DE GRUYTER OPEN

Nova Biotechnol Chim (2017) 16(1): 61-67
DOI: 10.1515/nbec-2017-0009

Nova Biotechnologica *et* Chimica

Rutin in buckwheat grain meal determined by UV photoacoustic spectroscopy and HPLC

Ottó Dóka[a,✉], Andrea Brunori[b], Rezső Schmidt[c], Dane Bicanic[d] and György Végvári[e,f]

[a] *Department of Physics and Chemistry. Faculty of Mechanical Engineering, Electrical Engineering and Informatics. Széchenyi István University. Egyetem sq. 1, Győr, H-9026, Hungary*

[b] *ENEA, CR Casaccia, SSPT-BIOAG-PROBIO, Via Anguillarese, 301, 00123 Santa Maria di Galeria, Roma, Italy*

[c] *Institute for Plant Production. Faculty of Agricultural and Food Sciences. Széchenyi István University. Mosonmagyaróvár Vár 2, H-9200, Hungary*

[d] *Laboratory of Biophysics, Wageningen University, Dreijenlaan 3, Wageningen, 6703 HA, The Netherlands*

[e] *Faculty of Horticultural Sciences, Szent István University, Budapest Xl. Villányi út 35-43, H-1118, Hungary*

[f] *Kaposvár University, Institute of Physiology, Biochemistry and Animal Health, Kaposvár Guba Sándor út 40, H-7400, Hungary*

Article info

Article history:
Received: 1st January 2017
Accepted: 19th May 2017

Keywords:
Buckwheat
Grain rutin content
UV photoacoustic spectroscopy

Abstract

A relatively novel approach for easy and quick determination of rutin in buckwheat grain is suggested. The rutin content of the grain in seven common buckwheat (*Fagopyrum esculentum*) and six Tartary buckwheat (*Fagopyrum tataricum*) varieties was investigated by means of UV photoacoustic spectroscopy and HPLC as reference method. The lowest content was found in 'Botan' and 'Bamby' varieties, while the highest values were obtained in the variety 'Emka'. Rutin content in grain of all Tartary buckwheat varieties was two orders of magnitude higher than in the other varieties. Rutin content in *F. esculentum* ranges between 9 and 36 mg/100 g dry weight as compared to 921 to 2 132 mg/100 g dry weight in *F. tataricum*. The UV photoacoustic spectroscopy data show rather good correlations of R^2=0.977 and R^2=0.980 with values obtained by HPLC data for all measured samples. Therefore, UV photoacoustic spectroscopy can be a cheap and quick method for determining rutin content in buckwheat grain.

Introduction

Flavonoids that constitute the largest group of phytonutrients are found in almost all fruits and vegetables. Like carotenoids, they are responsible for vivid and various colors in fruits and vegetables. One of them, rutin, occurs in high concentrations in buckwheat (*Fagopyrum esculentum*). Most rutin is accumulated in the inflorescence, stalks and upper leaves (Kreft *et al*. 2006). It is also found in leaves and petioles of *Rheum* species and *Asparagus*, in the fruits and flowers of pagoda tree, fruits and fruit rinds mainly of citrus fruits (like orange, grapes, lemon, lime) as well as in ash tree fruits and in berries such as mulberry and cranberries (Sánchez-Salcedo *et al*. 2015).

Buckwheat is a major source of natural rutin (Gupta *et al*. 2011). Rutin was detected in the whole buckwheat plant including leaves, flowers, stalks and seeds. Nevertheless, plants contain the highest concentration of rutin

✉ *Corresponding author:* doka.otto@sze.hu

at the beginning of flowering stage (Choi *et al.* 1996). However the amount of rutin at different parts of the plant is greatly dependent on plant species and its geographical origin (Kitabayashi *et al.* 1995). Regarding the varieties, the rutin content was much higher for *Fagopyrum tataricum* than for *Fagopyrum esculentum* (Fabjan *et al.* 2003; Park *et al.* 2004).

Buckwheat is mainly consumed as a seed. In addition to rutin, buckwheat is a rich source of starch and also contains many other valuable compounds such as proteins, antioxidative substances, trace elements and dietary fibre (Park *et al.* 2000; Bonafaccia *et al.* 2003). Whole buckwheat groats contain 55 % starch, 12 % protein, 4 % lipid, 2 % soluble carbohydrates, 7 % total dietary fibre, 2 % ash and 18 % other components (organic acids, phenolic compounds, tannins, etc.) (Eggum *et al.* 1980; Thacker *et al.* 1983; Vojtíšková *et al.* 2012).

Rutin is an important food component beneficial for human health since it affects positively the capillary fragility and permeability (Kamalakkannan and Stanely Mainzen 2006). It has a protective effect against development of diabetes and mitigates the effects on the consequences of diabetes (Je *et al.* 2002; Srinivasan *et al.* 2005). Moreover, it has antioxidative and anti-platelet formation properties as well (Afanas'eva *et al.* 2001; Sheu *et al.* 2004). In addition, it has a mitigating effect on cardiovascular diseases (He *et al.* 1995), it has also anticancer activity (Giménez-Bastida and Zieliński 2015) and antimutagenic activity (Brindzova *et al.* 2009).

Many sensitive analytical methods for determination of rutin have been developed including capillary electrophoresis (Lu *et al.* 2008; Zhang *et al.* 2013), chemiluminescence (Cheng *et al.* 2013), Fourier transform infrared spectroscopy (FTIR) and UV-VIS spectrophotometry (Rajan and Muthukrishnana 2013; Xu *et al.* 2010) and high-performance liquid chromatography (HPLC) (Deineka *et al.* 2004; Šatínský *et al.* 2013). However, some of the above mentioned methods, for example the HPLC, are time-consuming, expensive, need complicated pretreatments (e.g. multisolvent extraction) and require trained technicians to conduct the study. Here we suggest detection of rutin by photoacoustic spectroscopy (PAS). This technique offers several advantages above other techniques: it is non-destructive, it requires a minimum of preparation and can be readily applied to samples that are difficult to analyze like optically opaque powders (Yasa *et al.* 1982; Dóka *et al.* 2004). The main objective of the research undertaken in this work was to explore the feasibility of PAS for quantification of rutin content of thirteen whole grain buckwheat meals. The data obtained was compared to those acquired from the same samples by HPLC that served as golden standard.

Experimental

Fagopyrum esculentum varieties were grown in the summer of 2005 at Camigliatello Silano on the high plain of Sila in the Region of Calabria (Southern Italy). Grain samples were obtained from both, an agronomic trial for variety assessment and seed multiplication plots. In addition, six *F. tataricum* varieties grown on the nearby massif of Pollino in the Region of Basilicata, were included.

Grains of the following varieties were analysed:

i.) *F. esculentum* varieties 'Bamby', 'Emka', 'Jana', 'Kora', 'Botan', 'Hruszkowska', 'Lena' from an agronomic trial in Camigliatello Silano;

ii.) *F. tataricum* varieties 'Donan', 'Golden', 'Ishisoba', 'Chumoa', 'Hei Qiao-4' and 'Hei Feng' from an agronomic trial on the massif of Pollino.

HPLC analysis

The quantification of rutin was carried out by Waters HPLC (Waters corp., Milford, Massachusetts, USA) equipped with a plus auto-sampler, a binary HPLC pump and a dual absorbance detector operating at 350 nm wavelength. Whole meal was prepared from the grain using a Foss Tecator Cyclotec 1093 sample mill. Samples of 1 g each were extracted with 10 mL methanol for 24 h at room temperature and in darkness. The volume of methanol for *F. tataricum* varieties was doubled (20 mL) because of the expected higher rutin content.

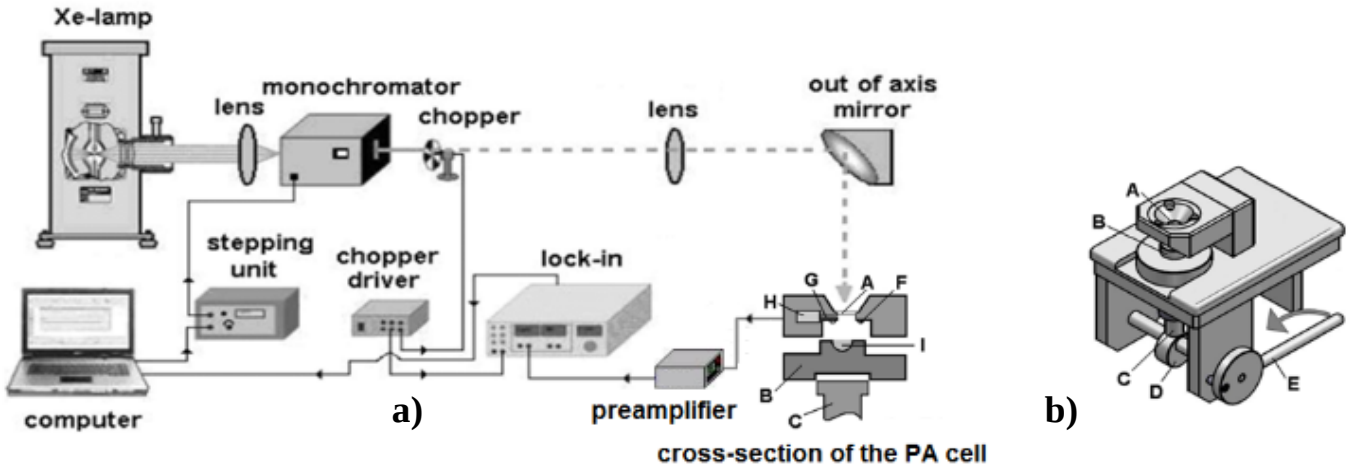

Fig. 1. UV-PAS system for measurement of rutin. a) The scheme of the self-made system and cross-section of the PA cell. A – transparent quartz window; B – sample holder; C – metal rod; D – eccentric wheel; E – lever of the sample holder; F – O-ring; G – capillary; H – microphone; I – sample. b) detailed view to the holder and the detector.

At the end of the extraction period the vials were kept for 5 minutes in an Elma Transsonic T420 ultrasonic bath operating at a frequency of 35 kHz. The methanol fraction was recovered by centrifuging for 5 min at 15 000 rpm (Hettich, Tuttlingen, Germany). Before analysis the methanol fraction was filtered by passing through a Millex HN 0.45 syringe-driven filter unit (Millipore corp., Billerica, Massachusetts, USA). The active content was separated on a Symmetry C18 5 µm 4.6×150 column. The analytical process was assisted by Empower 2TM software. The analysing conditions were: 2.5 % acetic acid in water (350 mL), MeOH (50 mL) and acetonitrile (100 mL) as a mobile phase. The flow-rate was 1 mL $min^{-1}$and pressure on the column 1 750±10 psi. Twenty µL of seed extract in methanol was used for rutin determination. Two replicated analyses were run for each sample. Reference was made to a solution of rutin hydrate [153-18-4] containing 2.65 mg dissolved in 25 mL of methanol (HPLC quality). Results were expressed in mg of rutin per 100 g of grain dry weight (DW).

UV photoacoustic spectroscopy (UV-PAS)

In the UV-PAS the sample to be investigated is irradiated by a modulated beam. The fraction of the energy absorbed by the sample is converted to heat, while the temperature of sample oscillates periodically at a frequency identical to that of the modulated radiation. Generated thermal waves eventually reach sample's surface and cause a periodic heating and cooling of the contacting layer of the surrounding gas. Finally, the expansions and contractions of the gas give rise to acoustic waves; these are detected as a voltage (termed PA signal) by a microphone. The optical and thermal parameters of sample and the contacting gas all play a decisive role in the generation of PA signal. In order to eliminate the variation of the output power on the emission wavelength of the power on the emission wavelength of the illuminating source it is customary to normalize the PA signal from the sample to that obtained (under identical experimental conditions) from the carbon black powder acting as a strongly absorbing reference sample. Such normalized signal ratio is expressed in arbitrary units (a. u.).

Four major parts of the self-made PA spectrometer used in this study are the 1000 W Xe lamp (Oriel Technology), the monochromator (Jobin-Yvon, H-10, spectral resolution of 16 nm), a modulator and a self-made PA cell (Fig. 1). After passing through the monochromator, the collimated beam of mechanically chopped (17 Hz) radiation was collected by a quartz lens and focussed into the PA cell. Radiation enters the PA cell (Fig. 1) through a quartz window half inch in diameter.

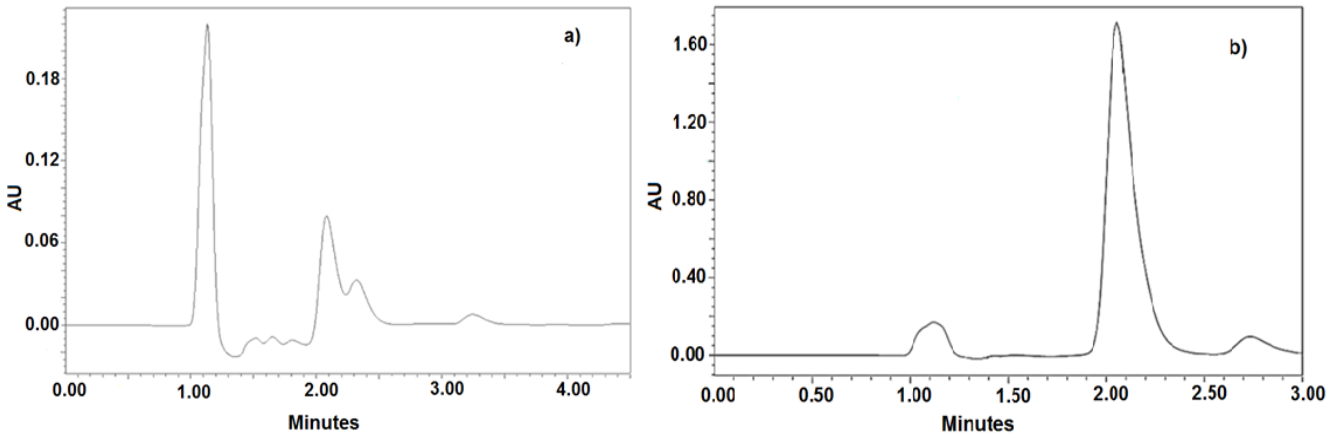

Fig. 2. Characteristic HPLC chromatograms for extraction from Jana *F.esculentum* (a) and Chumoa *F. tataricum* (b) showing that rutin content of the former group is more than 30-times higher. The chromatographic peak of rutin elutes about 2.08 min of running time for the total extract.

The sampling volume and a miniature (4.2 x 4.75 mm) microphone (Sennheiser KE 4-211-2) are acoustically coupled by means of a 3 mm long capillary. The PA cell is sealed (closed) when the lever of the sample holder is rotated by 180°. In such a case the metal rod presses the sample holder against the O-ring which, due to the eccentricity of the wheel, seals the PA detector. The buckwheat meal was loaded into a semispherical cavity made in the sample holder. The PA signal was processed by a preamplifier (Stanford SR552) and a dual phase lock-in amplifier (Stanford SR530) connected to the computer. With each sample the PA signal was recorded in the range of 250 to 600 nm. Three independent measurements with each sample have been performed.

Results

HPLC measurements

HPLC chromatograms were obtained for samples of *F. esculentum* and *F. tataricum* varieties (Fig. 2). Peaks assigned to rutin appeared in the chromatograms after 2.08 min of running time for the complete extract. As expected the content of rutin in the grain of *F. tataricum* was over 30-times higher when compared to that of F. *esculentum* (Table 1).

An appreciable variation in the rutin content of the grains was apparent among varieties of *F. esculentum*, ranging from 9.0 mg for the variety 'Botan' to 36.2 mg for 'Emka' (in 100 g grain DW). Similarly, in *F. tataricum* varieties it varied between 921 mg ('Chumoa') and 2 132 mg ('Donan').

UV-PA measurements

The same buckwheat grain samples were also measured using UV-PAS. The spectra between 250 nm and 600 nm were recorded using the PA spectrometer described in the Methods part. The obtained data (Figs. 3a,b) were normalized to the power output of Xe lamp. The spectra feature two specific peaks in the investigated range. One of them centered at 275 nm due to the protein content of the samples, while the other (stronger) peak at 378 nm (Fig. 3b) can be attributed to rutin in samples. However, the PA signal increases across the entire wavelength range in proportion to the content of rutin in the sample.

The net normalized PA signals at 378 nm plotted versus the rutin content for *F. esculentum* (Fig. 4a) and *F. tataricum* (Fig. 4b) varieties obtained by HPLC is presented in Fig. 4. The correlations of data by the two methods are linear with rather good determination coefficients for samples of both varieties (R^2=0.977 and R^2=0.980, respectively).

Table 1. Rutin content of buckwheat grain of seven *F. esculentum* varieties grown at Camigliatello Silano (Region of Calabria) and six *F. tataricum* varieties grown at Terranova del Pollino (Region of Basilicata) measured by HPLC.

Varieties	Rutin (mg/100 g dry weight)		
	Replicated analysis		Average±STDV
	1st	2nd	
	F. esculentum		
Bamby	9.2	10.1	9.7±0.06
Botan	9.6	8.3	9.0±0.93
Emka	38.0	34.4	36.2±2.61
Hruszkowska	22.4	21.6	22.0±0.52
Jana	22.4	21.0	21.7±1.04
Kora	23.6	21.8	22.7±1.32
Lena	29.0	29.7	29.3±0.45
	F. tataricum		
Donan	2 115.7	2 148.5	2 132.1±23.16
Golden	2 107.9	2 059.4	2 083.7±34.29
Ishishoba	1 762.1	1 660.5	1 711.3±71.84
Hei Feng	1 001.1	1 024.2	1 012.6±11.53
Hei Qiao-4	1 265.6	1 280.8	1 273.2±7.61
Chumoa	908.8	927.5	918.2±9.35

Discussion

UV-PAS was tested as a possible new anaytical method for quick and simple measurement of rutin content. The PA spectra obtained for the two varieties are very similar reflecting to relatively small differences in the content of rutin in the samples (up to only 27 mg/100 g). This latter fact explains the small slope of the calibration curve. Since rutin content in *F. tataricum* is two orders of magnitude higher than that in *F. esculentum*, the measured spectra have a more pronounced absorption at the given analytical wavelength. For *F. esculentum* varieties the PA signal can be increased by using a more powerful laser at the analytical wavelength.

Our parallel HPLC measurements confirmed that rutin content varies from about 10 mg to a few thousands milligrams per 100 g dry weight in the whole grain meal of buckwheat varieties. Calculated LOD value suggests that the method can be used successfully in the analyzed buckwheat varieties that likely can be extended to even another one. The other parts of the plant (root, stem, leaf, flower) have rutin content between 90 and 380 mg/100 g (Choi *et al.* 1996) and so the obtained LOD is enough to quantify the rutin content in different parts of the plant.

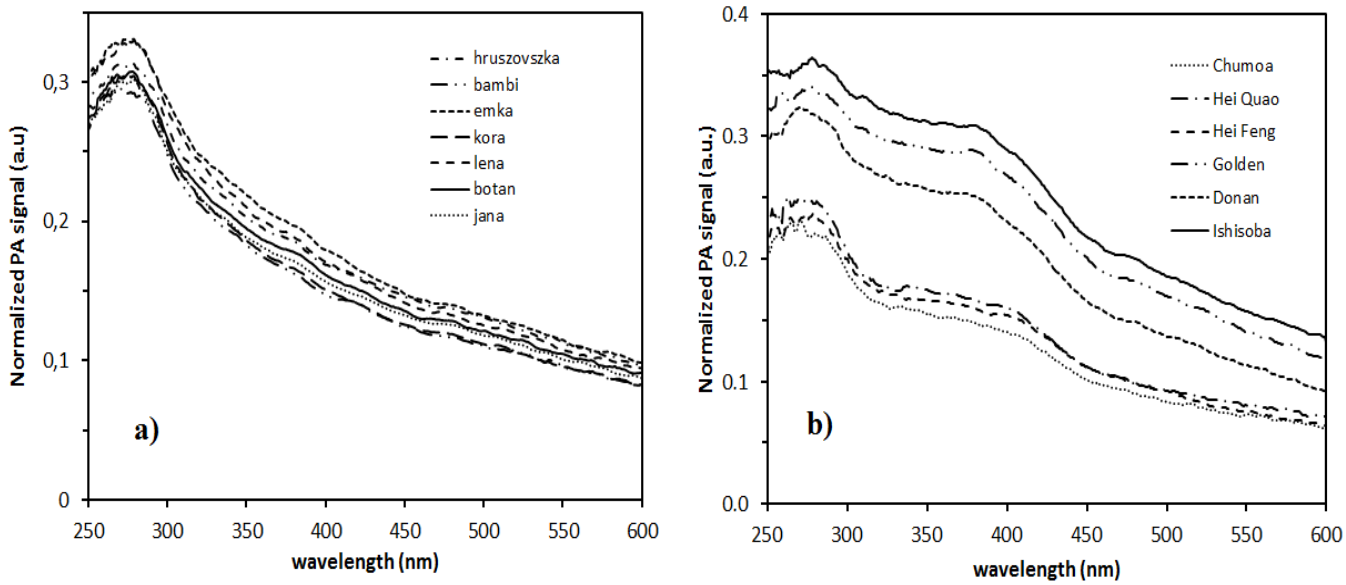

Fig. 3. Normalized photoacoustic spectra of seven *Fagopyrum esculentum* (a) and of six *Fagopyrum tataricum* (b) varieties between 250 and 600 nm.

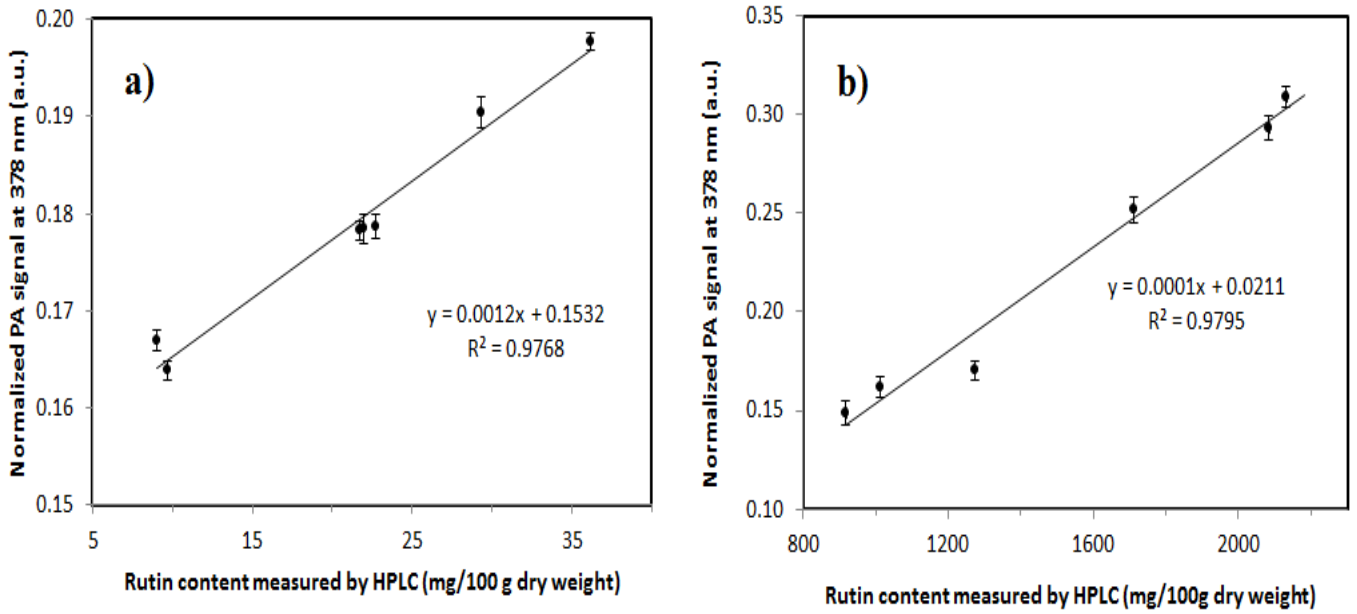

Fig. 4. The normalized PA signal (at 378 nm and 17 Hz) plotted versus the rutin content of *Fagopyrum esculentum* (a) and *Fagopyrum tataricum* (b) as determined by HPLC. Data represents the averages and the standard deviations of triplicate measurements.

Conclusions

UV-PAS was explored as a candidate analytical tool for rapid and simple quantification of rutin in buckwheat. In both of the tested varieties the magnitude of measured PA signal appears to be linearly dependent on the rutin content of the samples. Once a calibration curve is constructed using samples of known rutin content, buckwheat samples can routinely be analyzed directly. As no pre-treatment is required, the time for completing the analysis is reduced quite significantly.

References

Afanas'eva IB, Ostrakhovitch EA, Mikhal'chik EV, Ibragimova GA, Korkina LG (2001) Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals. Biochem. Pharmacol. 61: 677-684.

Bonafaccia G, Marocchini M, Kreft I (2003) Composition and technological properties of the flour and bran common and tartary buckwheat. Food Chem.: 80: 9-15.

Brindzova L, Mikulasova M, Takacsova M, Mosovska S, Opattova A (2009) Evaluation of the mutagenicity and antimutagenicity of extracts from oat, buckwheat and wheat bran in the Salmonella/microsome assay. J. Food Compos. Anal. 22: 87-90.

Cheng C, Huang Y, Wang J, Zheng B, Yuang H, Xiao D (2013) Anodic electrogenerated chemiluminescence behavior of graphite-like carbon nitride and its sensing for rutin. Anal. Chem. 85: 2601-2605.

Choi BH, Kim SL, Kim SK (1996) Rutin and functional ingredients of buckwheat and their variations. Korean J. Crop. Sci. 41: 69-93.

Dóka O, Bicanic D, Dicko MH, Slingerland M (2004) Photoacoustic approach to direct determination of the total phenolic content in red sorghum flours. J. Agric. Food Chem. 52: 2133-2136.

Deineka VI, Grigor'ev AM, Staroverov VM (2004) HPLC analysis of flavonoids: determining rutin in plant extracts. Pharm. Chem. J. 38: 487-489.

Eggum BO, Kreft I, Javornik B (1980) Chemical composition and protein quality of buckwheat (*Fagopyrum esculentum* Moench). Plant Food Hum. Nutr. 30: 175-179.

Fabjan N, Rode J, Kosir IJ, Wang Z, Kreft I (2003) Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercetin. J. Agr. Food Chem. 51: 6452-6455.

Gupta N, Sharma SK, Rana JC, Chauhan RS (2011) Expression of flavonoid biosynthesis genes *vis-a-vis* rutin content variation in different growth stages of *Fagopyrum species*. J. Plant Physiol. 168: 2117-2123.

Giménez-Bastida JA, Zieliński H (2015) Buckwheat as a functional food and its effects on health. J. Agr. Food Chem. 63: 7896-7913.

He J, Klag MJ, Whelton PK, Mo JP, Chen JY, Qian MG, Mo PS, He GQ (1995) Oats and buckwheat intake and cardiovascular disease risk factors in an ethnic minority of China. Am. J. Clin. Nutr. 61: 366-372.

Je HD, Shin CY, Park SY, Yim SH, Kum C, Huh IH, Kim JH, Sohn UD (2002) Combination of vitamin C and rutin on neuropathy and lung damage of diabetes mellitus rats. Arch. Pharm. Res. 25: 184-190.

Kamalakkannan N, Stanely Mainzen PP (2006) Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozoticin-induced diabetic wistar rats. Basic Clin. Pharmacol. 98: 97-103.

Kitabayashi H, Ujihara A, Hirose T, Minami M (1995) Varietal differences and heritability for rutin content in common buckwheat, *Fagopyrum esculentum* Moench. Breeding Sci. 45: 75-79.

Kreft I, Fabjan N, Yasumoto K (2006) Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. Food Chem. 98: 508-512.

Lu QH, Ba CD, Chen DY (2008) Investigating noncovalent interactions of rutin-serum albumin by capillary electrophoresis-frontal analysis. J. Pharm. Biomed. Anal. 47: 888-891.

Park BJ, Park JI, Kwang JC, Park CH (2004) Comparison in rutin content in seed and plant of tartary buckwheat (*Fagopyrum tataricum*). *In:* Proceeding of the 9th International Symposium on Buckwheat. Prague, Czech Republic, August 18–22, 2004, 626-629.

Park CH, Kim YB, Choi YS, Heo K, Kim SL, Lee KC, Chang KJ, Lee HB (2000) Rutin content in food products processed from groats, leaves and flowers of buckwheat. Fagopyrum. 17: 63-66.

Rajan T, Muthukrishnana S (2013) Characterization of phenolic compounds in *Pseudarthria viscida* root extract by HPLC and FT-IR analysis. Asian J. Pharm. Clin. Res. 6: 274-276.

Sánchez-Salcedo EM, Mena P, García-Viguera C, Martínez JJ, Hernández F (2015) Phytochemical evaluation of white *(Morus alba L.)* and black *(Morus nigra L.)* mulberry fruits, a starting point for the assessment of their beneficial properties. J. Func. Foods. 12: 399-408.

Šatínský D, Jägerová K, Havlíková L, Solich P (2013) A new and fast HPLC method for determination of rutin, troxerutin, diosmin and hesperidin in food supplements using fused-core column technology. Food Anal. Methods. 6: 1353-1360.

Sheu JR, Hsiao G, Chou PH, Shen MY, Chou DS (2004) Mechanisms involved in the antiplatelet activity of rutin, a glycoside of the flavonoid quercetin, in human platelets. J. Agr. Food Chem. 52: 4414-4418.

Srinivasan K, Kaul CL, Ramarao P (2005) Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. Indian J. Pharmacol. 37: 327-328.

Thacker PA, Anderson DM, Bowland JP (1983) Chemical composition and nutritive value of buckwheat cultivars for laboratory rats. Can. J. Anim. Sci. 63: 949-956.

Xu H, Li Y, Tang HW, Liu CM, Wu QS (2010) Determination of rutin with UV-VIS spectrophotometric and laser-induced fluorimetric detections using a nonscanning spectrometer. Anal. Lett. 43: 893-904.

Yasa ZA, Jackson WB, Amer NM (1982) Photothermal spectroscopy of scattering media. Appl. Opt. 21: 21-31.

Vojtíšková P, Kmentová K, Kubáň V, Kráčmar S (2012) Chemical composition of buckwheat plant (*Fagopyrum esculentum*) and selected buckwheat products. J. Microbiol. Biotechnol. Food Sci. 1: 1011-1019.

Zhang F, Qi X, Zou M, Li J (2013) Analysis of rutin from *Lespedeza virgata* (Thunb.) DC. by microwave-assisted extraction and capillary electrophoresis. J. Chem. 2013: Article ID 324294.