Journal of Cereal Science 70 (2016) 214–220

Contents lists available at ScienceDirect

Journal of Cereal Science

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

In vitro potential of phenolic phytochemicals from black rice on starch digestibility and rheological behaviors

Ji Soo An [a], In Young Bae [b], Sang-Ik Han [c], Sung-Joon Lee [d], Hyeon Gyu Lee [a, *]

[a] Department of Food and Nutrition, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Republic of Korea
[b] Department of Food & Fermentation, Far East University, Gamgok, Eumseong, Chungbuk, 369-700, Republic of Korea
[c] Department of Functional Crop, National Institute of Crop Science (NICS), Rural Development Administration (RDA), Jeompiljaero, Miryang, Gyeongnam, 627-803, Republic of Korea
[d] Department of Food Bioscience & Technology, College of Life Science & Biotechnology, Korea University, Seoul, 133-791, Republic of Korea

ARTICLE INFO

Article history:
Received 5 March 2016
Received in revised form
12 June 2016
Accepted 20 June 2016
Available online 21 June 2016

Keywords:
Black rice flour
Phenolic compound
In vitro starch digestibility
Rheology

ABSTRACT

Various black rice materials (black rice flour, BF; dietary fiber-enriched extract, DE; phenolic-enriched extract, PE) were prepared from black rice, and their effects on *in vitro* starch digestibility were investigated in a wheat flour gel model. Specifically, the *in vitro* digestive behavior of the gel samples was continuously monitored from a rheological point of view. BF and DE did not inhibit digestive enzymes; however, PE exhibited IC_{50} values of 24.12 mg/mL and 0.03 mg/mL against α-amylase and α-glucosidase, respectively. In addition, the predicted glycemic index (pGI) values of the gels with BF, DE, and PE at 20% replacement for wheat flour were as follows: control (wheat flour) ≈ BF > DE > PE. Moreover, a significant decrease in the *in vitro* viscosities of gels during intestinal digestion was observed in the order of BF, DE, and PE. As a result, PE showed the highest suppression effect on starch hydrolysis by inhibiting digestive enzyme. These results support phenolic compounds as more critical factors compared to dietary fiber for retarding *in vitro* starch digestibility of starch-based foods prepared with black rice.

1. Introduction

Black rice (*Oryza sativa* L., 'Josaengheugchal') has a coloring component (e.g. cyanidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside) and is high in nutritional value as a source of iron, vitamin E, and antioxidants (e.g. anthocyanins) (Zhang et al., 2006). Moreover, phenolic compounds and dietary fibers are much more abundant in black rice than white rice (Shen et al., 2009). Recently, it has been reported that the essential phytochemicals in grains such as rice have many beneficial effects on human health such as reducing the risk of cardiovascular disease, type 2 diabetes, and some cancers (Liu, 2007). Especially, natural antioxidants including phenolic compounds reduce the risk of diabetes since they can inhibit the activity of intestinal α-glucosidase and pancreatic α-amylase (Ranilla et al., 2010). On the other hand, dietary fibers incorporated into starch-based foods increase the viscosity of food products and delay the accessibility of starch granules to digestive enzymes in the human digestive system (Angioloni and Collar, 2011).

Although the effects of phenolic compounds and dietary fibers on reducing the risk of diabetes have been reported previously (Bae et al., 2016; Jun et al., 2014; Liu, 2007), it is important to understand which components of black rice are more effective in delaying starch digestibility. Therefore, the objectives of this study were to investigate the effects of various black rice materials (black rice flour, BF; dietary fiber-enriched extract, DE; phenolic-enriched extract, PE) on *in vitro* starch digestibility in a wheat flour gel system and to assess how these materials affect starch-digestive enzyme activity and *in vitro* rheology.

2. Materials and methods

2.1. Materials

Black rice (*Oryza sativa* L., Josaengheugchal) produced in Jindo in 2014 was obtained from the Rural Development Administration (RDA), Korea. The black rice was stored in a polyethylene bag (Cleanwrap Co., Gimhae, Korea) at 25 °C until analysis. All-purpose wheat flour (CJ Co. Ltd., Seoul, Korea) was obtained from a commercial source.

* Corresponding author.
E-mail address: hyeonlee@hanyang.ac.kr (H.G. Lee).

http://dx.doi.org/10.1016/j.jcs.2016.06.010
0733-5210/

The total dietary fiber assay kit (TDF-100A), α-amylase from porcine pancreas (A3176), pancreatin from porcine pancreas (P7545), bile extract porcine (B8631), amyloglucosidase (A9913), soluble starch (S9765), heat-stable α-amylase (A3306), celite (C8656), 3,5-dinitrosalicyclic acid (D0550), 4-nitrophenyl-alpha-D-glucopyranoside (pNPG, N1377), Folin & Ciocalteu's phenol reagent (F9252), gallic acid (G7384), acarbose (A8980), and catechin (C1788) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The total starch kit (K-TSTA) and glucose oxidase-peroxidase assay kit (GOPOD, K-GLUC) were purchased from Megazyme International Ireland Ltd. (Bray, Ireland). α-Glucosidase (*Bacillus stearothermophilus*; E-TSAGL) was purchased from Megazyme International Ireland Ltd. (Dublin, Ireland). Other chemicals were analytical grade.

2.2. Preparation of DE and PE

Black rice was ground in a blender (Hanil Food Co., Ltd., Korea), passed through a 100 mesh sieve and was used in further extract and analysis with the name of BF. DE was prepared according to the method described by Kim et al. (1997). BF (150 g) was gelatinized with 0.6% Termamyl (1 L) at 95 °C for 1 h to remove starch, followed by filtration. The residue was then washed three times with four volumes of heated water (100 °C). The residue was then washed with four volumes of 99.9% ethanol (preheated to 60 °C), followed by filtration. The residue was dried at 50 °C (forced convection dry oven, DAIHAN Scientific Co., Ltd., Seoul, Korea) overnight, passed through a 100 mesh sieve, and then stored in a polyethylene bag (Cleanwrap Co., Gimhae, Korea) at 4 °C until use. PE was prepared according to the method described by Kim et al. (2010a). The BF (1 g) and 70% ethanol (50 mL) were mixed for 24 h, followed by filtration. The extraction was then repeated once more. All supernatants were collected and evaporated at 40 °C for 1 h, followed by drying at 60 °C (DAIHAN Scientific Co., Ltd., Seoul, Korea) until all the solvents were removed and then stored at 4 °C until use.

2.3. Dietary fiber analysis

The amounts of soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) were determined according to the gravimetric enzymatic method as previously described by Prosky et al. (1988).

2.4. Total phenolic, total flavonoid, and total anthocyanin contents

The total phenolic content was determined by the Folin-Ciocalteu reagent method modified by Singleton and Rossi (1965). Briefly, 100 μL of the sample was transferred into a test tube and mixed with 2 mL of Na_2CO_3 (5%, w/v). After 3 min, 100 μL of Folin–Ciocalteu reagent (50%, v/v) was added to the mixture. After standing for 30 min at room temperature, the absorbance was measured at 750 nm. Gallic acid was used for constructing the standard curve. The total phenolic content was expressed in terms of gallic acid equivalents.

The total flavonoid content was determined according to the method modified by Zhishen et al. (1999). Briefly, 250 μL of the sample was transferred into a test tube and mixed with 75 μL of $NaNO_2$ (5%, w/v) and 1.25 mL distilled water. After 5 min, 150 μL $AlCl_3 \cdot 6H_2O$ (10%, w/v) was added. At 6 min, 500 μL of NaOH (1 M) and 275 μL distilled water was added to the mixture. The mixture was thoroughly mixed, and the absorbance was measured at 510 nm. Catechin was used for constructing the standard curve.

Measuring the total anthocyanin content was carried out as previously described by Giusti et al. (1999). Samples were dissolved in 0.025 M potassium chloride solution (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5), and the absorbance was measured at 510 and 700 nm. Data were expressed as milligrams of anthocyanins (cyanidin 3 glucoside, C3G) per 100 g of the fresh weight of seed powder using a molar extinction coefficient of 26,900, a molecular weight of 449, and an absorbance of A = [(A510-A700) at pH 1.0 − (A510-A700) at pH 4.5].

2.5. α-Amylase and α-glucosidase inhibitory activity

The α-amylase inhibitory activity of the black rice materials was determined according to a modification of the method of Ranilla et al. (2010). Briefly, 250 μL of sample and 125 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25 °C for 10 min. After pre-incubation, 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was stopped with 0.5 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 5 mL of distilled water, and the absorbance was measured at 540 nm. Acarbose was used as the positive control. The α-amylase inhibitory activity was calculated as follows:

$$\text{Inhibition } (\%) = (1 - A_{sample} / A_{control}) \times 100$$

where A_{sample} and $A_{control}$ were defined as the absorbance of the sample and the control (acarbose), respectively.

The α-glucosidase inhibitory activity of the black rice extract was determined according to the method described by Kim et al. (2010b). Sample (50 μL) was mixed with *Bacillus stearothermophilus* α-glucosidase (50 μL, 0.5 U/mL) and 0.2 M potassium phosphate buffer (pH 6.8, 50 μL). After pre-incubating at 37 °C for 15 min, 3 mM pNPG (100 μL) was added. The reaction was incubated at 37 °C for 10 min and stopped with 0.1 M Na_2CO_3 (750 μL). 4-Nitrophenol absorption was measured at 405 nm. A solution without sample was used as the control. A solution without substrate was used as a blank. Acarbose was also assayed as a standard reference. The experiment was performed in triplicate. The percent inhibition of α-glucosidase was calculated as follows:

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

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.

2.6. In vitro starch digestion

Wheat flour in a gel was replaced with BF, DE, or PE at 10, 20, or 40% by weight, and their effects on *in vitro* starch digestibility were evaluated. The mixtures (3 g) of wheat flour and black rice materials (BF, DE, or PE) were dispersed in distilled water (10.71%, w/v). The suspensions were stirred continuously for 5 min and then heated in a boiling water bath for 10 min. The starch gels were placed on a plate in a steam cooker with a lid and steamed over boiling water for 40 min. These gels were subjected to *in vitro* starch digestion according to the method described by Minekus et al. (2014) with slight modifications.

The gel sample (5 g) containing the same amount of total starch was mixed with distilled water (15 mL) and stirred at 700 rpm for 30 min to break the gel structure. Then, 11 mL of SIF, 40 μL of 0.3 M $CaCl_2$, 0.15 mL of 1 M NaOH, and 1.31 mL of distilled water were added and incubated at 37 °C. Pancreatin and bile salt solution

(added at concentrations of 2 g (final 100 U/mL) of pancreatin and 0.435 g (final 10 mM) of bile extract in 12 mL of SIF, 7.5 mL) and amyloglucosidase (0.2 mL per gram of starch in sample) were added. Simulated intestinal fluid (SIF) electrolyte stock solution was made up of 6.8 mL of 0.5 M KCl, 0.8 mL of 0.5 M KH_2PO_4, 42.5 mL of 1 M $NaHCO_3$, 9.6 mL of 2 M NaCl, and 1.1 mL of 0.15 M $MgCl_2(H_2O)_6$ in 400 mL of distilled water. The pH was adjusted to 6.0 with 1 N HCl. Aliquots (0.1 mL) were collected at different times (30, 60, 90, 120, and 180 min) during digestion and mixed with 1.4 mL ethanol. These solutions were centrifuged (600 × *g* for 3 min), and the released glucose content of the supernatants was measured using the GOPOD kit at 510 nm. The levels of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were measured after intestinal digestion for 20, 120, and 180 min, respectively.

2.7. Predicted glycemic index (pGI)

The digestion kinetics and pGI of the gels were determined according to the procedure established by Goñi et al. (1997). The starch hydrolysis kinetics was calculated using the equation: $[C = C^{\infty}(1 - e^{-kt})]$, where C, C^{∞}, and k denote the hydrolysis degree at each time, the extent of maximum hydrolysis, and the kinetic constant, respectively. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh white bread). The predicted glycemic index (pGI) was calculated using the equation: $pGI = 39.71 + 0.549HI$.

2.8. In vitro rheological monitoring during starch digestion

Rheological monitoring during *in vitro* starch digestion was performed according to the method by Kim et al. (2015) using a starch pasting cell attached to a controlled-stress rheometer (AR1500ex, TA Instruments Co., New Castle, DE, USA).

The mixtures (2.4 g) of wheat flour and black rice materials (BF, DE, or PE) were mixed with 20 mL of simulated salivary fluid (SSF) electrolyte stock solution. For cooking, the samples were heated to 90 °C at a rate of 12 °C/min, held at 90 °C for 2.5 min, and cooled to 37 °C at a rate of 12 °C/min. Then, 0.6 mL of porcine pancreatic α-amylase was added at 37 °C and incubated for 30 s. Next, 0.3 mL of pepsin was added, the pH was adjusted to 2.0 with 6 N HCl, and the mixture was incubated at 37 °C for 30 min. Then, 0.6 mL of pancreatin solution and 311.3 μL of amyloglucosidase per gram of starch in the sample were added to the mixture, the pH was adjusted to 6.0 with 1 M NaOH, and the mixture was incubated for 3 h. The reaction of the *in vitro* starch digestion was carried out in an aluminum canister surrounded by a temperature chamber (37 °C). The impeller was rotated at a speed of 160 rpm in the samples, and the viscosity change during the *in vitro* digestion was continuously monitored.

2.9. Statistical analysis

All analyses were performed at least in triplicate, and their values were expressed as mean ± standard deviation. Statistical analyses were carried out with Duncan's multiple range test ($P < 0.05$) using SPSS statistical software (Version 21.0 software, Chicago, IL, USA).

3. Results and discussion

3.1. Composition of black rice materials

The chemical compositions of various black rice materials (dry basis) are given in Table 1. BF, DE, and PE contained 69.68%, 0.47%, and 0.69% starch, respectively. DE exhibited the highest TDF and IDF, whereas PE exhibited the highest total phenolic, flavonoid, and anthocyanin contents. DE exhibited significantly higher TDF and IDF contents compared to the other materials ($P < 0.05$). The contents of TDF and IDF in DE were about four- and five-fold higher than those in BF, respectively. However, the total phenolic, flavonoid, and anthocyanin contents were not detected in DE. On the other hand, the amounts of total phenolic, flavonoid, and anthocyanin in PE were drastically increased by five-fold higher than those in BF. Zhang et al. (2006) reported that black rice is more abundant in anthocyanin and other phenolic compounds compared to that of white rice, and these phytochemical compounds are generally accumulated in the pericarp or bran of rice kernels.

3.2. Inhibitory effects of black rice materials against α-amylase and α-glucosidase

The main source of glucose in the diet is derived from starch hydrolysis through the human digestive system, and this reaction is initiated by pancreatic α-amylase (Tarling et al., 2008). In addition, α-glucosidase has been recognized as a therapeutic target for the regulation of postprandial hyperglycemia since it acts on the final step of starch hydrolysis, resulting in glucose release (McDougall and Stewart, 2005). Thus, the inhibitory effects of black rice materials against α-amylase and α-glucosidase were investigated and compared with those of acarbose (standard reference for the enzyme inhibitor).

As shown in Fig. 1, the inhibitory effects of PE against both enzymes increased in a concentration-dependent manner ($P < 0.05$), whereas BF and DE did not inhibit the enzyme activity (data not shown). PE had a weaker inhibitory effect in α-amylase; however, it had a better inhibitory effect in α-glucosidase compared with acarbose (Table 2). Especially, it should be noted that PE (IC_{50} of 0.03 ± 0.00 μg/mL) showed much stronger inhibition against α-glucosidase compared to that of acarbose (IC_{50} of 1.40 ± 0.00 μg/mL). Our results are in agreement with those of previous studies, which have indicated that plant-derived phenolic phytochemicals have stronger inhibition activity against α-glucosidase than α-amylase (Dong et al., 2012; Yilmazer-Musa et al., 2012). It is well-know that the plant-derived polyphenol extracts effectively inhibit α-amylase activity and intestinal α-glucosidase activity (Matsui et al., 2001). Also, phenolics and flavonoids from a wide range of food or medical plant sources could be effective α-glucosidase inhibitors (Kwon et al., 2008). Moreover, the high correlation between α-glucosidase inhibitory activity and phenolic content was previously reported (Apostolidis and Lee, 2010).

3.3. In vitro starch digestibility

The effects of BF, DE, and PE from black rice on *in vitro* starch digestion in wheat flour gels were investigated by measuring the released glucose contents during starch hydrolysis. Glucose release curves measured the reducing sugars degraded from starch due to a digestive enzyme. Fig. 2 shows the starch hydrolysis curves of BF-, DE-, and PE-incorporated gels compared with that of the control. Overall, starch hydrolysis increased sharply up to 30 min and then gradually increased for 180 min. The amount of released glucose decreased with increasing level of wheat flour replacement in the gels. In particular, the amount of released glucose in DE and PE was significantly decreased in a dose-dependent manner ($P < 0.05$). Especially, the glucose released content of the gels with BF, DE, and PE at 20% replacement for wheat flour were as follows: control (wheat flour) ≈ BF > DE > PE. Navarro-González et al. (2011) reported that *in vitro* starch digestibility of dietary fiber and phenolic

Table 1
Chemical compositions (dry basis) of various black rice materials (black rice flour, BF; dietary fiber-enriched extract, DE; phenolic-enriched extract, PE).

Content	BF	DE	PE
Starch (%)	69.68 ± 2.86[a]	0.47 ± 0.17[b]	0.69 ± 0.04[b]
TDF (%)	18.26 ± 2.61[b]	69.30 ± 3.30[a]	5.78 ± 0.00[c]
SDF (%)	4.71 ± 2.26[ns]	2.33 ± 0.00[ns]	1.23 ± 1.50[ns]
IDF (%)	13.55 ± 1.31[b]	66.97 ± 3.30[a]	4.55 ± 1.50[c]
Total phenolic (mg GAE/g flour)	105.91 ± 6.32[b]	ND	539.34 ± 32.18[a]
Total flavonoid (mg CE/g flour)	94.74 ± 4.89[b]	ND	482.48 ± 24.89[a]
Total anthocyanin (mg C3G/g flour)	72.57 ± 5.14[b]	ND	369.57 ± 26.19[a]

Mean values with different letters in the same row differ significantly ($p < 0.05$). ND means not detected. TDF, SDF, and IDF mean total dietary fiber, soluble dietary fiber, and insoluble dietary fiber, respectively.

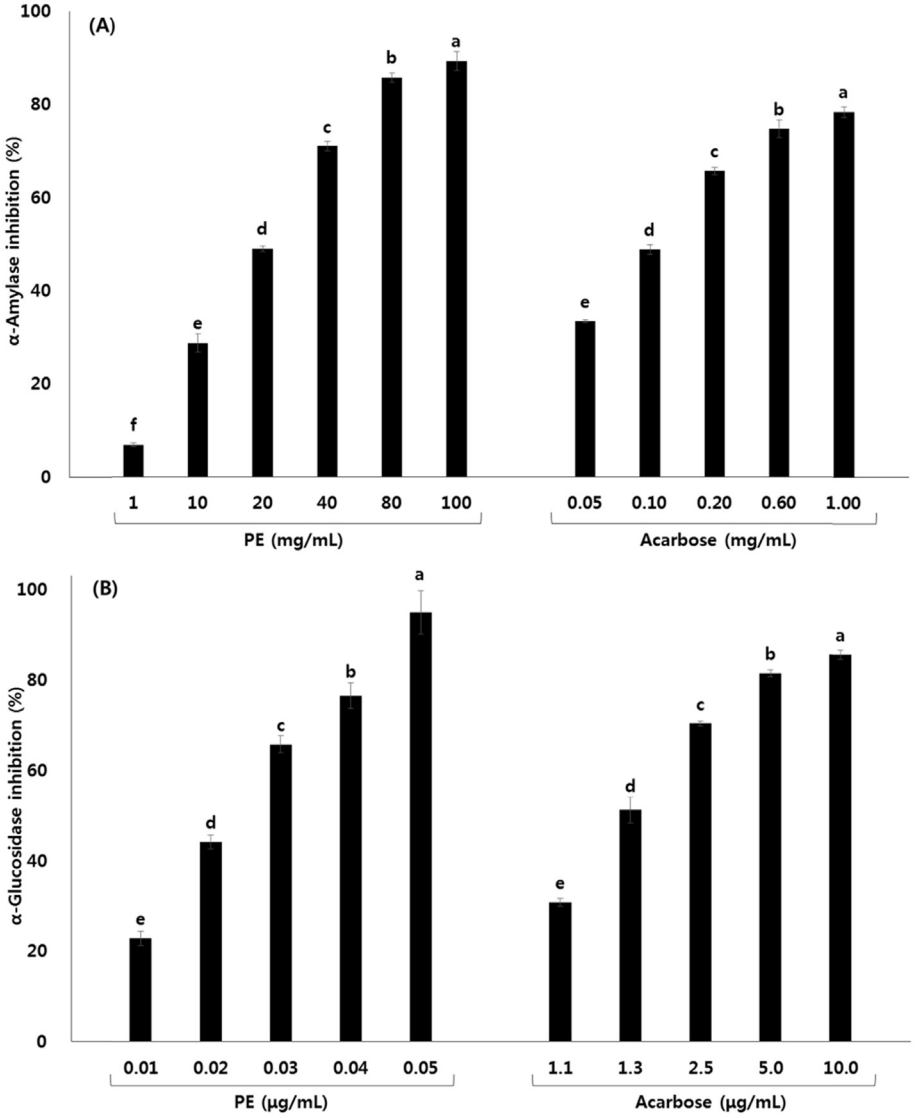

Fig. 1. Inhibitory effects of phenolic-enriched extract (PE) from black rice and acarbose (standard reference) against α-amylase (A) and α-glucosidase (B).

compounds has been significantly reduced. Also, Bae et al. (2016) showed that the amount of released glucose decreased significantly in noodles containing flavonoid extract and rutin-enriched flavonoid extract compared to those of buckwheat flour and dietary extracts.

The amounts of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) are presented in Table 3. All black rice material-incorporated wheat flours showed the same values of SDS with increasing level of wheat flour replacement in the gels ($P < 0.05$). Compared to the control gel, BF showed no significant difference in the value of RDS ($P < 0.05$), but it showed higher value of RS with increasing level of wheat flour replacement in the gels ($P < 0.05$). In addition, DE and PE showed lower values of RDS but very high levels of RS with increasing level of wheat flour replacement in the gels ($P < 0.05$). Specifically, the 40% DE and PE gels showed the lowest ratio of RDS (32% and 33%, respectively) and

Table 2
Values of 50% inhibitory concentrations (IC_{50}) of various black rice materials (black rice flour, BF; dietary fiber-enriched extract, DE; phenolic-enriched extract, PE) against α-amylase and α-glucosidase.

Sample	50% Inhibitory concentration (IC_{50})	
	α-Amylase (mg/mL)	α-Glucosidase (μg/mL)
BF	ND	ND
DE	ND	ND
PE	24.12 ± 0.36[a]	0.03 ± 0.00[b]
Acarbose	0.14 ± 0.00[b]	1.40 ± 0.00[a]

Mean values with different letters in the same column differ significantly ($p < 0.05$). ND means not detected.

the highest rates of RS (55% and 56%, respectively). RDS is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine, which leads to fast 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 diseases (Miao et al., 2011). Previous studies have shown that the added fiber reinforcement materials in bread and cake controlled starch digestibility in a concentration-dependent manner (Angioloni and Collar, 2011; Jun et al., 2014). In addition, dietary fibers that are combined in the products make it possible to limit the gelatinization of starch and the hydration, delaying the degradation of starch hydrolysis by the enzyme (Thondre et al., 2010). Also, the food-derived active ingredients such asphenol compounds have the ability to control blood glucose level (Thondre, 2013).

The pGI values of the gel samples with BF, DE, and PE were investigated depending on the level of wheat flour replacement. As shown in Table 3, the calculated pGI values of the BF, DE, and PE gels changed from 84.81 → 79.23, 80.27 → 70.70, and 77.85 → 69.77, respectively, with increasing replacement level (10%→40%) of black rice materials. The pGI values of the gels with BF replacement of 10%–20% were not significantly lower than those of the control wheat flour gels ($p > 0.05$). In the DE gels, a significant difference in pGI values was detected when DE was replaced with more than 20% wheat flour ($P < 0.05$). However, the pGI values of the PE gels dramatically decreased for all replacement levels ($P < 0.05$). Furthermore, the pGI values of the gels with 20% replacement black rice materials were as follows: control ≈ BF > DE > PE. Thus, PE had the highest suppression effect on starch hydrolysis among the black rice materials used in this study with an *in vitro* method. Based on these results, the suppressive effects of black rice materials on starch digestibility in wheat flour replacement gels is more highly related to the phenolic content and less to the dietary fiber content of black rice flour. This result was in agreement with the finding of Bae et al. (2016), who reported that the RDS amounts and pGI values of noodles with flavonoid extracts from buckwheat were more significantly reduced compared to those of the noodles with dietary fiber extracts in *in vitro* starch digestion. However, buckwheat flour and dietary fiber extract did not significantly decrease the pGI value of the noodles. As a result, for lowering the pGI with black rice materials, PE was more effective than DE in this study.

3.4. In vitro rheology during starch digestion

Pasting curves of wheat flour replaced with 20% BF, DE, and PE are shown in Fig. 3. Fig. 3(a) shows the viscosity of the suspension during the heating and cooling of the black rice materials. The RVA curves shifted down, indicating that the decreases in peak and final viscosity at 20% replacement were as follows: Control > BF > PE > DE. Peak viscosity, breakdown, setback, and final viscosity ranged from 0.58 to 2.20 pa·s, 0.09–0.52 pa·s, 0.09–3.66 pa·s, and 0.03–1.57 pa·s, respectively. BF had a peak viscosity and final viscosity similar to the control, whereas DE

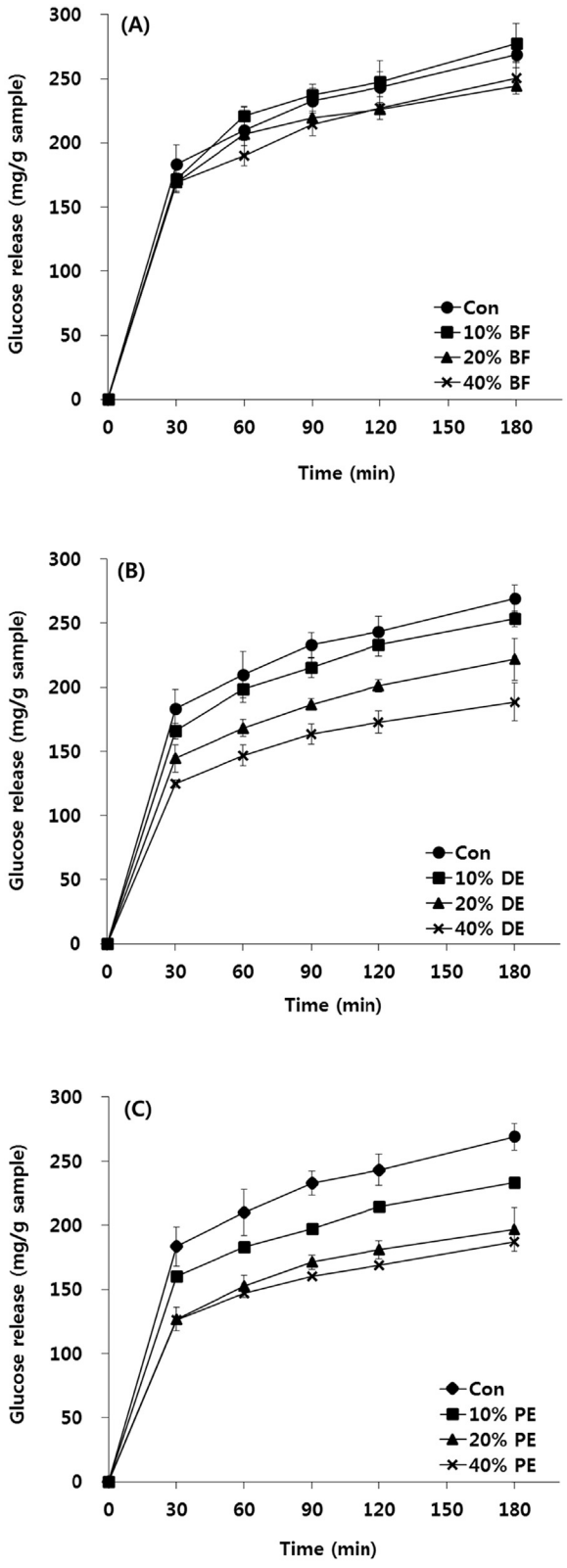

Fig. 2. Effects of various black rice materials at different replacement levels (10–40%) on the glucose release curves of wheat flour gels ((A) black rice flour, BF; (B) dietary fiber-enriched extract, DE; (C) phenolic-enriched extract, PE).

Table 3
Starch digestion fractions and predicted glycemic indexes (pGI) of wheat flour gels with various black rice materials (black rice flour, BF; dietary fiber-enriched extract, DE; phenolic-enriched extract, PE) at different replacement levels (10–40%) under *in vitro* starch digestion.

Replacement (%)		Starch digestion fractions (%)			pGI
		RDS	SDS	RS	
BF	10	44.49 ± 2.51^{ab}	19.57 ± 1.89^{a}	35.94 ± 4.32^{e}	84.81 ± 0.47^{a}
	20	43.86 ± 0.31^{ab}	14.65 ± 0.15^{ab}	41.49 ± 0.45^{cd}	81.76 ± 0.92^{bc}
	40	43.76 ± 1.97^{ab}	15.03 ± 2.45^{ab}	41.21 ± 2.33^{cde}	79.23 ± 1.63^{c}
DE	10	42.93 ± 1.57^{ab}	17.50 ± 3.56^{ab}	39.57 ± 2.35^{de}	80.27 ± 0.56^{bc}
	20	37.42 ± 2.78^{c}	14.68 ± 1.78^{ab}	47.90 ± 1.26^{b}	75.00 ± 1.71^{d}
	40	32.35 ± 0.70^{d}	12.38 ± 2.17^{ab}	55.27 ± 2.27^{a}	70.70 ± 2.18^{e}
PE	10	41.47 ± 0.73^{bc}	14.06 ± 1.08^{ab}	44.46 ± 0.45^{bc}	77.85 ± 1.56^{c}
	20	32.84 ± 2.37^{d}	13.99 ± 4.00^{ab}	53.17 ± 1.83^{a}	71.92 ± 1.68^{e}
	40	32.61 ± 3.14^{d}	11.17 ± 3.70^{b}	56.22 ± 1.42^{a}	69.77 ± 1.26^{e}
Control		47.49 ± 3.93^{a}	15.53 ± 1.29^{ab}	36.98 ± 3.11^{e}	83.26 ± 0.68^{ab}

Mean values with different letters in the same column differ significantly ($p < 0.05$). RDS, SDS, and RS mean rapidly digestible starch, slowly digestible starch, and resistant starch, respectively.

replacement before and after adding α-amylase in the oral step were as follows: control (3.49 Pa s→0.26 Pa s) > BF (2.35 Pa s→0.16 Pa s) > PE (0.45 Pa s→0.19 Pa s) > DE (0.08 Pa s→0.06 Pa s). In the gastric step with pepsin for 30 min, the viscosities of the samples decreased in the initial period (PE > DE > BF > control) and then became constant. In the small intestine, where starch digestion mainly occurs, all samples showed a decreased viscosity until 22 min after addition of pancreatic enzymes. The viscosities of the control and BF samples were further reduced; however, the viscosities of the DE and PE samples were constant or slightly reduced, showing similar patterns with the glucose release curves in Fig. 2. Moreover, viscosity crossover was observed in the DE and PE samples during intestinal digestion, in agreement with the results of Kim et al. (2015). As demonstrated above, the *in vitro* viscosities of all of the samples were reduced under simulated oral, gastric, and intestinal conditions. After cooking, DE and PE containing more non-starch portions were low in viscosity compared to the other samples.

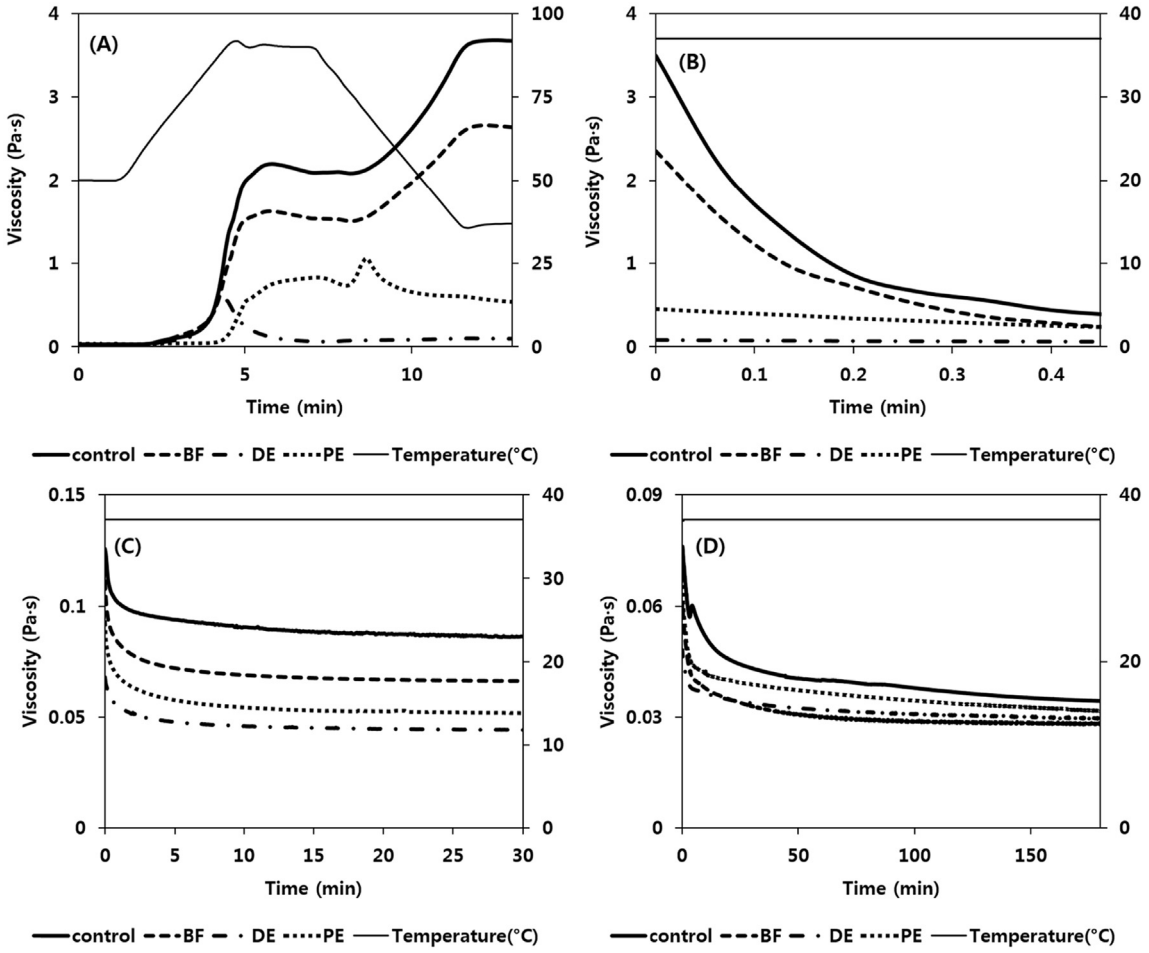

Fig. 3. *In vitro* rheological changes of wheat flour gels with various black rice materials (black rice flour, BF; dietary fiber-enriched extract, DE; phenolic-enriched extract, PE) at a replacement level of 20% under simulated digestion conditions ((A) before digestion, (B) oral, (C) gastric, and (D) intestinal digestion).

showed the lowest values for all of the pasting parameters ($P < 0.05$).

The *in vitro* rheological behavior in the samples with BF, DE, and PE replacing wheat flour at 20% by weight was monitored under simulated oral, gastric, and intestinal digestion (Fig. 3(B–D)). The viscosities of the samples with black rice materials at 20% However, viscosity crossover was observed during intestinal digestion. These results were correlated with the higher amount of released glucose in the BF. As a result, phenolic compounds were more effective than dietary fiber. Generally, dietary fiber appeared to delay digestion due to the viscosity. However, the dietary fiber derived from black rice extract used in this study had no effect on

the increase in viscosity, whereas effective digestion was delayed based on phenolic compounds. Therefore, the suppressive effects of black rice materials on starch digestibility were more related to phenolic compounds than dietary fiber.

In conclusion, BF and DE did not inhibit digestive enzymes; however, PE effectively inhibited α-amylase activity and intestinal α-glucosidase activity. The pGI values of the gels with BF and DE were not significantly different than the control gel; however, PE effectively inhibited starch hydrolysis in the gels and finally resulted in a lowered pGI. In addition, the *in vitro* viscosities of all of the digested samples were distinctly reduced under the simulated oral-gastric-intestinal conditions. After cooking, DE and PE containing more non-starch portions were low in viscosity compared to the other samples. However, viscosity crossover was observed during intestinal digestion. These results were correlated with the higher amount of released glucose in the BF. Based on these results, PE showed the highest suppression effect on starch hydrolysis by inhibiting digestive enzymes. The results support phenolic compounds as more critical factors than dietary fiber for retarding *in vitro* starch digestibility of starch-based foods prepared with black rice.

Acknowledgments

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of specialty rice cultivar based on rice starch and its materials for commercialization, Project No. PJ01125305)" Rural Development Administration, Republic of Korea.

References

Angioloni, A., Collar, C., 2011. Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. LWT – Food Sci. Technol. 44, 747–758.

Apostolidis, E., Lee, C.M., 2010. *In vitro* potential of ascophyllum nodosum phenolic antioxidant-mediated alpha-glucosidase and alpha-amylase inhibition. J. Food Sci. 75, H97–H102.

Bae, I.Y., Choi, A.S., Lee, H.G., 2016. Impact of buckwheat flavonoids on *in vitro* starch digestibility and noodle making property. Cereal Chem. 93, 299–305.

Dong, H.-Q., Li, M., Zhu, F., Liu, F.-L., Huang, J.-B., 2012. Inhibitory potential of trilobatin from *Lithocarpus polystachyus* Rehd against α-glucosidase and α-amylase linked to type 2 diabetes. Food Chem. 130, 261–266.

Giusti, M.M., Rodríguez-Saona, L.E., Wrolstad, R.E., 1999. Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. J. Agric. Food Chem. 47, 4631–4637.

Goñi, I., Garcia-Alonso, A., Saura-Calixto, F., 1997. A starch hydrolysis procedure to estimate glycemic index. Nutr. Res. 17, 427–437.

Jun, Y., Bae, I.Y., Lee, S., Lee, H.G., 2014. Utilisation of preharvest dropped apple peels as a flour substitute for a lower glycaemic index and higher fibre cake. Int. J. Food Sci. Nutr. 65, 62–68.

Kim, D.J., Oh, S.K., Yoon, M.R., Chun, A.R., Hong, H.C., Lee, J.S., Kim, Y.K., 2010a. Antioxidant compounds and antioxidant activities of the 70% ethanol extracts from brown and milled rice by cultivar. J. Korean Soc. Food Sci. Nutr. 39, 467–473.

Kim, H.J., L, J., Ko, S.H., Lee, S.Y., 2015. Rheological behaviors of white and brown rice flours during *in-vitro* simulation of starch digestion. Korean J. Food Sci. Technol. 47, 793–796.

Kim, J.S., Kwon, Y.S., J, W., Kim, T.Y., Sun, J., Yu, C.Y., Kim, M.J., 2010b. Rhus verniciflua stokes flavonoid extracts have anti-oxidant, anti-microbial and α-glucosidase inhibitory effect. Food Chem. 120, 539–543.

Kim, Y.S., Ha, T.Y., Lee, S.H., Lee, H.Y., 1997. Effects of rice bran dietary fiber extract on gelatinization and retrogradation of wheat flour. Korean J. Food Sci. Technol. 29, 464–469.

Kwon, Y.I., Apostolidis, E., Shetty, K., 2008. *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour. Technol. 99, 2981–2988.

Liu, R.H., 2007. Whole grain phytochemicals and health. J. Cereal Sci. 46, 207–219.

Matsui, T., Ueda, T., Oki, T., Sugita, K., Terahara, N., Matsumoto, K., 2001. α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. J. Agric. Food Chem. 49, 1948–1951.

McDougall, G.J., Stewart, D., 2005. The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors 23, 189–195.

Miao, M., Jiang, B., Zhang, T., Jin, Z., Mu, W., 2011. Impact of mild acid hydrolysis on structure and digestion properties of waxy maize starch. Food Chem. 126, 506–513.

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, D.J., Menard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S.J., Weitschies, W., Brodkorb, A., 2014. A standardised static *in vitro* digestion method suitable for food – an international consensus. Food Funct. 5, 1113–1124.

Navarro-González, I., García-Valverde, V., García-Alonso, J., Periago, M.J., 2011. Chemical profile, functional and antioxidant properties of tomato peel fiber. Food Res. Int. 44, 1528–1535.

Prosky, L., Asp, N.G., Schweizer, T.F., DeVries, J.W., Furda, I., 1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. J. Assoc. Off. Anal. Chem. 71, 1017–1023.

Ranilla, L.G., Kwon, Y.I., Apostolidis, E., Shetty, K., 2010. 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. Bioresour. Technol. 101, 4676–4689.

Shen, Y., Jin, L., Xiao, P., Lu, Y., Bao, J., 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. J. Cereal Sci. 49, 106–111.

Singleton, V., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Tarling, C.A., Woods, K., Zhang, R., Brastianos, H.C., Brayer, G.D., Andersen, R.J., Withers, S.G., 2008. The search for novel human pancreatic α-amylase inhibitors: high-throughput screening of terrestrial and marine natural product extracts. Eur. J. Chem. Biol. 9, 433–438.

Thondre, P.S., 2013. Food-based ingredients to modulate blood glucose. Adv. Food Nutr. Res. 70, 181–227.

Thondre, P.S., Monro, J.A., Mishra, S., Henry, C.J.K., 2010. High molecular weight barley β-glucan decreases particle breakdown in chapattis (Indian flat breads) during *in vitro* digestion. Food Res. Int. 43, 1476–1481.

Yilmazer-Musa, M., Griffith, A.M., Michels, A.J., Schneider, E., Frei, B., 2012. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. J. Agric. Food Chem. 60, 8924–8929.

Zhang, M.W., Guo, B.J., Zhang, R.F., Chi, J.W., Wei, Z.C., Xu, Z.H., Zhang, Y., Tang, X.J., 2006. Separation, purification and identification of antioxidant compositions in black rice. Agric. Sci. China 5, 431–440.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559.