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Suppressive effect of extruded adzuki beans (*Vigna angularis*) on hyperglycemia after sucrose loading in rats

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ABSTRACT

Extrusion process has been widely used for the development of many functional foods. The aim of this study was to assess the effect of extrusion process on antioxidant and α-glucosidases inhibition properties of adzuki beans. The results showed that there were no significant differences of polysaccharide content, protein content, and total flavonoid content (TFC) between adzuki beans extract (ABE) and extruded adzuki beans extract (EABE). However, a significant decrease in the total phenolic content (TPC) was observed. The antioxidant activity determined by a method based on the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical-scavenging activity also decreased significantly after extrusion. This study, for the first time, identified that extruded adzuki beans protein (10 mg/mL) significantly inhibited rat intestinal α-glucosidases (60.44%). Further animal study revealed that the oral intake of EABE (300 mg/kg) significantly reduced postprandial blood glucose by 15.6% and 30.9% following sucrose challenge in the normal and streptozocin-treated rats, respectively. The results demonstrated that the potential for the extruded food products with the improved antidiabetic activity from adzuki beans.

1. Introduction

Extrusion cooking, low cost and very efficient technology in food processing, has been used in the production of breakfast cereals, baby foods, snack foods, pasta products, pet foods and instant powders (Chang et al., 2011). Extrusion cooking has been applied to modify the structure, to improve the solubility, and to increase the soluble fiber content of fibrous materials such as plant cell-wall rich materials, bran and hulls of various cereals and legumes. During extrusion cooking, a variety of steps, including feed transport, mixing, heating, forming, and drying, occur within a short time. The food components in the extruder barrel experience high temperature, shear, and pressure during extrusion cooking (Gaosong and Vasanthan, 2000; Ralet et al., 1993; Rouilly et al., 2006). Extrusion cooking can affect and change the nature of many food constituents like proteins and polyphenols, by changing physical and chemical properties. Several studies have shown that extrusion processing significantly reduces phenolic compounds in food products. Repo-Carrasco-Valencia et al. (2009) reported that the level of total phenolic acid decreased 80.3% after extrusion in kiwicha. Similarly, Delgado-Licon et al. (2009) observed a significant decrease in the total polyphenols and antioxidant activity during extrusion of bean/corn mixture. They observed that the decrease in bioactive compounds was dependent on process condition. Phenolic compounds during extrusion may undergo decarboxylation due to high barrel temperature and high moisture content may promote polymerization of phenols and tannins leading to reduced extractability and antioxidant activity.

Interest in glucosidase inhibitors is growing because of its implications for the management of diabetes mellitus (DM). DM is a serious metabolic disorder that affects approximately 4% of the population worldwide and is expected to increase to affect 5.4% by 2025 (Yao et al., 2008). Acting as a key enzyme for carbohydrate digestion, intestinal α-glucosidase is one of the glucosidases located at the epithelium of the small intestine. α-Glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type 2 diabetes mellitus (Krentz and Bailey, 2005; Lebovitz, 1998). The inhibition on intestinal α-glucosidases would delay the digestion and absorption of carbohydrates and consequently suppress the postprandial hyperglycemia (Puls et al., 1977).

Adzuki beans have been a subject of extensive investigation due to their biological activities. Recently, they have been recommended as suitable foods for diabetic patients due to their proteins and phenolic compounds (Lin and Lai, 2006; Yao et al., 2011), which may offer extra benefits for the amelioration of diabetes. Itoh et al. (2004) reported that adzuki beans possess inhibition activity against α-glucosidase in streptozotocin (STZ)-induced diabetic rats. Cian et al. (2011) examined the effects of extrusion processing on the antioxidant and antihypertenstive activities of maize, and

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reported that the extrusion process can enhance the bioactivities compared with the maize control. However, apparently no studies have applied the extrusion processing technique to modify the antidiabetic activities of cereals or legumes. The present study was therefore carried out to (i) quantify the polysaccharide content, protein content, total phenolic content (TPC) and total flavonoid content (TFC) in extruded adzuki beans and (ii) assess their relative antioxidant and α-glucosidase inhibitory activities.

2. Materials and methods

2.1. Materials

Adzuki beans were provided by the Chinese National Genebank (Beijing, China). Adzuki beans coats, accounting for 9% of the whole beans, was removed in a grain polish machine. Rat intestinal acetone powder and streptozotocin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acarbose was purchased from Bayer Health Care Pharmaceuticals, Inc. (USA). All chemicals used were of analytical grade and were obtained from Beijing Chemical Reagent (Beijing, China).

2.2. Extrusion

Extrusion was carried out in laboratory twin screw extruder typed DS56 (Saixin machinery, Shandong, China) with the following operation parameters: feed speed 20 g/min, screw speed 160 rpm, moisture content 16% (wet base), temperature settings for the feeding zone to die zone 80–110–150–135–80 °C.

2.3. Chemical analyses

Adzuki beans and extruded adzuki beans were ground in a laboratory mill and passed through a sieve (80 mesh). The polysaccharide content was determined using the phenol–sulphuric acid method with D-glucose as a reference (Du et al., 1956; Kozarski et al., 2011). Samples were hydrolyzed with 2 M TFA at 100 °C overnight, followed by evaporation to dryness. Residual TFA was removed by evaporation with 0.5 mL of methanol, and the final residue was dissolved in 0.5 mL of water.

The protein compositions of adzuki beans and extruded adzuki beans were determined according to the methods of AOAC 14.108 (AOAC, 1990).

For the total phenolic content (TPC) and total flavonoid content (TFC) determination of adzuki beans and extruded adzuki beans, 1 g samples were extracted in 20 mL of 60% ethanol for 2 h at room temperature. TPC was measured using the Folin–Ciocalteu method as previously described by Zhou et al. (2009) and modified by Yao et al. (2012). Briefly, 50 μL of the extract was mixed in 5 mL of distilled deionized water followed by the addition of 500 μL of 1 M Folin–Ciocalteu reagent and 500 μL of a 20% (w/v) Na_2CO_3 solution. The mixture was thoroughly mixed and allowed to stand for 60 min at room temperature before the absorbance was measured at 765 nm (Bio-Rad Smart Spec Plus Spectrophotometer, Hercules, USA). Quantification was performed with respect to the standard curve of gallic acid. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram. TFC was measured using the colorimetric method as previously described by Zou et al. (2004) with some modifications. The extract (0.5 mL) was mixed in 2 mL of distilled water followed by the addition of 0.15 mL of a 5% (w/v) $NaNO_2$ solution. After 6 min, 0.15 mL of a 10% $AlCl_3$ solution was added to the mixture and the solution was then allowed to stand for 6 min followed by the addition of 2 mL of a 4% NaOH solution. Water was immediately added to bring the final volume to 5 mL, and the mixture was then thoroughly mixed and allowed to stand for an additional 15 min before the absorbance was measured. The results were expressed as milligrams of catechin equivalent (CE) per gram.

2.4. Evaluation of total antioxidant activity and α-glucosidase inhibitory activity

For the antioxidant and α-glucosidase activities evaluation of adzuki beans and extruded adzuki beans, 1 g samples were extracted in 20 mL of distilled water for 2 h at room temperature. The DPPH radical-scavenging activity was determined using the method reported by Yen and Chen (1995). DPPH (100 μM) was dissolved in 96% ethanol. The extract was dissolved in ethanol in a ratio of 1:3. The DPPH solution (1 mL) was mixed with 1 mL of the extract solution. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm after 10 min. The results were expressed in micromoles of Trolox equivalents (TE) per gram.

The α-glucosidase inhibitory activity was determined as previously described with slight modifications (Nishioka et al., 1998; Yao et al., 2010). The inhibition activity of α-glucosidase (1 unit/mL) was assayed using 50 μL of extracts with varying concentrations incubated with 100 μL of 0.1 M phosphate buffer (pH 7.0) in 96-well plates at 37 °C for 10 min. After preincubation, 50 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 7.0) was added to each well at varying time intervals. The reaction mixtures were incubated at 37 °C for 5 min. The absorbance readings were recorded at 490 nm on a microplate reader before and after incubation (BioRad, IMAX, Hercules, USA). The results were expressed as a percent of α-glucosidase inhibition and calculated according to the following equation: % inhibition = $Abs^{control} - Abs^{extract} \times 100/Abs^{control}$.

2.5. Isolation of crude polysaccharide

Ten grams of adzuki beans or extruded adzuki beans were extracted by 200 mL water. The extracted solution was centrifuged at 5000 rpm for 10 min, and the supernatant was concentrated to about 100 mL. The associated proteins in the products were removed using trichloroacetic acid (TCA) deproteinizing method (Wang et al., 2011). After overnight disposal, the solution was centrifuged at 3000 rpm for 10 min. Supernatant were collected and then freeze-dried to give the crude polysaccharide extract.

2.6. Isolation of crude protein

Protein isolates from adzuki beans and extruded adzuki beans were prepared using the method described by Li et al. (2010) with some modification. Five percent (w/v) of beans flour slurry was adjusted to pH 9.5 with 1 M NaOH at room temperature, mixed round for 1 h and centrifuged for 15 min at 2000 × *g*. Then the pH was adjusted to 4.5 with 1 M HCl to precipitate the protein. The proteins were recovered by centrifugation at 2000 × *g* for 15 min followed by removal of the supernatant. Protein curd was washed with distilled water and the curd was re-dispersed in distilled water. The washed precipitate was collected and immediately put into freeze dryer.

2.7. Animals

Male SD rats were obtained from Department of Laboratory Animal Science Center (Beijing, China). All rats were housed individually in stainless steel wire-bottom cages in an air-conditioned room kept at controlled ambient temperature (22 ± 1 °C), humidity (50 ± 10%) and a 12-h light/dark cycle. The experiment was carried out according to the European Community Guidelines for the Use

of Experimental Animals and approved by the Peking University Committee on Animal Care and Use.

After 2 weeks of quarantine, rats were administrated with STZ (45 mg/kg, bw) by intraperitoneal injection for five consecutive days to induce moderate level of diabetes. The STZ was dissolved in ice-cold citrate buffer and injected immediately. Five days after STZ injection, rats showed elevated levels of fasting blood glucose and were randomly assigned to different treatment groups.

For the sucrose loading evaluation of adzuki beans and extruded adzuki beans, one kilogram of adzuki beans or extruded adzuki beans were extracted by 20 L water. After vacuum filtration, the supernatants were concentrated under reduced pressure in a rotary evaporator at 50 °C. After freeze-drying, the sample powder was stored at −20 °C until analysis.

All rats were fasted for 14 h with free access to water before the experiment. The fasted rats were divided into 8 groups: group I: normal rats ($n = 10$); group II: normal rats given 50 mg acarbose/kg ($n = 10$); group III: normal rats given 200 mg adzuki beans extract (ABE)/kg ($n = 10$); group IV: normal rats given 200 mg extruded adzuki beans extract (EABE)/kg ($n = 10$); group V: diabetic rats ($n = 10$); group VI: diabetic rats given 50 mg acarbose/kg ($n = 10$); group VII: diabetic rats given 200 mg ABE/kg ($n = 10$); group VIII: diabetic rats given 200 mg EABE/kg ($n = 10$). After 30 min, fasting animals were given sucrose orally (2 g/kg). Approximately 5 μL of whole blood samples were collected from the tail vein of each mouse. The blood samples were acquired at 0 (just before the sucrose administration), 30, 60 and 120 min after the starch ingestion. Blood glucose levels were measured with a glucose analyzer (ACCU-CHEK Active, Roche, Shanghai, China).

2.8. Statistical analysis

All values were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) followed by the post hoc LSD test on SPSS Statistical Software (SPSS version 16.0, SPSS, Chicago, IL, USA)

3. Results and discussion

3.1. Polysaccharide content, protein content, total phenolic content (TPC) and total flavonoid content (TFC)

Extrusion cooking increased polysaccharide content and protein content by 17.39% and 7.86% compared with that of the unprocessed adzuki beans. The results are in agreement with those observed by Anguita et al. (2006), who reported that extrusion processing promotes starch hydrolysis and increases in the amount of soluble non-starch polysaccharides as well as modifies the physicochemical properties. The loss of protein may be due to phenolic compounds with protein molecules forms hydrophobic surface.

TPC and TFC in extruded adzuki beans were reduced by 41.56% and 25.00% compared with that of the unprocessed adzuki beans. In current study, extrusion variables resulted in degradation of phenolic compounds similar to that reported for extruded common beans and oat extrudates by 20% and 50%, respectively (Zadernowski et al., 1999; Korus et al., 2007). Dlamini et al. (2007) also observed that extrusion cooking significantly reduces total phenols and tannins for both whole and decorticated sorghums. It has been stated that high temperature during extrusion can alter molecular structure of phenolic compounds and either reduce their chemical reactivity or decrease their extractability due to a certain degree of polymerization (Alonso et al., 2000). Anuonye et al. (2010) observed that extrusion cooking of soybean had little change in isoflavone content but significant changed isoflavone profile. White et al. (2010) investigated the changes in the flavonoid contents of cranberry pomace/corn starch blends during extrusion cooking. They found significant losses (46–64%) in flavonoid contents of extrudates and losses were higher at higher barrel temperature. They deduced that flavonoid contents losses are mainly due to processing temperature and moisture content.

Table 1
Polysaccharide content, protein content, total phenolic contents (TPC), total flavonoid content (TFC), anti-DPPH radical activity and α-glucosidase inhibitory activity of adzuki beans extract (ABE) and extruded adzuki beans extract (EABE).

	ABE	EABE
Polysaccharide content (mg/g)	135.72 ± 10.17b	159.32 ± 8.24a
Protein content[a] (%)	19.46 ± 0.08b	20.99 ± 0.10a
TPC (mg GAE/g)	0.77 ± 0.03a	0.45 ± 0.02b
TFC (mg CE/g)	0.16 ± 0.01a	0.12 ± 0.01a
DPPH	0.86 ± 0.07a	0.50 ± 0.04b
α-Glucosidase inhibitory activity (%)	16.31 ± 1.02b	66.48 ± 0.67a

Data are expressed as mean ± standard deviation of triplicate samples.
The anti-DPPH capacity was expressed as μM TE/g.
Values in the same row sharing different letters expressed as significantly different ($P < 0.05$).
[a] Expressed as % of dry weight.

3.2. Antioxidant activity

Extrusion cooking reduced antioxidant activities in extruded adzuki beans by 41.86% compared with that of the unprocessed adzuki beans (Table 1). The losses we observed consistent with those reported by Hamama and Nawar (1991), who observed that the loss of antioxidants has been attributed to both evaporation and decomposition at elevated temperatures. Zadernowski et al. (1999) also stated that the disadvantage of natural antioxidants is their low resistance to high temperatures since heating over 80 °C destroys their antioxidant properties. Decreased antioxidant activity could be attributable to the effect of extrusion on (1) breaking complex polyphenols into low molecular weight phenolic compounds with scavenging activity, (2) interaction of the phenolics with protein under heat treatment, and (3) formation of Maillard reaction products. High temperature extrusion promotes the Maillard reaction and formation of brown compounds that may have had an effect on the total antioxidant capacities of the extrudates (Anese et al., 1999). Additionally, the potential binding of phenolic compounds to the protein matrix may account for the decreased in the total antioxidant capacities observed on those formulations extruded at die temperatures of 140 °C compared to their raw samples. At high-protein concentration, complex interactions and cross-linking of different protein molecules with phenolic compounds forms hydrophobic surface (Mcmanus et al., 1985).

Antioxidant activity of extruded products is dependent not only on the level of bioactive compounds but also on the composition of bioactive compounds. Korus et al. (2007) observed a lower antioxidant activity for dark-red beans compared to black brown and cream colored beans, even though dark-red beans extrudates exhibited higher total phenolic content compared to black brown and cream colored beans extrudates. Oomah et al. (2005) reported that the presence of total phenolic and flavonol in common beans are the major contributors to the total antiradical activity, accounting for about 40–71% and 20–39%, respectively whereas the contribution from the flavonoid, anthocyanin and tartaric esters is minimal.

3.3. α-Glucosidase inhibition activities

Extrusion cooking increased antidiabetic activities in extruded adzuki beans by 307.60% compared with that of the unprocessed adzuki beans (Table 1). The original adzuki beans extract was prepared from hot-water extraction, red coats, it was initially

Table 2
α-Glucosidase inhibitory activities of polysaccharide and protein from adzuki beans and extruded adzuki beans.

	α-Glucosidase inhibitory activity (%)
Polysaccharide from adzuki beans	6.33 ± 0.12c
Polysaccharide from extruded adzuki beans	35.47 ± 2.74b
Protein from adzuki beans	1.66 ± 0.05c
Protein from extruded adzuki beans	60.44 ± 5.68a
Acarbose	82.73 ± 7.89a

Values in the same row sharing different letters expressed as significantly different ($P < 0.05$).

speculated that the active inhibiting constituents in adzuki beans may be anthocyanins and phenolic compounds (Itoh et al., 2009), since previous studies showed that anthocyanins and phenolic compounds suppress the postprandial blood glucose by inhibiting α-glucosidase (Matsui et al., 2001; Fukuda et al., 2004). However, the extruded adzuki beans without coats were tested. Interestingly, the EABE also showed strong inhibition of rat α-glucosidases. These results strongly suggest that there should be constituents other than anthocyanins or phenolic compounds in adzuki beans that inhibit α-glucosidases. Hence, polysaccharide and protein were selected for further α-glucosidases inhibition experiments. The protein from extruded adzuki beans was the most active (60.44%), which was 36.41 times higher than the protein from adzuki beans (Table 2).

3.4. Effect of extrusion on the protein composition of adzuki beans

Postprandial blood glucose variation was measured after loading sucrose to the normal rats (Fig. 1A). In the control group, blood glucose level was an average of 10.4 mmol/L at 30 min after the sucrose load. In the group that received EABE along with sucrose, the 30 min post-load glucose level was only 8.79 mmol/L on an average. This indicates the potency of EABE extract to significantly suppress high sucrose diet associated postprandial glucose elevation. Compared to control, the whole glycemic response is reduced by 15.6% on EABE treatment and 22.6% on acarbose treatment.

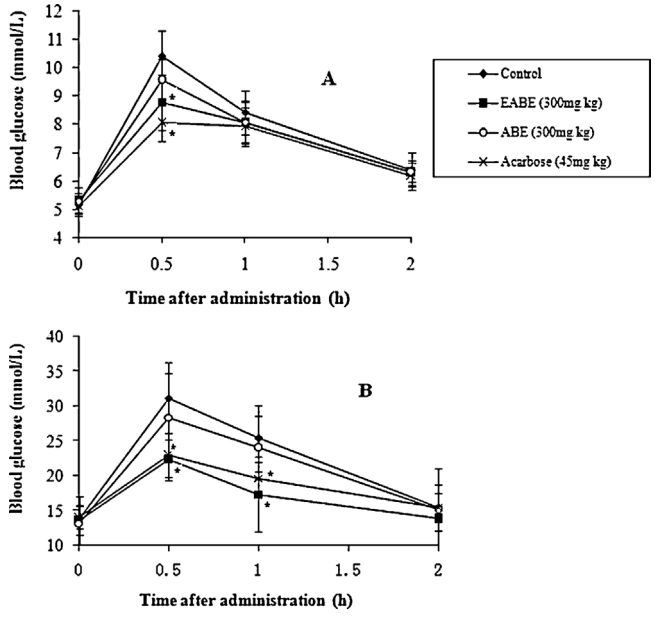

Fig. 1. (A) Inhibitory effects of ABE and EABE on blood glucose after sucrose loading in normal rats. (B) Inhibitory effects of ABE and EABE on blood glucose after sucrose loading in diabetic rats. *$P < 0.05$ compared to the control group.

Postprandial blood glucose variation was measured after loading sucrose to the diabetic rats (Fig. 1B). In the control group, blood glucose level increased to an average of 31.2 mmol/L 30 min after sucrose administration. However, the rise of the post-load blood glucose has been significantly impeded in EABE group. Similar kind of suppression effect was observed in the group that received acarbose as the positive control along with sucrose. Compared to control, the whole glycemic response is reduced by 28.4% and 23.0% when treated with 300 mg/kg body weight of EABE and 20 mg/kg body weight of acarbose, respectively. Presently, there is growing interest in herbal remedies for the treatment of diabetes mellitus. More than 400 plants with glucose-lowering effects are known. Itoh et al. (2009) have observed that gave 500 mg/kg hot-water extracts of adzuki to the non-diabetic and diabetic mice could suppressed the development of hyperglycemia and hyperinsulinemia for four weeks treatment. We also observed that mung bean sprout (2 g/kg) and mung bean seed coat (3 g/kg) lowered blood glucose and at the same time improved glucose tolerance and increased insulin immunoreactive levels in diabetic mice after 5 weeks treatment (Yao et al., 2008).

In summary, the present study found that polysaccharide and protein from extruded adzuki beans significantly inhibited rat intestinal α-glucosidases in vitro. Administration of EABE to normal rats and STZ-induced diabetic rats could improve of postprandial hyperglycaemia after sucrose loading. Therefore, EABE will have the potential to be further explored as functional food in the prevention of diabetes.

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