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α-Amylase Inhibitory Activity from Nut Seed Skin Polyphenols. 1. Purification and Characterization of Almond Seed Skin Polyphenols

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ABSTRACT: Using α-amylase inhibition as a separation guide, polyphenolic compounds from almond (*Prunus dulcis*) seed skin were purified using ultrafiltration and Sephadex LH-20 and ODS columns. The purified fraction specifically and strongly inhibited α-amylase; the IC_{50} value was 2.2 μg/mL for pig pancreatic α-amylase. The fraction contained about 62% of the total polyphenols, 33.8% flavanol-type tannins and 30% procyanidins. Oral administration of the polyphenol fraction to rats fed corn starch significantly suppressed an increase in blood glucose levels and area under the curve (AUC), in a dose-dependent manner. High-resolution MALDI-TOF mass spectra showed that the structure of this sample is a series of polyflavan-3-ol polymers composed of catechin/epicatechin units and gallocatechin/epigallocatechin units up to 11-mer with several interflavanoid ether linkages. The results suggest almond seed skin contains highly polymerized polyphenols with strong α-amylase inhibitory activity, which retard absorption of carbohydrate.

KEYWORDS: *almond seed skin, polyphenol, amylase inhibitor, oral carbohydrate tolerance test*

INTRODUCTION

Diabetes is a major worldwide public health problem with the number of patients increasing greatly in the past 50 years in both developed and developing nations.[1] In Japan, about 8.9 million people are diabetic and about 13.2 million people are prediabetic (conditional report of 2007 from the Ministry of Health, Labor and Welfare of Japan). Maintenance of healthy blood glucose levels is important in diabetic patients, and blood glucose concentration is greatly affected by dietary carbohydrates. Studies suggest that postprandial hyperglycemia is an important contributory factor in the development of atherosclerosis and cardiovascular disease.[2−4] Therefore, control of postprandial plasma glucose levels is important, and various α-glucosidase or α-amylase inhibitors have been used to inhibit excess energy supply to control blood glucose and to prevent or treat obesity and diabetes. Acarbose and voglibose, strong α-glycosidase inhibitors, are typical therapeutic agents used to control postprandial glucose concentration and are used in the treatment of type 2 diabetes.[5−7] Wheat and kidney bean proteins are α-amylase inhibitors[8,9] and are used as a functional food to prevent diabetes. We screened different plants for inhibitors of carbohydrate-hydrolyzing enzymes: we discovered polyphenols extracted from nut seed skin inhibited α-amylase. We previously reported that polyphenols isolated from chestnut astringent skin inhibited α-amylase activity, retarded absorption of carbohydrate, and reduced postprandial hyperglycemia in rats and humans.[10−12] In this study, we report on polyphenols isolated from almond seed skin with α-amylase inhibitory activity and discuss their characterization.

Almond is a deciduous tree, growing 4−10 m in height, and is related to stone fruit trees, such as peach, plum, and cherry. Almond trees are cultivated in warm and arid regions, especially California in the United States and in southern Europe, Spain, France, and Italy. Almond seed skin is generated in large quantities as a waste product during industrial seed skin-free pea processing. The skin represents approximately 4% of the total almond weight but contains about 60% of the total polyphenols present in the nut.[13] We isolated polyphenols from almond seed skin, an inexpensive functional food material, using 70% aqueous acetone. α-Amylase inhibition was used to guide the separation. The effect of the separated polyphenols on carbohydrate absorption was also investigated.

MATERIALS AND METHODS

Materials. Roasted almond (*Prunus dulcis*) seed skin was obtained from Chuon Co., Ltd. (Matsuyama, Japan). α-Amylases from porcine pancreas and human saliva, pancreatic lipase (Type VI-S) from porcine pancreas, intestinal acetone powder from rat, cellulase from *Aspergillus niger*, Folin−Ciocalteu reagent, (+)-catechin, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Japan (Tokyo, Japan). α-Amylase from *Bacillus* sp. was obtained from Nacalai Co. (Kyoto, Japan). Glucoamylase from *Rhizopus* sp. was obtained from Seikagaku Biobusiness Co. (Tokyo, Japan). Cyanidin chloride was obtained from ChromaDex (Irvine, CA, USA). Trolox was obtained from Calbiochem (Darmstadt, Germany). Sephadex LH-20 was obtained from GE Healthcare Japan (Tokyo, Japan). Ultrafilters were obtained from ADVANTEC (Tokyo, Japan). Octadecylsilyl silica gel (ODS) was obtained from Yamazen Co. (Osaka, Japan).

Preparation of Almond Seed Skin Extract. Roasted almond seed skins were milled in a Millser-600D (Iwatani Co., Tokyo, Japan), and the skins (1 g) were added to 10 mL of water or 70% aqueous

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Table 1. Extract of Almond Seed Skin[a]

extract solution	dry wt (mg)	polyphenol (mg)	α-amylase inhibition U/mg	α-amylase inhibition total U	DPPH (TE μmol/mg)
water	99.6	11.7	0.613	61.1	1.02
70% methanol	21.1	1.06	0.223	4.71	0.542
70% ethanol	40.5	3.11	0.156	6.30	0.619
70% acetone	20.7	3.54	5.75	119	0.982
70% acetonitrile	9.1	1.03	1.86	16.9	2.54

[a]Results are expressed as the mean of three assays.

organic solvent (methanol, ethanol, acetone, or acetonitrile) followed by shaking at 40 °C for 24 h. The mixture was filtered using gauzes; the filtrate was then concentrated under reduced pressure and lyophilized.

Separation of Polyphenols from Almond Seed Skin Extract. Ten grams of roasted and milled almond seed skins was added to 70% aqueous acetone (100 mL), followed by shaking at 40 °C for 24 h. The mixture was filtered through gauze layers, and the filtrate was concentrated under reduced pressure and lyophilized to generate acetone extract. The acetone extract (2000 mg) was added to 1000 mL of 70% aqueous ethanol, followed by stirring at 40 °C for 1 h. The mixture was centrifuged at 3000*g* for 10 min, and the supernatant was fractionated stepwise using Ultrafilters (diameter, 90 mm; molecular weight cutoff, 200, 50, and 10 kDa). Each fraction was concentrated under reduced pressure and lyophilized. The 200−50 kDa fraction contained most of the strong α-amylase inhibitory activity, and this was dissolved in water before being applied to a Sephadex LH-20 column (300 mm × 35 mm i.d.) equilibrated with water. The adsorbed compounds were eluted stepwise with 500 mL of 30, 40, 50, 60, and 80% aqueous acetone after washing with water (1000 mL). The eluates were concentrated under reduced pressure and lyophilized. The 50−60% aqueous acetone fraction contained most of the strongest α-amylase inhibitory activity and was dissolved in water before being applied to an ODS column (175 mm × 18 mm i.d.) equilibrated with water. The active compounds were eluted with a linear gradient of 20−50% aqueous methanol (200 mL−200 mL) after washing with water (100 mL) and 20% aqueous methanol (100 mL). The eluate containing high α-amylase inhibitory activity was concentrated and lyophilized. During purification, α-amylase inhibition and the amount of polyphenol were monitored.

Assay Methods. α-Amylase activity was determined by measuring the reducing power of released oligosaccharide from soluble starch using the method of Miller[14] with the following minor modifications:[10] the assay system consisted of 100 mM sodium phosphate, pH 6.8, 17 mM NaCl, 5 mg of soluble starch, 100 μL of inhibitor solution, and 10 μL of enzyme solution in a total volume of 1 mL. After incubation at 37 °C for 30 min, the reaction was stopped by the addition of 0.1 mL of 2 N NaOH and 0.1 mL of color reagent (4.4 μmol of 3,5-dinitrosalicylic acid, 106 μmol of potassium sodium (+)-tartrate tetrahydrate, and 40 μmol of NaOH), followed by 3 min of incubation at 100 °C and subsequent absorbance measurement at 540 nm.

Maltase activity was determined using maltose as the substrate, and glucose produced in the reaction was measured with a commercial assay kit (Glucose C II-test, Wako Pure Chemical Industries, Ltd., Osaka, Japan).[15,16] Lipase activity was determined by measuring the rate of release of oleic acid from trioleoylglycerol.[17] The rate of enzyme inhibition was calculated as a percentage of the control (without inhibitor) using the formula

$$\text{enzyme inhibition (\%)} = [(A_0 - A_i)/A_0] \times 100$$

where A_i = activity with inhibitor and A_0 = control activity (activity without inhibitor). One unit of enzyme inhibition (U) was expressed by the weight of the IC_{50} value per milliliter.

Polyphenols (total phenolics) were determined using the Folin−Ciocalteu method and (+)-catechin as the standard.[18,19] In addition, flavan-3-ol concentration was determined using the vanillin assay,[20] and procyanidins were determined using the butanolic−HCl assay[21] using (+)-catechin and cyanidin chloride as standards, respectively.

Antioxidant activities were determined using the DPPH radical scavenging capacity assay. The DPPH radical scavenging capacity of each fraction was determined according to the method of Miliauskas.[22] The radical scavenging activity of the samples or trolox calibration solutions against stable DPPH radicals was determined spectrophotometrically, and the activities were expressed as micromoles trolox equivalents (TE) per milligram sample.

Analysis of Polyphenol by MALDI-TOF/MS. The sample (0.5 mg of the purified fraction) was dissolved in 0.5 mL of acetone. About 10 mg of 2,4,6-trihydroxyacetophenone (THAP; Wako Pure Chemical Industries, Ltd.) as a matrix was dissolved in 1 mL of acetone/Milli-Q water at a ratio of 4:1 (v/v). The sample and matrix solutions were then mixed with a ratio of 10:1 (v/v), and the resulting 1 μL was placed on a target plate and dried in air. MALDI-TOF mass spectra were collected on a JMS-S-3000 MALDI Spiral TOF mass spectrometer (JEOL Ltd., Tokyo, Japan). Ions generated by irradiation with a 349 nm Nd:YLF laser were accelerated at 20 kV. The ions then passed along a spiral ion trajectory with a flight length of approximately 17 m. The settings of delay time and grid voltage were optimized to maintain constant at ΔM = 0.02−0.03 Da over the range of m/z 800−3000. Mass calibration was made with a poly(methyl methacrylate) (PMMA) standard (peak-top molecular weight, M_p = 1310) purchased from Polymer Laboratories (Church Stretton, UK). Three mass spectra for each sample were collected.

Oral Carbohydrate Tolerance Test in Rats. The experimental animal protocol was approved by the Animal Study Committee of Ehime University. Male Wistar King rats, weighing 250−300 g, were starved overnight (15 h) and divided into two groups. The test group received 2.5 mL of carbohydrate solution (2 g/kg body weight) containing the almond seed skin polyphenols, whereas the control group received carbohydrate solution only. The solutions were fed via a stomach tube. After administration, blood samples were collected from the tail vein or artery at regular intervals. Blood glucose was measured using a blood glucose test meter, GLUCOCARD (Arkray Inc. Kyoto, Japan).[10] After a 1 week interval, the test and control groups were switched and the experiment was repeated.

Statistical Analysis. Results are expressed as the mean ± SE. The statistical significance of differences with and without (control) the samples were assessed using the paired Student's *t* test.

RESULTS AND DISCUSSION

Extraction of Compounds with α-Amylase Inhibition from Almond Seed Skin. Roasted almond seed skin was extracted with water and aqueous organic solvent (70% aqueous methanol, ethanol, acetone, or acetonitrile). We found that water was the most effective solvent, producing the highest freeze-dried weight and total polyphenol content (Table 1). DPPH radical scavenging capacity was effectively extracted with 70% aqueous acetonitrile; however, the highest α-amylase inhibitory activity was extracted with 70% aqueous acetone. The specific α-amylase inhibitory activity of the aqueous acetone extract was about 9-fold higher than the water extract and 3-fold higher than the aqueous acetonitrile extract. α-Amylase inhibitory activity and DPPH radical scavenging

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Table 2. Filtration of Almond Seed Skin Extract by Ultrafilters[a]

filtration	dry wt (mg)	polyphenol (mg)	α-amylase inhibition U/mg	α-amylase inhibition total kU	DPPH (TE μmol/mg)
<200 kDa	415	158	39.9	16.5	3.51
200−50 kDa	345	150	43.3	14.9	3.28
50−10 kDa	238	57.5	1.52	0.36	1.59
>10 kDa	450	8.6	0.20	0.09	0.98

[a]Results are expressed as the mean of three assays.

capacity were not effectively extracted with 70% aqueous methanol and ethanol. Garrido et al. reported that methanol/HCl (1000:1 v/v) was the most effective solvent mixture for antioxidant extraction from almond skin.[23,24] However, we found that the specific α-amylase inhibitory activity of the methanol/HCl extract was about 40% less than that of the aqueous acetone extract (data not shown). α-Amylase inhibitory activity was not effectively extracted with nonpolar solvents, such as hexane, ethyl acetate, and chloroform: the specific α-amylase inhibitory activities were 0.61, 0.26, and 0.25 U/mg dry weight, respectively. α-Amylase inhibitory activity was also not effectively extracted with 100% acetone (0.34 U/mg dry weight). Therefore, we used 70% aqueous acetone to extract from almond seed skin followed by concentration under reduced pressure before lyophilization to generate an acetone extract.

Fractionation of Compounds with α-Amylase Inhibitory Activity from Almond Seed Skin Extract. The acetone extract was then dissolved in 70% aqueous ethanol and fractionated using Ultrafilters. All fractions contained α-amylase inhibitory activity; however, high α-amylase inhibitory activity was observed in the 200−50 kDa fraction (Table 2). The specific α-amylase inhibitory activity in the 200−50 kDa fraction was about 28-fold higher than in the 50−10 kDa fraction and 222-fold higher than in the <10 kDa fraction. High α-amylase inhibitory activity was also present in the >200 kDa fraction. High DPPH radical scavenging capacity was observed in the >200 kDa and 200−50 kDa fractions. The sum of the total α-amylase inhibition of each filtration fraction was about 32 kU, which was about 3 times higher than the acetone extract (11.6 kU) (Table 4). It is suggested that compounds within the acetone extract might interfere with α-amylase inhibition.

Although the molecular standards of the ultrafiltration membranes are globular proteins, the results suggest the α-amylase inhibitory compounds are high-polymer substances. Similar results were observed using chestnut astringent skin extract (high α-amylase inhibitory activities were observed in the 300−100 kDa fraction).[12] We do not know why high α-amylase inhibitory activities are observed at high molecular weight such as >50 kDa. It is unlikely that the degree of polymerization of the catechin/epicatechin unit would be over 175. There is little possibility of an aggregation of polyphenols, because samples were dissolved in 70% aqueous ethanol and centrifuged before the clear supernatant was fractionated, stepwise, using Ultrafilters. Further studies are needed to clarify the molecular weight of the fractions containing high α-amylase inhibitory activities.

Gu et al. reported that there is a strong relationship between α-amylase inhibition and the degree of polymerization of procyanidins.[25] Polyphenols from blueberries and raspberries (70% acetone extracts) have weak α-amylase inhibitory activity: 1.89 and 0.38 U/mg dry weight, respectively. When these polyphenols were separated using Ultrafilters, >90% of the material was observed in the <10 kDa fractions (data not shown). These results also suggested that polyphenols with high α-amylase inhibitory activity might be high-polymer substances.

Purification of Compounds with α-Amylase Inhibitiory Activity from Almond Seed Skin Extract. The 200−50 kDa fraction, which contained the highest amount of specific α-amylase inhibitory activity, was applied to a Sephadex LH-20 column. α-Amylase inhibitory activities were adsorbed in the column and eluted, stepwise, with aqueous acetone after washing with water. All aqueous acetone eluted fractions contained α-amylase inhibitory activity (Table 3). α-Amylase inhibitory activities were not detected in the nonadsorbed fraction (the water-eluted fraction). Most active materials with high α-amylase inhibitory activity were observed in the 50−60% acetone fraction; the α-amylase inhibitory activity was about 6 times greater in the 50−60% acetone fraction compared with the 200−50 kDa fraction (Table2). The highest DPPH radical scavenging capacity was also observed in the 50−60% acetone fraction. This fraction was applied to an ODS column, and about 74% of α-amylase inhibitory activity was adsorbed to the column. The α-amylase inhibitory activity and polyphenol were eluted broadly and together using a linear gradient of 20−50% aqueous methanol, as shown in Figure 1. Fractions 10−30 were pooled, concentrated, and lyophilized to generate a purified polyphenol fraction.

Table 3. Sephadex LH-20 Column Separation of 200−50 kDa Fraction[a]

elution	dry weight (mg)	polyphenol (mg)	α-amylase inhibition U/mg	α-amylase inhibition total kU	DPPH (TE μmol/mg)
water	55.5	5.54	nd[b]	nd	0.671
30% acetone	26.4	16.6	12.9	0.341	4.05
40% acetone	47.9	28.6	71.9	3.44	4.40
50% acetone	97.5	54.9	102	9.95	5.31
60% acetone	25.8	14.3	260	6.71	5.62
80% acetone	33.8	5.64	70.9	2.40	2.25

[a]Results are expressed as the mean of three assays. [b]nd, not detectable.

Purification steps and yields at each step are summarized in Table 4. During purification, each fraction inhibited α-amylase activity in a dose-dependent manner (Figure 2). The specific α-amylase inhibitory activity was increased about 79-fold, and the IC_{50} value was 2.2 μg/mL. However, α-glucosidase inhibitory activity and DPPH radical scavenging capacity were increased only about 7.2- and 2.5-fold, respectively. The yield of α-amylase inhibitory activity was about 21%, but the yields of dry weight, polyphenol, α-glucosidase inhibitory activity, and DPPH radical scavenging capacity were only about 0.27, 0.67,

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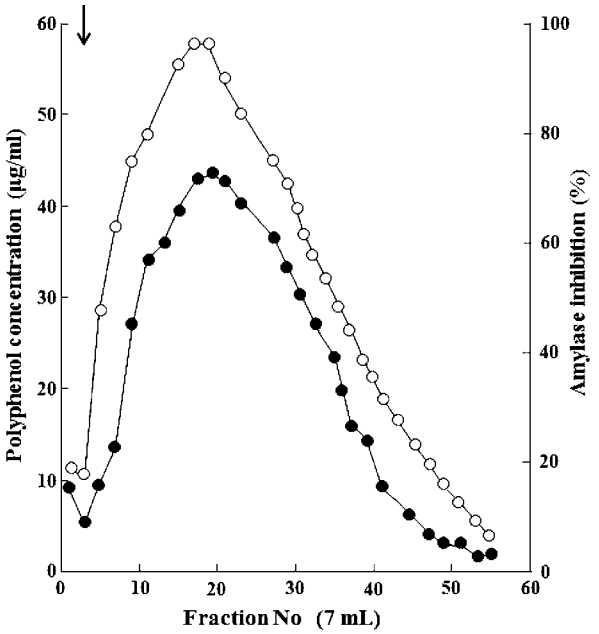

Figure 1. ODS column chromatography of the polyphenol fraction obtained from the Sephadex LH-20 fraction. The 50−60% acetone fraction was applied to the column. The column was washed with water and 20% aqueous methanol and developed with a linear gradient using 20−50% aqueous methanol (arrow). α-Amylase inhibitory activity (○) and polyphenol concentration (●) were determined.

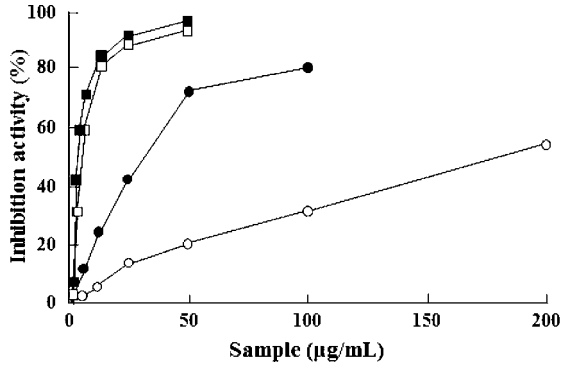

Figure 2. Effect of increased concentration of samples (acetone extract (○), the 200−50 kDa filtration fraction (●), the 50−60% acetone fraction from Sephadex LH-20 (□), and the ODS fraction (■)) on α-amylase inhibitory activity. Results are expressed as the mean of three assays.

Table 5. Enzyme Inhibition by the Purified Polyphenol Fraction[a]

enzyme inhibition	IC_{50} value (μg/mL)
α-amylase from pig pancreas	2.20
α-amylase from human saliva	2.74
α-amylase from *Bacillus* sp.	49.5
glucoamylase from *Rizopus* sp.	>1000
maltase from rat intestine	468
sucrase from rat intestine	627
cellulase from *A. niger*	584
lipase from pig pancreas	570

[a]Results are expressed as the mean of three assays.

2.0, and 0.67%, respectively. These results suggested that the α-amylase inhibitory activity was specifically purified by these purification steps. The amount of total polyphenols in each fraction was determined using catechin as a standard according to the Folin−Ciocalteu method. The total polyphenol content of the purified fraction was about 62% of the dry weight. The content of flavan-3-ol and procyanidins in each fraction was determined using the vanillin assay and the butanolic−HCl assay, respectively. In the purified fraction, they were about 34 and 30% of the dry weight, respectively (data not shown).

Properties of the Purified Polyphenol Fraction from Almond Seed Skin. Table 5 lists some of the enzymes inhibited by the purified fraction. Pig pancreatic and human saliva α-amylase were strongly inhibited. α-Amylase from *Bacillus* sp. was inhibited in a concentration-dependent manner (data not shown), and the IC_{50} value was about 23 times higher compared with that of pig pancreatic α-amylase. α-Glucosidase, bacterial cellulase, and pig pancreatic lipase were weakly inhibited; the IC_{50} values of the fraction for maltase, sucrase, cellulase, and lipase activities were about 210, 285, 265, and 260 times higher than that of pig pancreatic α-amylase, respectively. The purified fraction did not inhibit bacterial glucoamylase activity up to 1000 μg/mL. These results suggested that the purified fraction specifically and strongly inhibited mammalian digestive α-amylase.

Figure 3 shows MALDI mass spectra of the purified fraction. The broad-band mass spectrum (Figure 3A) shows a series of peaks exhibiting a mass increment of ca. 288 Da, corresponding to what is known about the structure of the catechin/epicatechin with a repeat unit. The data may indicate a series of polyflavan-3-ol polymers, termed the B-type linkage.[26,27] A degree of polymerization of this series could be confirmed from 2-mer (m/z ca. 600) to at least 11-mer (m/z ca. 3195) (data not shown).

The narrow-band mass spectrum at m/z 1170−1200 (Figure 3B) expands the tetramer region of Figure 3A, where the peak observed at m/z 1177.2589 is in excellent agreement with the theoretical mass value of tetramer of catechin/epicatechin ($[M + Na]^+$: m/z 1177.2590). The peaks at intervals of ca. 2 Da lower (m/z 1171.2230, 1173.2332, and 1175.2474) suggest the presence of interflavanoid (A-type) ether linkages between two flavan-3-ol units.[24] The peak at m/z 1193.2541 is 15.9952 Da

Table 4. Purification Step of Almond Seed Skin Polyphenol[a]

purification	dry wt (mg)	polyphenol (mg)	α-amylase inhibition		maltase inhibition (U/mg)	DPPH (TE μmol/mg)
			U/mg	total kU		
acetone extract	2000	504	5.78	11.6	0.296	2.20
filtration (200−50 kDa)	345	154	43.3	14.9	0.793	3.28
Sephadex LH-20 (50−60% acetone)	29.7	16.4	260	7.72	2.32	5.60
ODS column	5.45	3.36	455	2.48	2.14	5.43

[a]Results are expressed as the mean of three assays.

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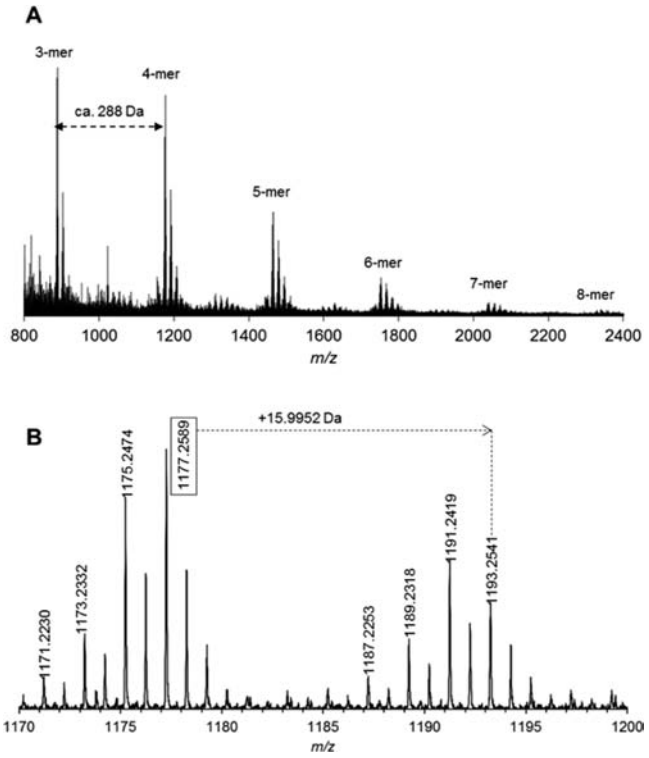

Figure 3. MALDI-TOF positive ion mode spectrum of the purified polyphenol fraction from almond seed skin.

higher than that of polyflavan-3-ol tetramer. This mass difference corresponds to the mass of oxygen (15.9949 Da), suggesting the presence of an additional hydroxyl unit, that is, gallocatechin/epigallocatechin.[24] Krueger et al.[28] have reported the MALDI mass spectra of grape seed extract, and they have assigned the peak at m/z 1193 as a trimer of polyflavan-3-ol having two galloyl esters and a theoretical mass of 1193.2175 Da. High mass accuracy of the MALDI Spiral TOF mass spectrometer, however, clearly denies the possibility of this structure in our samples because of the mass differences in ca. 0.036 Da. The peaks at m/z 1187−1191 with 2 Da intervals also indicate the presence of A-type ether linkages. According to this, we could confirm that the structure of polyflavan-3-ols in this sample are condensates of catechin/epicatechin units and gallocatechin/epigallocatechin units up to 11-mer with several numbers of A-type ether linkages.

Effect of Almond Seed Skin Polyphenol on Carbohydrate Absorption. We examined whether the amylase inhibition observed in vitro could exert an inhibitory effect on carbohydrate absorption in vivo. In control rats, blood glucose levels increased from a baseline of 56.0 ± 2.98 mg/dL at 0 min to a peak of 98.0 ± 3.73 mg/dL (increased blood glucose value 42.0 ± 3.28 mg/dL) at 60 min after corn starch administration (2 g/kg body weight). When the almond seed skin extract was given with corn starch, the peak was delayed at 60−120 min (Figure 4A). The area under the curve (AUC) was calculated geometrically accounting only for the area under the curve of incremental blood glucose levels up to 180 min. The AUC for the extract administration was not significantly decreased (Figure 4B). When the 200−50 kDa filtration fraction was given with corn starch, the rise in blood glucose was suppressed and the AUC was decreased by about 34%, although this was not significant. The rise in blood glucose was suppressed in a dose-dependent manner when the Sephadex LH-20 fraction was given with corn starch (Figure 5A). The AUC for the Sephadex LH-20 fraction administration was also found to decrease with increasing concentrations of this fraction, compared to administration of corn starch alone (Figure 5B). The percent ratios of the AUC against the control (corn starch alone) calculated from Figures 4 and 5 were replotted against the addition of units of α-amylase inhibition (Figure 6). The percent ratio of the AUC was linearly decreased as α-amylase inhibition increased: 50% inhibition occurred at about 55.4 kU/kg body weight. This result suggests that the suppression of starch absorption by the almond seed skin extracts mainly results from the α-amylase inhibition.

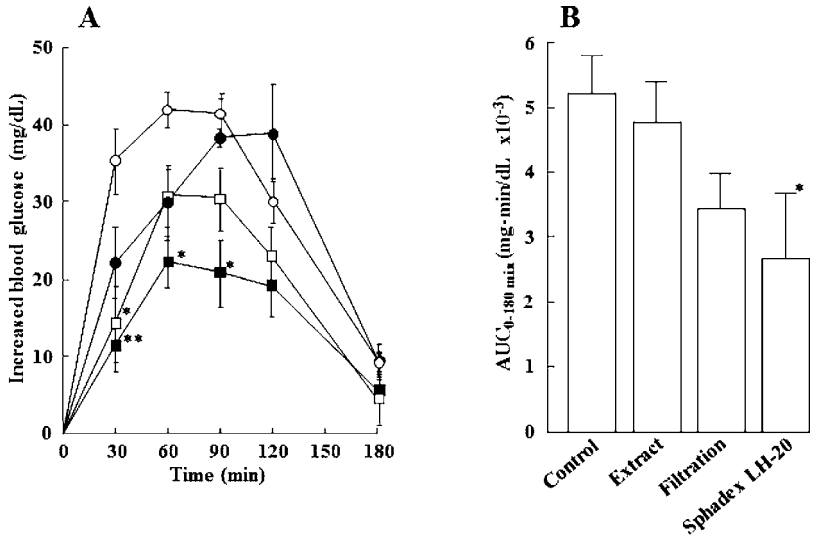

Figure 4. Effects of the polyphenol fractions on increased blood glucose concentration (A) and AUC (B) in rats. (A) Rats were fasted for 15 h before 200 mg/kg body weight of the polyphenol fractions obtained from the purification step (acetone extract (●), the 200−50 kDa filtration fraction (□), and the 50−60% acetone fraction from Sephadex LH-20 (■)) and corn starch (2 g/kg body weight) were administered. For the control, rats were given only corn starch (2 g/kg body weight) (○). (B) $AUC_{0-180\ min}$ is the area under the curve of the incremental blood glucose level up to 180 min. Results are expressed as the mean ± SE $n = 5$. (*) $P < 0.05$ and (**) $p < 0.01$ versus control.

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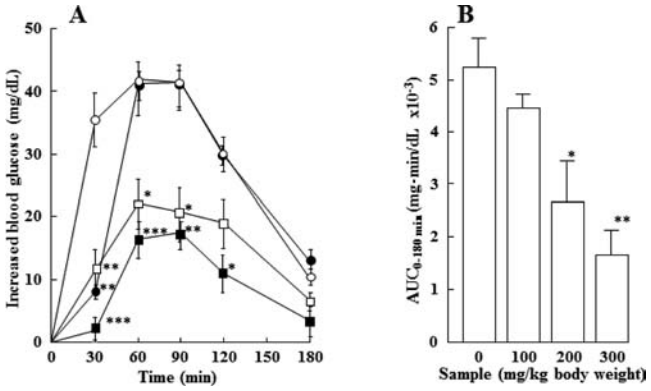

Figure 5. Effects of the 50−60% acetone fraction from Sephadex LH-20 on increased blood glucose concentrations (A) and the AUC (B) in rats. (A) Rats were fasted for 15 h before the 50−60% acetone fraction from Sephadex LH-20 (100 (●), 200 (□), and 300 (■) mg/kg body weight) and corn starch (2 g/kg body weight) were administered. For the control, rats were given only corn starch (2 g/kg body weight) (○). (B) $AUC_{0-180\ min}$ is the area under the curve of the incremental blood glucose level up to 180 min. Results are expressed as the mean ± SE $n = 5$. (∗) $P < 0.05$, (∗∗) $p < 0.01$, and (∗∗∗) $p < 0.001$ versus control.

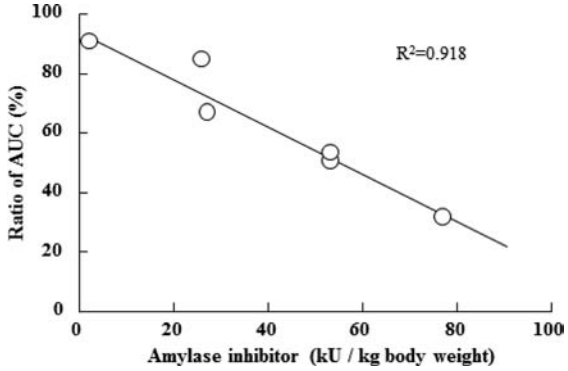

Figure 6. Effect of the addition of α-amylase inhibitor on the percent ratios of the AUC. The percent ratios of the AUC against the contro1 calculated from Figures 4 and 5 were replotted against the addition of α-amylase inhibition units.

Polyphenolic compounds are widely distributed in plants, including nuts skins. Although the weight of the skin is very little compared to the total nut, it contains a large ratio of the total polyphenols present in the nut. Milbury et al. reported that approximately 60% of almond phenolics were present in the seed skin.[13] Kornsteiner et al. suggested about 81% of almond phenolics occurred in the seed skin.[29] Polyphenols are not essential for the survival of seeds, and the role of these polyphenols might be to defend seeds against a variety of herbivores and pathogenic microbes. They might produce an unpalatable bitter taste to prevent seeds from being eaten and inhibit digestive enzymes to prevent seeds from being digested.[30] Polyphenols are also produced to enhance the seed's ability to fight disease or damage, such as oxidation. Therefore, almond seed skin may contain bioactive polyphenols. In fact, almond seed skin polyphenols have an antioxidant capacity and antimicrobial potential.[31−37] However, the α-amylase inhibitory activity of polyphenols from almond seed skin has not yet been reported. In the past 10 years, many investigators reported that almond seed skin contains a variety of polyphenols. They examined the low molecular weight polyphenols (under 1000 Da), but the high molecular weight polyphenols have not been well studied. In this paper, we focused on the high molecular weight polyphenols from almond seed skin, because the specific α-amylase inhibitory activity of the high molecular weight polyphenols was stronger than that of the low molecular weight polyphenols (Table 2).

In mammals, α-amylase is a key enzyme in dietary carbohydrate absorption, catalyzing the first step in carbohydrate digestion by transforming starch to oligosaccharides. The oligosaccharides are further hydrolyzed by α-glucosidase, such as maltase. After these steps, the resulting product, glucose, is absorbed into the small intestine and the blood glucose concentration is increased. If these enzymes are inhibited, dietary carbohydrate absorption is also inhibited, and the subsequent increase in blood glucose concentration is inhibited. Thus, many inhibitors of carbohydrate digestive enzymes have been marketed for the regulation of blood glucose concentration. In vitro studies have shown that many plant polyphenols inhibited carbohydrate-hydrolyzing enzymes, including polyphenols from chestnut astringent skin,[12] evergreen mangrove seeds,[38] Japanese horse chestnut,[39] and berry.[40] In conclusion, almond seed skin contains highly polymerized polyphenols, such as the polymerized procyanidins, which have strong α-amylase inhibition, retard carbohydrate absorption, and reduce postprandial hyperglycemia. Our results suggest the potential usefulness of highly polymerized polyphenols from almond seed skin as a source for nutraceutical factors.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AUC, area under the curve; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC_{50}, concentration of inhibitor required to inhibit 50% of enzyme activity; TE, trolox equivalents

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