Optimized preparation of anthocyanin-rich extract from black rice and its effects on in vitro digestibility

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Abstract The procedure for obtaining anthocyanin-enriched extracts from black rice was optimized by response surface methodology, and the effects of the optimized extract on in vitro starch digestibility were investigated in a wheat flour gel model. The experimental results were well-described by a polynomial multiple regression model ($R^2 = 0.8812$, $p = 0.0546$) with regard to anthocyanin content in anthocyanin-enriched extracts from black rice. The optimal conditions for obtaining anthocyanin-enriched extracts from black rice were 50.78% ethanol and 1 N HCl (0.60 mL), yielding a predicted anthocyanin content of 624.27 mg cyanidin 3 glucoside extract. The optimized anthocyanin-enriched extract was a stronger inhibitor of α -glucosidase than acarbose. Furthermore, the predicted glycemic index values of gels prepared with the optimized extract were significantly lower than that of wheat flour gel. These results indicate that the optimized extract suppressed starch hydrolysis by inhibiting digestive enzymes.

Keywords α -Glucosidase inhibition · Anthocyanin · Black rice · In vitro starch digestibility · Predicted glycemic index

Introduction

Pigmented varieties of rice (*Oryza sativa* L.) such as black rice, are good sources of fiber, minerals, phytochemicals, and basic nutrients [1]. In particular, pigmented rice varieties contain higher amount of phenolic compounds (e.g., cyanidin-3-glucoside, malvidin-3-glucoside, and peonidin-3-glucoside) than white rice [2]. The research of de Mira et al. [3] indicated that the total phenolic content of pigmented rice was four times greater than that of non-pigmented varieties. Moreover, red and purple rice varieties were found to have higher total phenolic, flavonoid, and antioxidant activities than lighter varieties, such as white and brown rice [4].

Black rice (*Oryza sativa* L., ‘Josaengheugchal’) has been considered an important health-promoting crop due to high anthocyanin content of its aleurone layer. This variety has been introduced into many other areas such as North America and Eastern Asia [5]. The main anthocyanin in black rice is cyanidin-3-glucoside, while a peonidin-3-glucoside is minor component [6]. Anthocyanins belong to a larger group of compounds collectively known as flavonoids, which comprise a subgroup of an even larger group of compounds known as polyphenolics. In nature, anthocyanins are responsible for the vibrant colors of flowers, fruits, and grains [7]. Anthocyanins are considered to be bioactive compounds, and their consumption has been linked to beneficial health effects [8]. Due to their reduction of risk of oxidative damage, anthocyanins were recently suggested as potential candidates for the treatment of cardiovascular disease, type 2 diabetes, and cancer [6].

Anthocyanins reduce starch digestion by inhibiting α -glucosidase activities [9]. McDougall et al. [10] tested the inhibitory activity of several extracts from soft fruit (e.g., blueberry, blackcurrant) against α -glucosidase and

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reported that the anthocyanin-rich fractions effectively inhibited α -glucosidase. Moreover, Yao et al. [11] found that anthocyanins isolated from black rice inhibited α -glucosidase. Therefore, anthocyanins are capable of inhibiting key enzymes such as α -glucosidase and α -amylase. As these enzymes have both been linked to postprandial hyperglycemia, their inhibition could be used in the treatment of type 2 diabetes [8].

The first step in isolating compounds from natural sources is extraction. However, the optimal extraction conditions differ among various plant materials because the extraction efficiency is influenced by several parameters, including the chemical nature of the sample, solvent used, agitation method, extraction time, solute/solvent ratio, and temperature [12]. Traditionally, organic solvents such as absolute methanol, ethanol, and acetone have been used to extract anthocyanins [13]. Ethanol is desirable because it is less toxic than methanol [14]. Aqueous mixtures of solvents with small amounts of acid have also been used. Acidified organic solvents destroy the cell membrane, simultaneously dissolve anthocyanins, and stabilize the anthocyanins [15]. Therefore, the percentage of ethanol and the acidity of the organic solvent are important determinants of the extraction efficiency.

Therefore, our purpose in this study was to use response surface methodology to optimize the extraction parameters of ethanol percentage (10–90%) and 1 N HCl volume (0–1 mL) in the extraction solution, with the goal of obtaining anthocyanin-enriched extracts from black rice. In addition, the effects of the optimized anthocyanin-enriched extracts on in vitro starch digestibility in a wheat flour gel model was investigated.

Materials and methods

Materials

Black rice (Josaengheugchal, *Oryza sativa* L., harvested in 2014, Korea) was supplied from the Rural Development Administration (RDA) at Mirayng, South Korea. The black rice was stored at 25 °C until laboratory analysis. Wheat flour was purchased from CJ Co. (Seoul, Korea). Acarbose (A8980), amyloglucosidase (A9913), 4-nitrophenyl- α -D-glucopyranoside (pNPG, N1377), porcine bile extract (B8631), and porcine pancreas pancreatin (P7545) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). A total starch kit (K-TSTA), glucose oxidase–peroxidase assay kit (GOPOD, K-GLUC), and α -glucosidase (*Bacillus stearothermophilus*; E-TSAGL) were purchased from Megazyme International Ireland Ltd. (Dublin, Ireland). All reagents were of standard analytical grade.

Extraction methodology

Black rice was ground in a blender (Hanil Food Co., Ltd., Korea) and passed through a 100-mesh sieve. The resultant material, referred to hereafter as “BF”, was used in all further extractions and analyses. Anthocyanin-enriched extracts were extracted from BF according to the method described by Prior et al. [16] with slight modification. Briefly, 10 g of BF was mixed with 100 mL of ethanol at different percentages (10–90%) and with different volumes of 1 N HCl (0–1 mL) at 30 °C for 2 h (Table 1). All extracts were collected and filtered. The supernatants were then collected, evaporated at 35 °C, and dried at 60 °C in a forced-convection dry oven (DAIHAN Scientific Co., Ltd., Seoul, Korea) until all solvent was removed. The resultant powder was stored at 4 °C until use.

Total anthocyanin contents

Total anthocyanin content was determined by the methods of Giusti et al. [17]. In short, black rice extracts were separately diluted with 0.4 M sodium acetate buffer (pH 4.5) and 0.025 M potassium chloride solution (pH 1.0), and the absorbance values of both dilutions were measured at 700 and 510 nm. The results were expressed as milligrams of anthocyanin (cyanidin 3 glucoside, C3G) equivalent per gram of dry weight of the extract. Calculations were performed with the equation $A = [(A_{510} - A_{700} \text{ at pH } 1.0) - (A_{510} - A_{700} \text{ at pH } 4.5)]$ with a molar extinction coefficient of 26,900 and a molecular weight of 449.2 g/mol.

Experimental design and data analysis

The optimal conditions for preparing anthocyanin-enriched extracts from BF were determined by response surface methodology, consisting of 10 experimental points (fractional 2^2 factorial points, four star points, and two replicates at the center point) and a two-factor, five-level central composite design. The two independent variables examined were ethanol percentage (%), X_1 and 1 N HCl level (mL), X_2 in the extraction solution. The actual values of each variable were coded at five levels for statistical

Table 1 Coded and actual levels of independent variables used for central composite design

Variables	Coded level					ΔX
	−2	−1	0	1	2	
Ethanol percentage (%)	10	30	50	70	90	20
1 N HCl level (mL)	0.00	0.25	0.50	0.75	1.00	0.25

analysis (Table 1). The ranges of these independent values were predetermined from preliminary experiments. The dependent response (Y_i) was total anthocyanin content (Y). The quadratic model for predicting the optimal point was expressed with the following equation:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j>i}^3 \beta_{ij} X_i X_j$$

where β_0 , β_i , β_{ii} , and β_{ij} are the constant, linear, quadratic, and cross-product regression coefficients, respectively. Statistical Analysis System (SAS) 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis of the experimental data.

Inhibition of α -glucosidase

The α -glucosidase inhibitory abilities of the black rice extracts were measured according to the method of Kim et al. [18]. Briefly, α -glucosidase (0.5 U/mL, 50 μ L, from *Aspergillus niger*) was premixed with black rice extract (50 μ L); then, potassium phosphate buffer (0.2 M, pH 6.8, 50 μ L) was added, and the mixture was incubated at 37 °C for 15 min. pNPG (3 mM, 100 μ L) was added to the resulted solution, the mixture was incubated at 37 °C for 10 min and the reaction was terminated by the addition of Na_2CO_3 (0.1 M, 750 μ L). α -Glucosidase ability was determined by the measurement of 4-nitrophenol absorbance at 405 nm. Acarbose was also assayed as a standard reference. The inhibition rate of α -glucosidase was calculated as follows:

$$\text{Inhibition rate (\%)} = \left[1 - \frac{(A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \right] \times 100$$

where A_{sample} is the absorbance of the experimental sample, A_{blank} is the absorbance of the blank, and A_{control} is the absorbance of the control. IC_{50} was calculated as the concentration of anthocyanin-enriched extracts from black rice required to inhibit 50% of enzyme activity.

In vitro starch digestion

Wheat flour in a gel was substituted with the optimized anthocyanin-enriched extract at 10, 15, or 20% by weight, and the effects of the extract on in vitro starch digestibility were observed. The wheat flour gel (10.71%, w/v) was prepared with the mixtures (3 g) of wheat flour and the optimized anthocyanin-enriched extracts adding distilled water (25 mL). The suspensions were heated for 10 min in a boiling water bath, and the gels were steamed over boiling water for 40 min in steam-cooker.

The in vitro starch digestion of the gels was then analyzed by method of Minekus et al. [19] with slight modification. Briefly, each gel sample (5 g) was mixed with

distilled water (15 mL) and stirred at 700 rpm for 30 min to disrupt the gel structure. Then, simulated intestinal fluid (SIF; 11 mL), CaCl_2 (0.3 M, 40 μ L), NaOH (1 M, 0.15 mL), and distilled water (1.31 mL) were added, and the reaction was incubated at 37 °C. Pancreatin, a bile salt solution, and amyloglucosidase (0.2 mL/g of starch in the sample, from *Aspergillus niger*) were then added. The SIF electrolyte stock solution consisted of KCl (0.5 M, 6.8 mL), KH_2PO_4 (0.5 M, 0.8 mL), NaHCO_3 (1 M, 42.5 mL), NaCl (2 M, 9.6 mL), and $\text{MgCl}_2(\text{H}_2\text{O})_6$ (0.15 M, 1.1 mL) in 400 mL of distilled water. Aliquots (0.1 mL) were collected at different time points (30, 60, 90, 120, and 180 min) and combined with 1.4 mL ethanol. These solutions were centrifuged ($600\times g$ for 3 min), the supernatants were collected, and a GOPOD kit was used to measure the released glucose content at 510 nm. From a nutritional point of view, most starches are classified into three major fractions according to the extents of digestion in the gastrointestinal tract which are rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). To determine the levels of RDS, SDS, and RS, the released glucose contents were measured after simulated intestinal digestion for 30, 120, and 180 min, respectively.

Predicted glycemic index (pGI)

The pGIs of the gels were determined by the procedure of Goni et al. [20] through the use of the following equation: $[C = C^\infty(1 - e^{-kt})]$, where C , C^∞ , and k indicate the hydrolysis degree at each time, the extent of maximum hydrolysis, and the kinetic constant, respectively. The hydrolysis index (HI) was calculated from the area under the hydrolysis curve of each sample, expressed as a percentage of the corresponding area of a reference sample (fresh white bread). The predicted glycemic index (pGI) was calculated with the Goni equation: $\text{pGI} = 39.71 + 0.549(\text{HI})$.

Identification of the anthocyanins in the optimized black rice extracts

LC/MS analysis was performed by the procedure of Hao et al. [21] with modifications. The anthocyanins in the optimized anthocyanin-enriched black rice extracts were identified by ultra-performance liquid chromatography/quadrupole-time of flight-mass spectrometry (UPLC/Q-TOF-MS). UPLC analysis was carried out on an ACQUITY^{TM} ultra-performance liquid chromatography system (Waters Corporation, Milford, MA, USA). Chromatographic separation was performed on a Cortecs 1.6 μ m column (2.1 $\times$ 100 mm, Waters Corporation, USA) at a column temperature of 20 °C. The mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). The gradient conditions at a flow rate of 0.6 mL/min were as

follows: 0–4 min, 99% A; 4–20 min, 99–1% A; 20–25 min, 1% A; 25–30 min, 1–99% A. The injection volume was 5 μ L. Mass analysis was performed on a Q-TOF SYNAPT G2 mass spectrometer (Waters MS Technologies, Manchester, UK). Spectra were recorded in ESI positive ion mode between m/z 50 and m/z 1200. The MS parameters were set as follows: capillary voltage, 3.1 kV; sampling cone voltage, 35.0 V; extraction cone voltage, 4.0 V; source temperature, 120 $^{\circ}$ C; desolvation temperature, 350 $^{\circ}$ C; cone gas flow, 100 L/h; and desolvation gas flow, 750 L/h.

Statistical analysis

All experiments were performed at least in triplicate. The values are expressed as means and standard deviations. All statistical analyses consisted of Duncan's multiple range test ($p < 0.05$) and were performed with SPSS statistical software (Version 21.0, Chicago, IL, USA).

Results and discussion

Optimization of the preparation of anthocyanin-enriched extracts from black rice

The effects of the two extraction conditions (ethanol percentage [X_1] and 1 N HCl volume [X_2]) on total anthocyanin content (Y) are shown in Table 2. The total anthocyanin contents under different extraction conditions varied significantly ($p < 0.05$) from 116.81 to 659.27 mg C3G/g of extract (Table 2). The mathematical relationship between the extraction conditions (X_i) and Y was obtained from regression analysis, as follows: $Y = -737.77^* + 36.45X_1^{**} + 1447.92X_2^* - 6.45X_1X_2 - 0.32 X_1^{2***} - 927.61X_2^{2*}$ (***,

**, and * indicate significance at $p < 0.01$, $p < 0.05$, and $p < 0.1$, respectively). This regression model was significant yielding an R^2 value of 0.8812 and a p value of 0.0546. Thus, the linear and quadratic coefficients X_1 and X_2 significantly influenced total anthocyanin content.

Response surfaces were plotted to investigate the effects of these parameters (ethanol percentage and 1 N HCl volume) and their interactions on total anthocyanin content (Fig. 1). The total anthocyanin content increased as the ethanol percentage increased from 40 to 60%, but decreased at other percentages. Likewise, the total anthocyanin content increased as the volume of 1 N HCl increased only within the range of 0.4–0.8 mL. In general, acidified organic solvents are good solutions for extracting anthocyanins from plant materials [15]. Such solvent systems destroy the cell membrane, simultaneously dissolve anthocyanins, and stabilize the anthocyanins. Moreover, extracted anthocyanins are most stable at strong acidic pH (< 3) and are relatively unstable at the weak acidic to neutral pH values of most foods [22]. Barnes et al. [23] systematically compared different solvents and acids for the extraction of anthocyanins. Acetone, methanol, and ethanol were found to be the most efficient in extracting blueberry anthocyanins, whereas trifluoroacetic acid and hydrochloric acid were best suited for solvent acidification. Among these three solvents, ethanol is the least toxic.

The highest total anthocyanin content was predicted to be achieved when the ethanol percentage was 50.78% and the 1 N HCl level was 0.60 mL. The maximum Y value predicted from these optimized conditions was 624.72 μ g C3G/g extract, which was close to the experimental Y value (638.23 ± 20.77 μ g C3G/g extract) obtained when the extraction was performed under the optimized conditions. According to the results of Abdel-Aal et al. [24],

Table 2 Anthocyanin contents determined from 10 experimental runs generated by central composite design

Run number	Independent variables ^a (X _n)				Response variables ^b (Y _n)	
	Coded variables		Process variables			Y
	X ₁	X ₂	X ₁	X ₂		
1	−1	−1	30	0.25	299.58 ± 40.54 ^{de}	
2	1	−1	70	0.25	496.96 ± 55.42 ^b	
3	−1	1	30	0.75	345.92 ± 85.63 ^{cd}	
4	1	1	70	0.75	414.22 ± 74.66 ^c	
5	0	0	50	0.50	646.08 ± 4.49 ^a	
6	0	0	50	0.50	659.27 ± 72.26 ^a	
7	−2	0	10	0.50	116.81 ± 17.45 ^f	
8	2	0	90	0.50	124.24 ± 16.58 ^f	
9	0	−2	50	0.00	244.14 ± 7.94 ^e	
10	0	2	50	1.00	558.74 ± 37.08 ^b	

Different letters indicate significant differences within samples ($p < 0.05$)

^a X_1 = Ethanol percentage (%), X_2 = 1 N HCl level (mL)

^b Y = Total anthocyanins (mg C3G/g flour)

Fig. 1 Effects of ethanol percentage and 1 N HCl level in extract solutions on the anthocyanin contents in the various extracts

black rice had a wide range of total anthocyanins depending upon cultivar and exhibited a range of 27.2–3276 $\mu\text{g/g}$.

α -Glucosidase inhibitory effects of the optimized extract from black rice

α -Glucosidase performs on the final step of starch hydrolysis, resulting in the release of glucose, and has thus been recognized as a therapeutic target for the regulation of postprandial hyperglycemia [10]. The ability of the optimized anthocyanin-enriched black rice extract to inhibit α -glucosidase was investigated with acarbose as a positive control.

As shown in Fig. 2, the optimized anthocyanin-enriched extract inhibited α -glucosidase in a concentration-dependent manner ($p < 0.05$). Furthermore, the optimized anthocyanin-enriched extract (IC_{50} of $0.02 \pm 0.00 \mu\text{g/mL}$) inhibited α -glucosidase much more strongly than did acarbose (IC_{50} of $1.40 \pm 0.00 \mu\text{g/mL}$). Our results are in agreement with previous findings that soft fruits can effectively inhibit α -glucosidase; this activity was shown to be present in the anthocyanin-rich fraction [10]. Moreover, Yao et al. [11] found that anthocyanins from black rice strongly inhibited α -glucosidase.

Effects of the optimized black rice extract on in vitro starch digestibility

To investigate the effects of the optimized anthocyanin-enriched extract from black rice on in vitro starch digestibility in wheat flour gels, the released glucose contents during starch hydrolysis were measured. Glucose

Fig. 2 Inhibitory effects of the optimized anthocyanin-enriched extracts from black rice and acarbose (standard reference) against α -glucosidase. Different letters on the bars are significantly different from each other ($p < 0.05$)

release curves were generated to measure the reducing sugars produced by enzyme-mediated starch digestion. Figure 3(A) displays the starch hydrolysis curves of the optimized anthocyanin-enriched extract-incorporating gels and the starch hydrolysis curve of the wheat flour gel. Overall, starch hydrolysis sharply increased for 30 min and then gradually increased until 180 min. The amount of released glucose decreased as the amount of optimized anthocyanin-enriched extract (wheat flour replacement) in the gel increased. Of particular note, the amount of released glucose in the gels with the optimized anthocyanin-enriched extract significantly decreased in a dose-dependent manner ($p < 0.05$). As mentioned above, the optimized anthocyanin-enriched extract from black rice in this study effectively inhibited α -glucosidase. Matsui et al. [9] found that anthocyanins from various plants could inhibit α -amylase and possibly suppress the increase in postprandial glucose level resulting from starch digestion. Also, in an animal model of T2DM, treatment with an anthocyanin (cyanidin 3-glucoside) significantly reduced blood glucose concentration and improved insulin sensitivity after an insulin tolerance test in male mice [25]. Therefore, it was thought that the optimized anthocyanin-enriched extract suppressed starch hydrolysis in the wheat flour gels by inhibiting starch-digesting enzymes.

The amounts of RDS, SDS, and RS are presented in Fig. 3(B). The RDS and SDS values of all optimized anthocyanin-enriched extract incorporating wheat flour gels were lowered with increasing amount of wheat flour replacement, while the RS values were higher than control (0% wheat flour replacement). RDS is rapidly digested and absorbed in the small intestine, resulting to rapid elevation of blood glucose and insulin levels. This sharp increase in blood glucose is associated with health complications such as obesity, diabetes, hyperglycemia, and cardiovascular

Fig. 3 Effects of the optimized anthocyanin-enriched extracts at different replacement levels (10–20%) on the glucose release (A), digestive starch fractions (B), and predicted glycemic index (pGI) of wheat flour gels (C). Different letters on the bars are significantly different from each other ($p < 0.05$)

disease [26]. Previous studies have demonstrated that food-derived active ingredients such as phenolic compounds can control blood glucose level [27]. In addition, fortification with anthocyanins from plant sources effectively reduced the digestion rate of bread [28]. As a result, anthocyanin-

enriched extract act as a non-competitive type inhibitor of starch digestive enzyme, resulting in slow digestion rates [29].

Next, the effects of the optimized anthocyanin-enriched extract wheat flour replacement on the pGI values of the gel samples were investigated. As shown in Fig. 3(C), the pGI values of the wheat flour gels with the optimized anthocyanin-enriched extract decreased significantly (from 74.08 to 67.34) as the replacement level increased from 10 to 20% ($p < 0.05$). These results suggest that the anthocyanin compounds in black rice cultivars were responsible for the ability of the wheat flour replacement gels to suppress starch digestion. Our data are in consistent with the findings of Ghosh et al. [30], who reported that different berry species (e.g., cranberry, strawberry, and blueberry) could potentially protect against diabetes. Furthermore, Tsuda et al. [31] reported that serum levels of glucose, insulin, and leptin were lower in mice fed a high-fat diet containing anthocyanins from purple corn (which is rich in cyanidin-3-glucoside) than in control mice fed a high-fat diet with no such anthocyanins. Cumulatively, these results support our findings that the optimized anthocyanin-enriched extract effectively lowered the pGI in an in vitro system by suppressing starch hydrolysis.

Identification of anthocyanins in the optimized extracts from black rice

LC/MS analysis was next used to identify the specific anthocyanins in the optimized anthocyanin-enriched extract from black rice. Twelve peaks were present on the chromatograms, which were sorted by the selected ion (m/z) on the LC/MS operating system (Fig. 4). Peak 1 (RT = 20.23 min) was identified as cyanidin-3-glucoside (C3G) by comparison with the standard. Inspection of the mass spectra revealed that the major ion of C3G had an m/z ratio of 449. Due to the loss of its glucoside group (162 m/z), C3G maintained an ion with an m/z ratio of 287. Peak 2 (RT = 20.56 min) was identified as peonidin-3-glucoside (P3G) by comparison with the standard. Inspection of the mass spectra revealed that the major ion of P3G had an m/z ratio of 463. Peak 3 (RT = 22.00 min), peak 4 (RT = 23.64 min), and peak 5 (RT = 25.00 min), which corresponded to unidentifiable ions, had m/z ratios of 363 (m/z), 149 (m/z), and 149 (m/z), respectively. HPLC analysis of the optimized anthocyanin-enriched extract from black rice revealed that C3G comprised 90% of the anthocyanins present and C3G was detected in black rice extract (878.54 μg C3G/g extract). In agreement with this, Lee [32] identified two anthocyanins in black rice (cyanidin-3-glucoside and peonidin-3-glucoside), and Hou et al. [5] also identified four anthocyanins (cyanidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-rutinoside, and cyanidin-

Fig. 4 MS spectra of the two major peaks obtained by analyzing the optimized anthocyanin-enriched extracts, and the structures of the corresponding compounds [(A) cyanidin-3-glucoside, (B) peonidin-3-glucoside]

3,5-diglucoside) in black rice. Moreover, Abdel-Aal et al. [24] found that cyanidin-3-glucoside comprised 80–100% of the total anthocyanins in black rice.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Zhang MW, Zhang RF, Zhang FX, Liu RH. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *J Agric Food Chem* 58: 7580–7587 (2010)
2. Yawadio R, Tanimori S, Morita N. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. *Food Chem* 101: 1616–1625 (2007)
3. de Mira NVM, Massaretto IL, Pascual CdSCI, Lanfer Marquez UM. Comparative study of phenolic compounds in different Brazilian rice (*Oryza sativa* L.) genotypes. *J Food Compos Anal* 22: 405–409 (2009)
4. Shao Y, Xu F, Sun X, Bao J, Beta T. Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). *J. Cereal Sci.* 59: 211–218 (2014)
5. Hou Z, Qin P, Zhang Y, Cui S, Ren G. Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. *Food Res Int* 50: 691–697 (2013)
6. Chen XQ, Nagao N, Itani T, Irifune K. Anti-oxidative analysis, and identification and quantification of anthocyanin pigments in different coloured rice. *Food Chem* 135: 2783–2788 (2012)
7. Kong J-M, Chia L-S, Goh N-K, Chia T-F, Brouillard R. Analysis and biological activities of anthocyanins. *Phytochemistry* 64: 923–933 (2003)
8. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technol* 101: 4676–4689 (2010)
9. Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K. α -Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. *J. Agric. Food Chem.* 49: 1948–1951 (2001)
10. McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit α -

- amylase and α -glucosidase. *J. Agric. Food Chem.* 53: 2760–2766 (2005)
11. Yao Y, Sang W, Zhou M, Ren G. Antioxidant and alpha-glucosidase inhibitory activity of colored grains in China. *J Agric Food Chem* 58: 770–774 (2010)
  12. Luthria DL. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. *Food Chem* 107: 745–752 (2008)
  13. Awika JM, Rooney LW, Waniska RD. Anthocyanins from black sorghum and their antioxidant properties. *Food Chem* 90: 293–301 (2005)
  14. Pascual-Teresa S, Santos-Buelga C, Rivas-Gonzalo JC. LC–MS analysis of anthocyanins from purple corn cob. *J Sci Food Agric* 82: 1003–1006 (2002)
  15. Naczek M, Shahidi F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J Pharm Biomed Anal* 41: 1523–1542 (2006)
  16. Prior RL, Wu XL, Gu LW, Hager TJ, Hager A, Howard LR. Whole berries versus berry anthocyanins: interactions with dietary fat levels in the C57BL/6J mouse model of obesity. *J. Agric. Food Chem.* 56: 647–653 (2008)
  17. Giusti MM, Rodríguez-Saona LE, Wrolstad RE. Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. *J. Agric. Food Chem.* 47: 4631–4637 (1999)
  18. Kim J-S, Kwon Y-S, Chun W-J, Kim T-Y, Sun J, Yu C-Y, Kim M-J. *Rhus verniciflua* stokes flavonoid extracts have anti-oxidant, anti-microbial and α -glucosidase inhibitory effect. *Food Chem* 120: 539–543 (2010)
  19. Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Carriere F, Boutrou R, Corredig M, Dupont D, Dufour C, Egger L, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie A, Marze S, McClements DJ, Menard O, Recio I, Santos CN, Singh RP, Vegarud GE, Wickham MS, Weitschies W, Brodtkorb A. A standardised static in vitro digestion method suitable for food—an international consensus. *Food Funct* 5: 1113–1124 (2014)
  20. Goñi I, Garcia-Alonso A, Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. *Nutr Res* 17: 427–437 (1997)
  21. Hao J, Zhu H, Liu S, Li H. Characterization of anthocyanins in fruit of *Kadsura coccinea* (Lem.) A.C. Smith by UPLC/Q-TOF-MS analysis and evaluation of stability of the major anthocyanins. *Food Anal Methods* 7: 1312–1322 (2014)
  22. Cabrita L, Fossen T, Andersen ØM. Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. *Food Chem* 68: 101–107 (2000)
  23. Barnes JS, Nguyen HP, Shen S, Schug KA. General method for extraction of blueberry anthocyanins and identification using high performance liquid chromatography–electrospray ionization-ion trap-time of flight-mass spectrometry. *J Chromatogr A* 1216: 4728–4735 (2009)
  24. Abdel-Aal E-SM, Young JC, Rabalski I. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *J Agric Food Chem* 54: 4696–4704 (2006)
  25. Sasaki R, Nishimura N, Hoshino H, Isa Y, Kadowaki M, Ichi T, Tanaka A, Nishiumi S, Fukuda I, Ashida H. Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to down-regulation of retinol binding protein 4 expression in diabetic mice. *Biochem Pharmacol* 74: 1619–1627 (2007)
  26. Miao M, Jiang B, Zhang T, Jin Z, Mu W. Impact of mild acid hydrolysis on structure and digestion properties of waxy maize starch. *Food Chem* 126: 506–513 (2011)
  27. Thondre PS. Chapter Five—Food-based ingredients to modulate blood glucose. pp. 181–227. In: advances in food and nutrition research. Jeyakumar H (ed). Academic Press: 181–227 (2013)
  28. Sui X, Zhang Y, Zhou W. Bread fortified with anthocyanin-rich extract from black rice as nutraceutical sources: Its quality attributes and in vitro digestibility. *Food Chem* 196: 910–916 (2016)
  29. Martinez-Gonzalez AI, Díaz-Sánchez ÁG, Rosa LA, Vargas-Requena CL, Bustos-Jaimes I. Polyphenolic compounds and digestive enzymes: In vitro non-covalent interactions. *Molecules* 22: 669–693 (2017)
  30. Ghosh D, Konishi T. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pac. J. Clin. Nutr.* 16: 200–208 (2007)
  31. Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O- β -D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J. Nutr.* 133: 2125–2130 (2003)
  32. Lee JH. Identification and quantification of anthocyanins from the grains of black rice (*Oryza sativa* L.) varieties. *Food Sci Biotechnol* 19: 391–397 (2010)