuk and Olech 2016).

Results and Discussion

The phenolic acid compositions of precooked buckwheat pasta were as follows: gallic, protocatechuic, gentisic, 4-OH-benzoic, vanillic, *trans*-caffeic, *cis*-caffeic, *trans*-p-coumaric, *cis*-p-coumaric, *trans*-ferulic (content lower than LOQ, but higher than LOD), *cis*-ferulic, and salicylic (Table 1, Fig. 1).

Optimization of extraction solvent was the first step of the experiment. In order to achieve this, 80 % aqueous ethanol and 80 % aqueous methanol were used. Eighty percent of EtOH appears to be the superior extractant for the isolation of analyzed compounds from buckwheat pasta. This solvent meets the requirements of "Green Chemistry" (Chemat et al. 2008) and offers several advantages, for example, insignificant toxicity and environmental

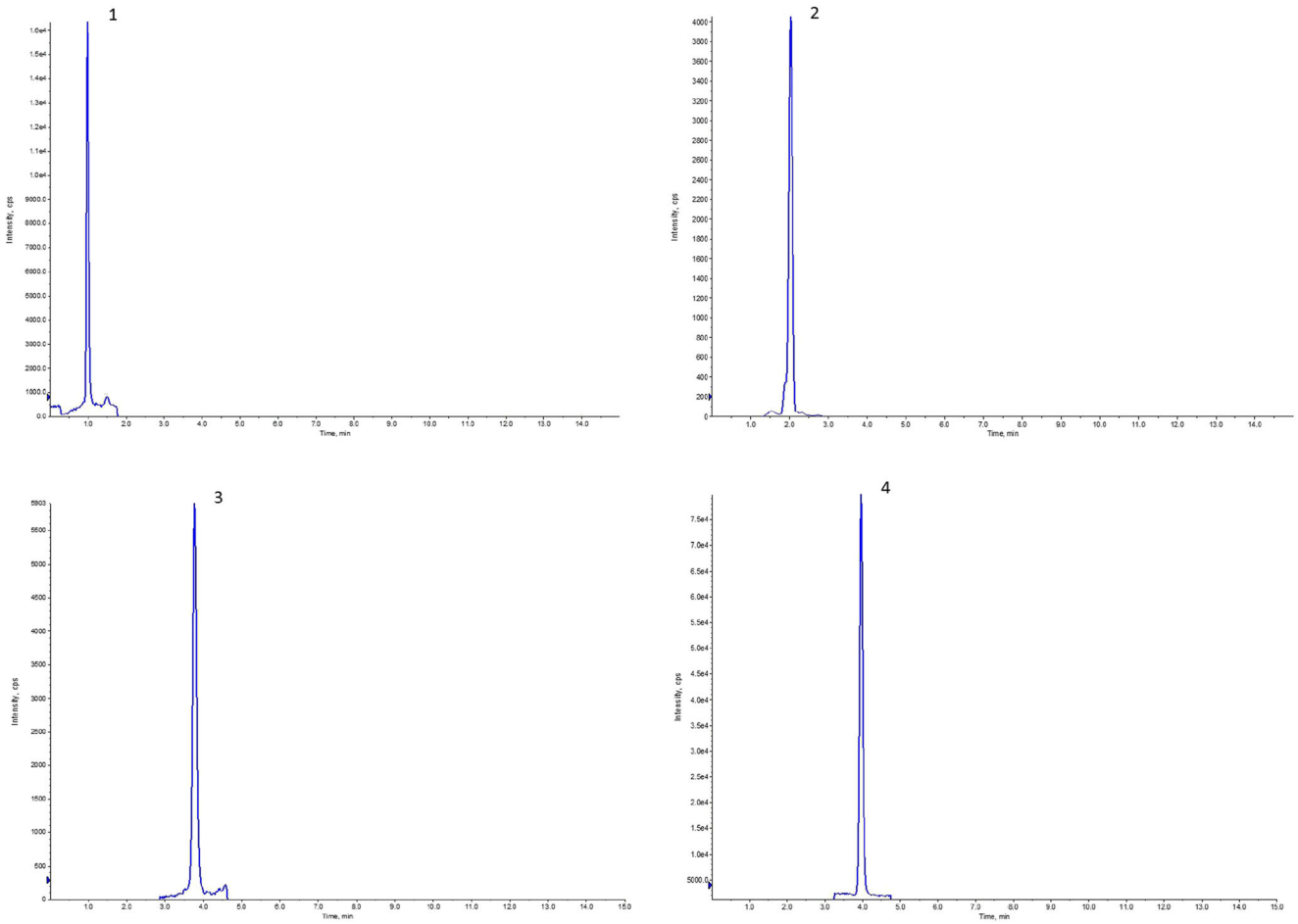

Fig. 1 LC-ESI-MS/MS chromatogram of phenolic acids from precooked buckwheat pasta. Extraction method: UAE, 60 °C, ultrasound frequency 20 kHz, power 100 W, time 40 min, 80 % aqueous ethanol. Acids: *1* gallic, *2* protocatechuic, *3* gentisic, *4* 4-OH-benzoic, *5* vanillic, *6* *trans*-caffeic, *7* *cis*-caffeic, *8* *trans*-p-coumaric, *9* *cis*-p-coumaric, *10* *trans*-ferulic, *11* *cis*-ferulic, *12* salicylic

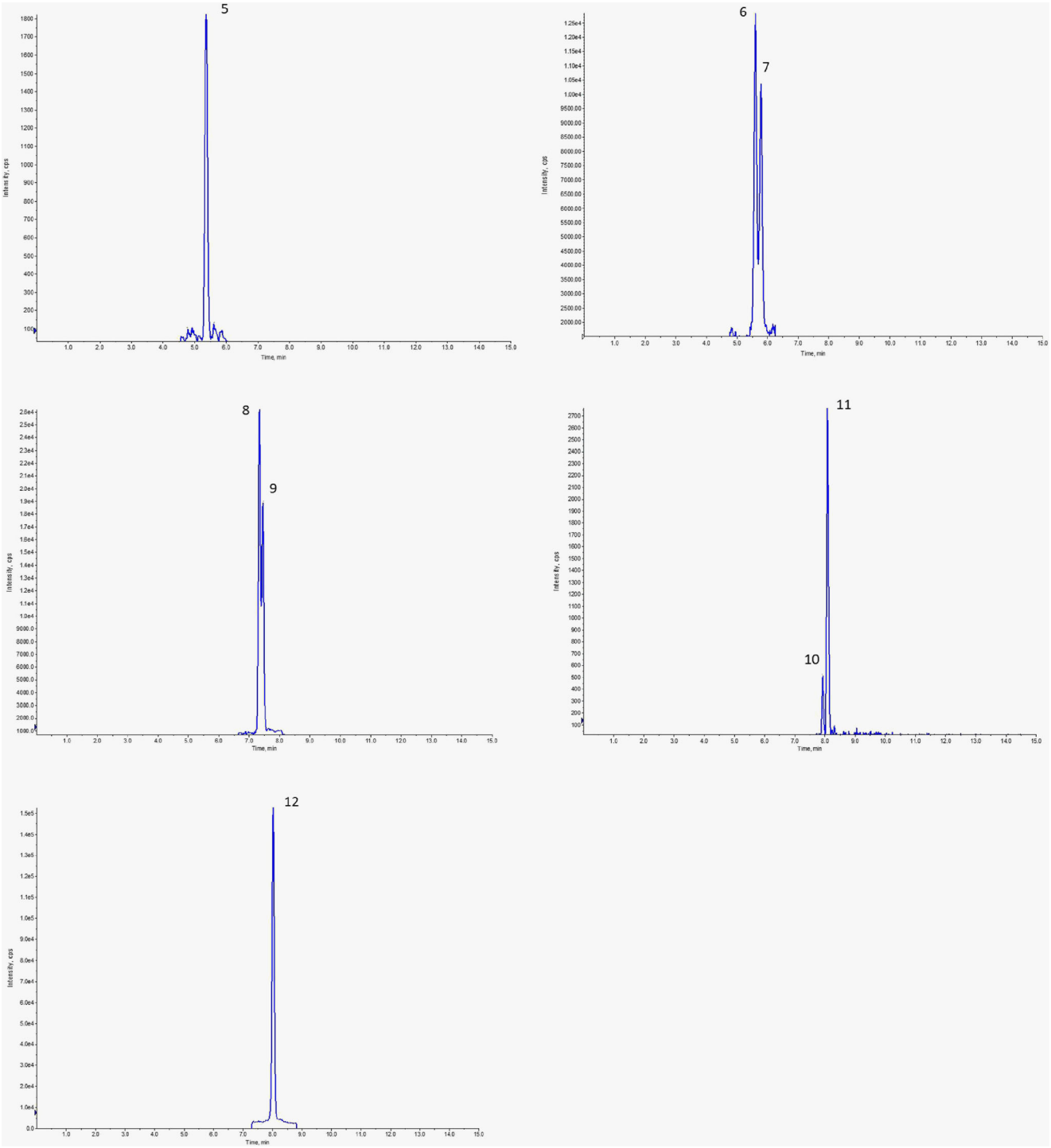

Fig. 1 (continued)

compatibility. Due to the polar nature of the phenolic acids, the addition of water (20 % *v*/*v*) to organic solvent increases the extraction yield (Oniszczuk and Olech 2016). Gallardo et al. reported slightly lower levels of phenolic compounds in aqueous extract compared to 80 % methanolic extract from buckwheat flour (Gallardo et al. 2006). In contrast, other researchers have found that 80 % methanol extracted 64 times more phenolic compounds than did water (Zielinski and Kozlowska 2000).

The next step was optimization of the extraction temperature using 80 % aqueous ethanol as extractant. The increase of temperature from 55 to 60 °C improves efficiency of the process, whereas the further increase of temperature reduces the efficiency of extraction. It can be supposed that higher

temperature (and cavitation in this condition) caused the degradation of phenolic acids (Boonkird et al. 2008).

The accuracy of the method was evaluated through recovery studies. The samples moistened with the solvent were spiked with known amounts of each standard solution (three concentration levels). Afterward, UAE was carried out using the same ways employed in the quantitative determination of phenolic compounds in the samples. The recoveries were in the range of 88.9 % (for *cis*-p-coumaric acid) to 97.3 % (for salicylic acid); therefore, UAE can be considered as an accurate method.

A short time of quantitative extraction of analyzed compounds results from the fact that polyphenols are presented in buckwheat seeds in the free form. In contrast, polyphenols of other cereal grains are primarily bound to cell wall components (Adom and Liu 2002). Such complexes are difficult to break down, and diffusion of solvent into these materials requires drastic extraction conditions and long extraction time (Oniszczuk et al. 2014).

Buckwheat products play an important role in the nutrition of people with gluten intolerance and other problems of the digestive track. Nowadays, people are looking for functional foods that are both tasty and having pro-healthy properties. The above research results indicated that extrusion cooking is a method, which provides a high content of active polyphenols in products. Precooked buckwheat pasta, due to the presence of high levels of phenolic acids, ensures health benefits, beyond the nutrition aspects. Extrusion cooking provides stable products with all nutritive components preserved or enhanced by the application of natural, biologically active compounds.

Conclusions

Precooked buckwheat pasta, due to the presence of high levels of phenolic acids, ensures health benefits, beyond the nutrition aspects. It contains natural polyphenolic antioxidants and therefore could be classified as convenience food. The phenolic acid compositions of the product were as follows: gallic, protocatechuic, gentisic, 4-OH-benzoic, vanillic, *trans*--caffeic, *cis*-caffeic, *trans*-p- coumaric, *cis*-p-coumaric, *trans*-ferulic, *cis*-ferulic, and salicylic.

The solvent type and extraction temperature influenced extraction yield under ultrasound treatment. The most effective conditions for the isolation of phenolic acids with the use of UAE were as follows: 80 % aqueous ethanol, 60 °C, ultrasound frequency 20 kHz, power 100 W, and time 40 min.

Compliance with Ethical Standards

Funding This study was supported by the Medical University of Lublin.

Conflict of Interest Anna Oniszczuk declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Not applicable.

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