ARTICLE

Blueberry extract inhibits carbohydrate-hydrolyzing enzymes and these inhibitory activities are not proanthocyanidin dependent

Bou-Hee Kang · Kenneth Racicot · Sarah J. Pilkenton · Young-In Kwon · Emmanouil Apostolidis

Abstract This study investigates the carbohydrate-hydrolyzing inhibitory potential of blueberry extract on carbohydrate-hydrolyzing enzymes and evaluates if the inhibitory activity is proanthocyanidin (PAC) or lower molecular weight phenolic dependent. Freeze-dried blueberry powder was extracted using acetone and subjected to C_{18} extraction (BAE). Low-molecular weight phenolics (BAE-LMW) and PACs (BAE-PAC) were separated from BAE with gel filtration chromatography using LH-20 column. Total phenolic content, PAC content, and phenolic profiles using HPLC, as well as rat α-glucosidase, sucrase, and maltase inhibitory activities, were determined for all samples. The rat alpha-glucosidase inhibitory activity of BAE (IC_{50} 0.390 mg/mL TP basis) was enhanced in BAE-LMW (IC_{50} 0.242 mg/mL TP basis) and reduced in BAE-PAC (IC_{50} 0.915 mg/mL TP basis). Similar trends were observed with maltase and sucrase inhibitory activities.

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Our findings suggest that blueberry acetone extract has inhibitory activity on carbohydrate-hydrolyzing enzymes and this effect is dependent on LMWs rather than PAC.

Keywords Alpha-glucosidase inhibition · Blueberry · Maltase inhibition · Proanthocyanidins · Sucrase inhibition

Introduction

Type 2 diabetes, once called non-insulin-dependent diabetes mellitus (NIDDIM), is a metabolic disorder that is characterized by high level of glucose in the blood. The disease is associated with high calorie diets as well as the consumption of high calorie sweetened foods. Worldwide, 347 million people have diabetes (WHO 2013) resulting in immense health care costs (ADA 2013a). Type 2 diabetes accounts for 90 % of diabetes cases (WHO 2013). In 2011, 79 million people in U.S.A. aged 20 years or older were classified as prediabetic (CDC 2013). The American Diabetes Association defines prediabetic individual as an individual with blood glucose levels higher than normal (impaired fasting glucose between 100 and 125 mg/dL, impaired glucose tolerance between 140 and 199 mg/dL, and HbA1c between 5.7 and 6.4 %) but not high enough to be considered diabetic (impaired fasting glucose between >126 mg/dL, impaired glucose tolerance between >200 mg/dL, and HbA1c between >6.5 %) (ADA 2013b). α-Glucosidase inhibitors, such as acarbose, are the only oral anti-diabetes agent approved for the treatment of pre-diabetes (Hanefeld 2007). Briefly, lower doses of acarbose have shown to have beneficial effect toward pre-diabetes management by delaying the absorption of carbohydrates from the gut (Hanefeld 2007). Taking into consideration that pre-diabetes is not a disease would be more desirable

to find natural components capable of reducing glucose absorption in the small intestinal environment, via inhibition of carbohydrate-hydrolyzing enzymes.

Dietary carbohydrates are digested by carbohydrate-hydrolyzing enzymes, resulting in monosaccharides, which are then absorbed through the small intestine. The carbohydrate-hydrolyzing enzymes break down polysaccharides, oligosaccharides, and disaccharides to monosaccharides. Alpha-glucosidases are expressed in small intestine and are a group of enzymes that hydrolyzes disaccharides such as sucrose, maltose, and lactose to monosaccharides. Therefore, inhibition of alpha-glucosidases (sucrase and maltase) can be an effective strategy to reduce the glucose absorption in the digestive tract (Krentz and Bailey 2005). It has been reported that certain natural products are potentially good inhibitors of carbohydrate-hydrolyzing enzymes with their effect being attributed to the unique phenolic phytochemicals (Kwon et al. 2006, 2007; Apostolidis et al. 2012).

Blueberries are great sources of various phenolic phytochemicals (Castrejón et al. 2008). Major cities of highbush blueberry production in USA include California, Mississippi, North Carolina, Oregon, and Washington (Strik and Yarborough 2005). More than half of all highbush blueberries are shipped to the fresh market, to keep pace with the ever-increasing demand. The rest are frozen, pureed, concentrated, canned, or dried to be used in a wide range of food products including yogurt, pastries, muffins, cereals, and health bars (U.S High Blueberry Council 2012). Previous reports evaluated a wide variety of different phenolic-derived blueberry health benefits. Some of the phenolic-derived health benefits of blueberries include anti-cancer activity(Adams et al. 2010), heart disease prevention (Basu et al. 2010), antioxidant activity (Wang et al. 2008), and anti-inflammatory effect (Paul et al. 2009). In addition, an animal trial using low-bush blueberries demonstrated that the phenolic-enriched fraction can enhance insulin sensitivity (Grace et al. 2009), which in turn can help manage Type 2 diabetes. A recent animal trial using obese and hyperglycemic C57BL/6 mice showed that administration of defatted soybean flour fortified with blueberry phenolics for 13 weeks reduces weight gain, improves glucose tolerance, and lowers fasting blood glucose levels (Roopchard et al. 2012). Another preliminary study showed that both high-bush and low-bush blueberries have a phenolic-dependent alpha-glucosidase inhibitory activity (Wang et al. 2012).

Phenolic phytochemicals, secondary metabolites of plant origin, are important components of human diet. Major phenolic compounds present in blueberries include phenolic acids (such as chlorogenic acid) (Gavrilova et al. 2011), anthocyanins (Hellstrom et al. 2007), and proanthocyanidins (Hellstrom et al. 2007). Proanthocyanidins

(PACs) are condensed tannins which consist of oligomeric and/or polymeric flavans and flavan-3-ols. PACs can be classified as A-type or B-type depending on two interflavan linkages (C–C and C–O). Blueberries contain both A- and B-type PACs. B-type PACs in blueberry consist of dimers and higher oligomers of flavan-3-ol units linked through the C4–C8 bond and/or the C4–C6 bond (Hellstrom et al. 2007). A-type PACs which are abundant in blueberries are flavan-3-ol units linked by additional ether bond between C2 and O7 (Gu et al. 2003) with B-type.

It has been reported that blueberries have potential for type 2 diabetes prevention via carbohydrate-hydrolyzing enzyme inhibition (Wang et al. 2012). However, the source of the responsible bioactivities is not clear. Identification of the bioactive fraction for the inhibition of carbohydrate-hydrolyzing enzymes will assist in better understanding of blueberries utilization for the prevention of type 2 diabetes. The aim of this study was (1) to evaluate the inhibitory activity of blueberry extract against rat alpha-glucosidase, sucrase, and maltase and (2) to determine the true source of the responsible bioactivities between PACs and LMW phenolics.

Materials and methods

Freeze-dried whole blueberry powder was obtained from the U.S. Highbush Blueberry Council (Foslom, CA). The freeze-dried powder was kept at −4 °C until use. All solvents used for research were of either ACS or HPLC grade and were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Rat intestinal acetone powder and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation

Five grams of blueberry powder were extracted with 100 mL of acetone solution which contained acetone, deionized water, and hydrochloric acid in the following volume ratio 70:29.9:0.01 for 2 h at room temperature with continuous stirring. After the extraction with acetone solution, extracts were vacuum filtered through a Whatman #2 (Whatman Inc.; Florham, NJ, USA) and evaporated to a final volume of 25 mL by a rotary evaporator (Fisher Scientific; Hanover Park, IL).The extract was stored at −4 °C until further testing.

C_{18} cartridge extraction

The resulting extract (25 mL) was subjected to solid phase extraction using C_{18} cartridge (DSC-18, 3 mL Tube, 500 mg; Supelco, Bellefonte, PA, USA). A subsample (2 mL) of the extract was loaded onto each pre-conditioned C_{18} cartridge,

followed by rinsing with deionized water to eliminate hydrophilic compounds. After washing, the hydrophobic compounds (including phenolic compounds) were eluted with 5 mL of 99:1 (v/v) methanol:acetic acid solution. The solvent was evaporated from the hydrophobic fraction using a rotary evaporator (Fisher Scientific; Hanover Park, IL, USA) at 60 °C to yield a final volume of 25 mL. The evaporated extract was stored at −4 °C until further testing.

LH-20 column extraction

Roto-evaporated acetone extract was subjected to gel filtration chromatography using LH-20 (Sigma Lipophilic Sephadex; St. Louis, MO, USA) column extraction to separate the low-molecular weight phenolic compounds from the high-molecular weight PACs. Five mL each of concentrated extracts was loaded onto 40 mL LH-20 column, which has been conditioned by washing with 200 mL of 30 % methanol. The column was washed with 500 mL of 30 % methanol to collect low-molecular weight phenolic compounds (such as phenolic acids, anthocyanin, and flavonoids) and with 150 mL of acetone:deionized water:acetic acid (70:29.9:0.01) (v/v), the PAC-containing fraction was eluted. Both resulting fractions were roto-evaporated by vacuum rotor evaporator (Fisher Scientific; Hanover Park, IL, USA) to final volume of 25 mL and then stored at 4 °C until further testing.

Total phenolic content determination

Total phenolic content of all extracts was determined by Folin–Ciocalteau's reagent (Sigma-Aldrich; St. Louis, MO, USA). Briefly, 1 mL of sample or standard was mixed with 1 mL of 95 % ethanol, 5 mL deionized water, and 1 mL of 1 N Folin–Ciocalteu reagent (Sigma-Aldrich; St. Louis, MO, USA) in test tubes. Then the tubes were incubated at room temperature for 5 min. After adding 1 mL of 5 % Na_2CO_3 into mixture, the mixture was stored at room temperature for 60 min in the absence of light. The absorbance was measured at 725 nm (U 2001 Spectrophotometer; Hitachi, Pleasanton, CA, USA) after vortexing. Gallic acid standard was prepared (15.625, 31.25, 62.5, 125, 250, and 500 μg/mL in ethanol) and used to establish the standard curve. Results were expressed as mg of gallic acid equivalents per gram of sample (DW).

Proanthocyanidin content determination

Total PAC contents were determined by the use of 4-dimethylaminocinnamaldehyde (DMAC) (Sigma-Aldrich; St. Louis, MO, USA). DMAC reagent was prepared with 1:1 6 N H_2SO_4 and 2 % DMAC (w/v) in methanol. 20 μL of samples/standard, 2,380 μL of methanol, and 100 μL of

DMAC reagent were added in cuvettes. The mixture was incubated at room temperature for 20 min. The absorbance of the mixture was observed at 640 nm (U 2001 Spectrophtometer; Hitachi, Pleasanton, CA, USA). The calibration curve was established using PAC type A standards (Chromadex; Irvine, CA, USA) at concentrations of 0, 30, 50, 80, and 100 μg/mL in ethanol. Results were expressed as mg of PAC type A equivalents per 1 mL of sample.

Enzyme mixture preparation

All samples were dissolved in water. A total of 0.3 g rat intestinal acetone powder was mixed with 9 mL of 0.9 % saline and sonicated (1510E-DTH12; Baransonic, Danbury, CT, USA) 12 times for 30 s at 4 °C. After centrifugation (10,000g, 20 min, 4 °C) (Accuspin Micro 17R; Fisher Scientific, Hanover Park, IL, USA) of the mixture, the supernatant was used as an enzyme mixture. 50 μL of sample or buffer and 100 μL of the enzyme mixture were added into a 96 microplate well.

Rat α-glucosidase inhibitory activity

After incubation at 37 °C for 10 min, 50 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) in 0.1 M sodium phosphate buffer (pH 6.8) was added into each microplate well. In blank, 0.1 M sodium phosphate buffer (pH 6.8) was added for substrate instead of 5 mM pNPG. After
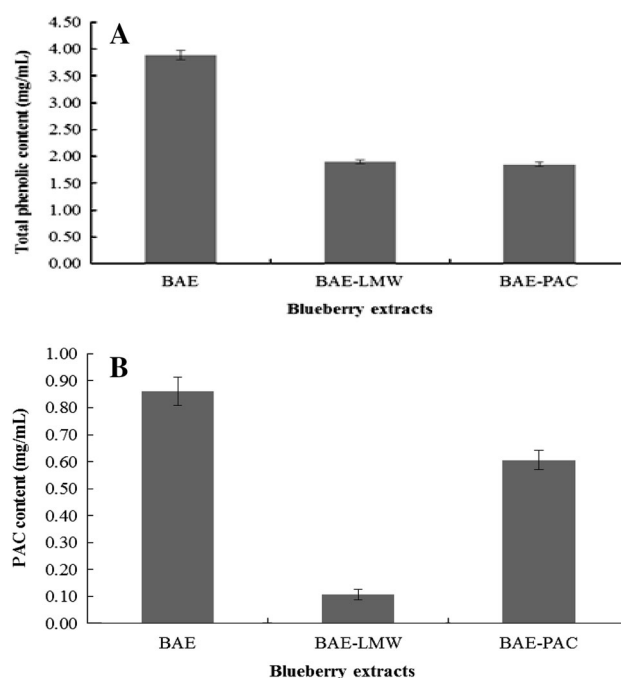
Fig. 1 Total phenolic contents (**a**) and PAC contents (**b**) of blueberry extract and resulting fractions

incubation for 30 min, the absorbance of each mixture was measured at 405 nm and compared to the control using a microplate reader (Molecular Devices; Thermo max, Ramsey, MN, USA). Rat α-glucosidase inhibitory activity was expressed by inhibition % and calculated as follows:

$$\text{Inhibition } (\%) = \left(\left[\frac{\Delta A_{405}^{\text{Control}} - \Delta A_{405}^{\text{Extract}}}{\left[\Delta A_{405}^{\text{Control}} \right]} \right] \right) \times 100.$$
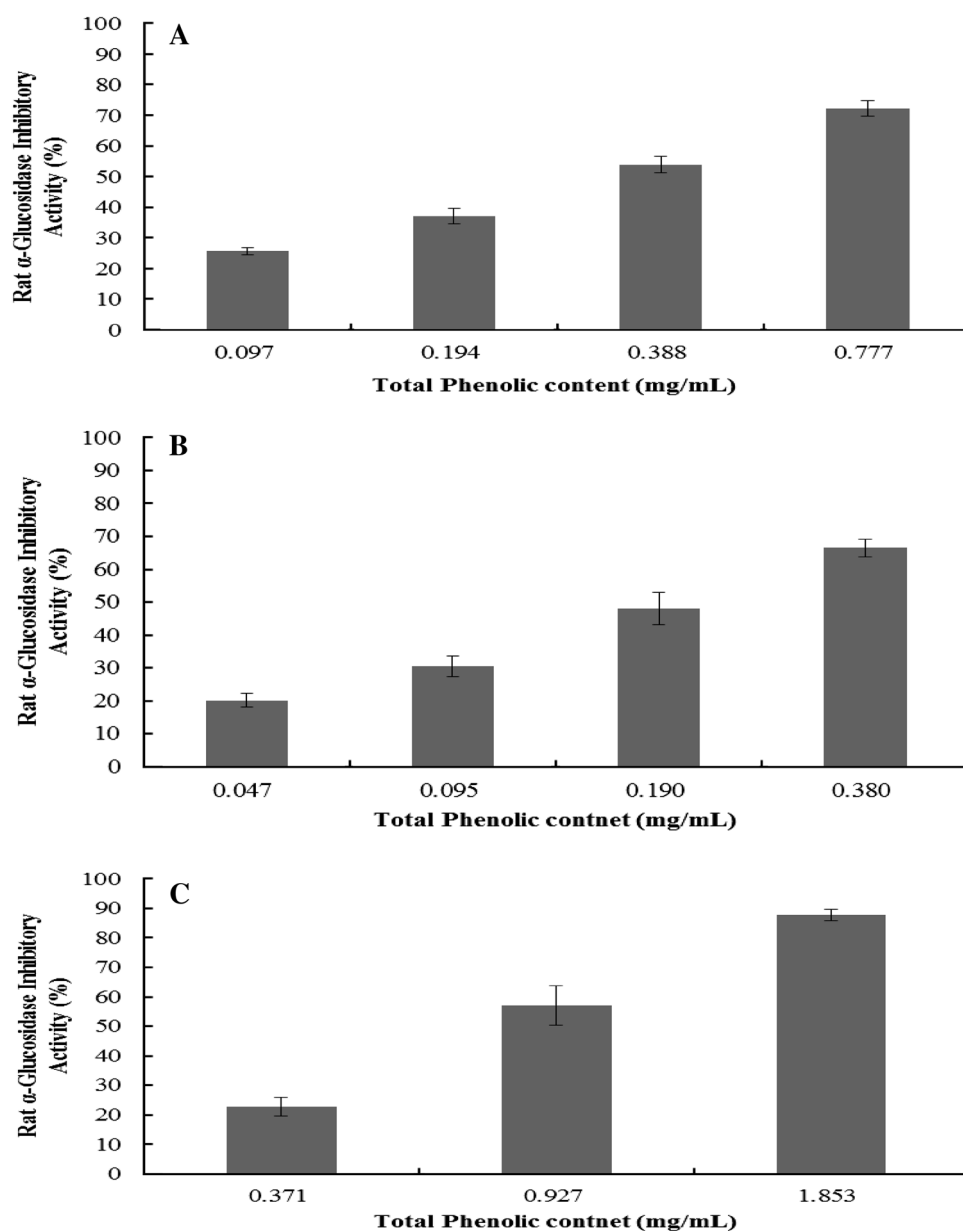
Maltase inhibitory activity

After incubation at 37 °C for 10 min, 50 μL of 200 mM maltose in 0.1 M sodium phosphate buffer was added into the microplate well. In blank, 0.1 M sodium phosphate buffer was added for substrate instead of 100 mM maltose solution. After incubation at 37 °C for 30 min, 10 μL of the reaction mixture was transferred into pre-incubated 200 μL glucose oxidase/peroxidase reagent for 10 min at 37 °C. After incubation, the reaction was stopped by the addition of 200 μL of 12 N H_2SO_4. The absorbance was measured at 540 nm using a microplate reader (Molecular Devices; Thermo max, Ramsey, MN, USA). Maltase inhibitory activity was expressed by inhibition % and calculated as follows:

$$\text{Inhibition } (\%) = \left(\left[\frac{\Delta A_{540}^{\text{Control}} - \Delta A_{540}^{\text{Extract}}}{\left[\Delta A_{540}^{\text{Control}} \right]} \right] \right) \times 100.$$

Fig. 2 Dose-dependent rat α-glucosidase inhibitory activities of blueberry extracts on total phenolic content basis. a BAE, b BAE-LMW, and c BAE-PAC
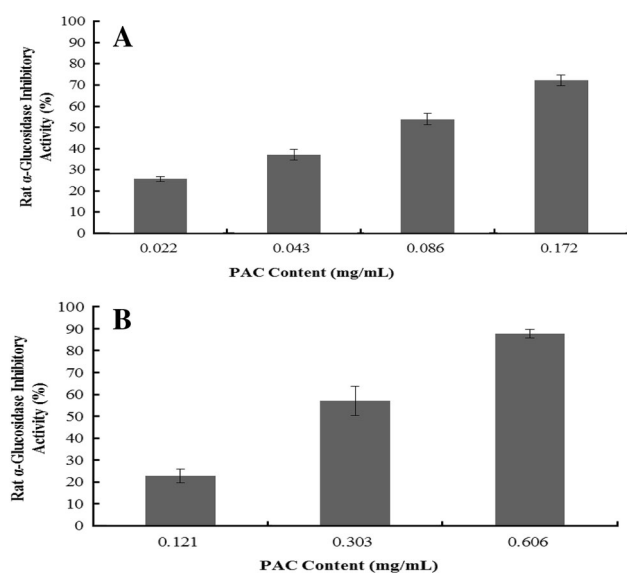
Fig. 3 Dose-dependent rat α-glucosidase inhibitory activities of blueberry extracts on PAC content basis. **a** BAE, **b** BAE-PAC

Table 1 IC_{50} values of blueberry extract and fractions against rat α-glucosidase, maltase, and sucrase (TP and PAC basis)

IC_{50} (mg/mL)	Acetone (extract)	Low MW fraction	PAC fraction
Rat α-glucosidase IC_{50}			
T.P	0.390	0.242	0.915
PAC	0.090	N/A	0.299
Maltase IC_{50}			
T.P	0.124	0.070	>1.853
PAC	0.026	N/A	>0.303
Sucrase IC_{50}			
T.P	0.027	0.016	0.889
PAC	0.006	N/A	0.291

Sucrase inhibitory activity

After incubation at 37 °C for 10 min, 50 μL of 200 mM sucrose in 0.1 M sodium phosphate buffer was added into the microplate well. In blank, 0.1 M sodium phosphate buffer was added for substrate instead of 200 mM sucrose solution. After incubation at 37 °C for 30 min, 25 μL of the reaction mixture was transferred into pre-incubated 100 μL glucose oxidase/peroxidase reagent for 10 min at 37 °C. After incubation, the reaction was stopped by the addition of 100 μL of 12 N H_2SO_4. The absorbance was measured at 540 nm using a microplate reader (Molecular Devices; Thermo max, Ramsey, MN, USA). Sucrase inhibitory activity was expressed by inhibition % and calculated as follows:

$$\text{Inhibition}\ (\%\ ) = \left(\left[\frac{\Delta A_{540}^{\text{Control}} - \Delta A_{540}^{\text{Extract}}}{\left[\Delta A_{540}^{\text{Control}} \right]} \right] \right) \times 100.$$

Phenolic profiling using HPLC

All samples were filtered using a 0.45 μm Fisherbrand® syringe filter (Thermo Fisher Sci, Pittsburg, PA). An injection volume of 20 μL of each sample was injected and analyzed using a reverse phase C-18 column (Agilent ZORBAX Extend C-18 column, 250 × 4.6 mm id.d, 5 μm particle size). The mobile phase consisted of solvent A (4 % phosphoric acid) and solvent B (acetonitrile). Gradient elution was used under linear gradient conditions starting with 95 % A, decreasing to 65 % A over a 70-min time period at a flow rate of 0.75 mL/min. Phenolic and anthocyanin profiles were observed at 254 and 520 nm, respectively.

Statistical analysis

All experiments were performed at least three times in triplicates. Analysis at every time point from each experiment was carried out in triplicates. Means, standard errors, standard deviations, and IC_{50} values were calculated using Microsoft Excel XP.

Results and discussion

Total phenolic content

The total phenolic content (Fig. 1a) of the resulting blueberry extracts was evaluated using Folin–Ciocalteau's assay. In blueberry extracts, the total phenolic contents of BAE, BAE-LMW and BAE-PAC were determined to be 3.88, 1.90, and 1.85 mg/mL, respectively. The total phenolic content results indicate that blueberry acetone extract contains both low MW phenolic compounds as well as PACs. Although the phenolic contents were similar between the BAE-LMW and BAE-PAC, the phenolic profiles were considered to be different. Demiray et al. (2009) previously reported that acetone is an appropriate solvent to extract phenolic compounds and that elution solvents used in LH-20 column can affect the profile of received fractions.

Proanthocyanidin content

The PAC content was determined in BAE, and the resulting BAE-PAC and BAE-LMW using the DMAC assay. Our findings show that the majority of PACs were eluted in the BAE-PAC fraction (Fig. 1b). More specifically, the

Fig. 4 Dose-dependent maltase inhibitory activities of blueberry extracts on total phenolic content basis. **a** BAE, **b** BAE-LMW, and **c** BAE-PAC
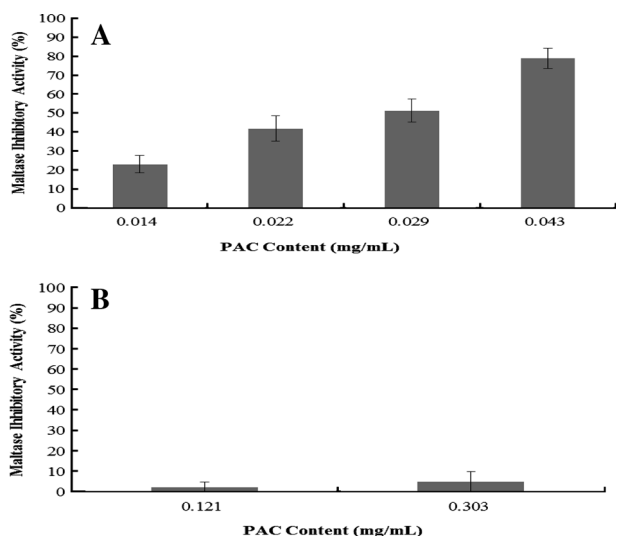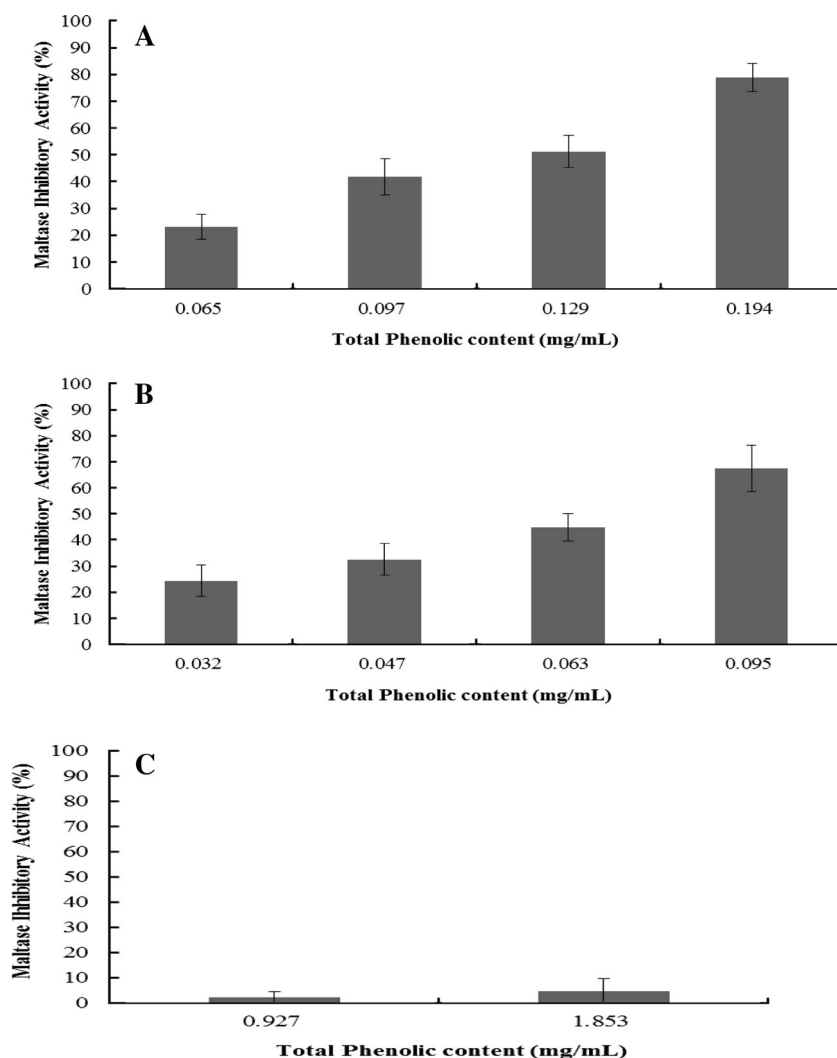
Fig. 5 Dose-dependent maltase inhibitory activities of blueberry extracts on PAC content basis. **a** BAE, **b** BAE-PAC


determined PAC contents were 0.86 mg/mL PAC A2 eq., 0.11 mg/mL PAC A2 eq. and 0.61 mg/mL PAC A2 eq., for BAE, BAE-LMW and BAE-PAC samples, respectively (Fig. 1b).

Previous reports demonstrated that LH-20 column is a suitable method for PAC purification (Jarkko and Pirjo 2008; Lee 2013). Lee (2013), evaluated five methods for PAC purification, and the results showed that size exclusion resins such as Toyopearl 40C and Sephadex LH-20 were the most suitable to purify PACs. Our findings are in agreement with previous reports, supporting that LH-20 column is suitable for PAC purification.

Rat α-glucosidase inhibitory activity

The dose-dependent rat α-glucosidase inhibitory activities of BAE, BAAE-LMW, and BAE-PAC were investigated (Figs. 2, 3). For comparison purposes and to determine

Fig. 6 Dose-dependent sucrase inhibitory activities of blueberry extracts on total phenolic content basis. **a** BAE, **b** BAE-LMW, and **c** BAE-PAC
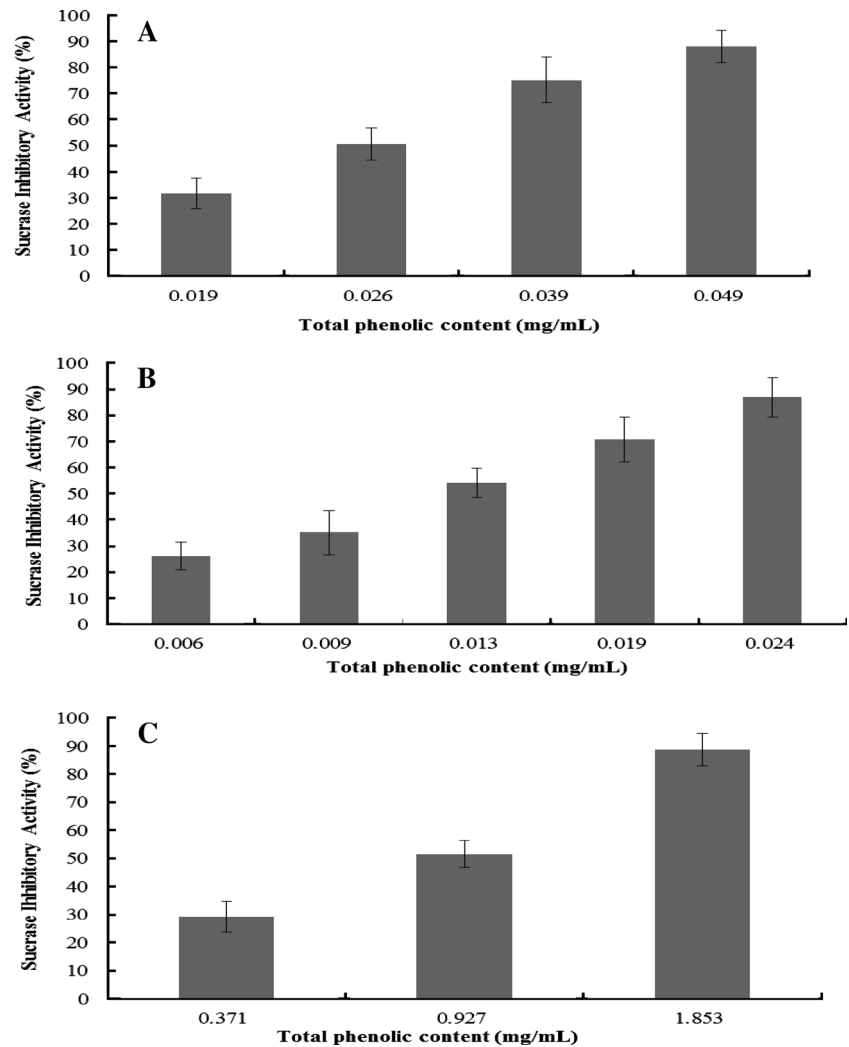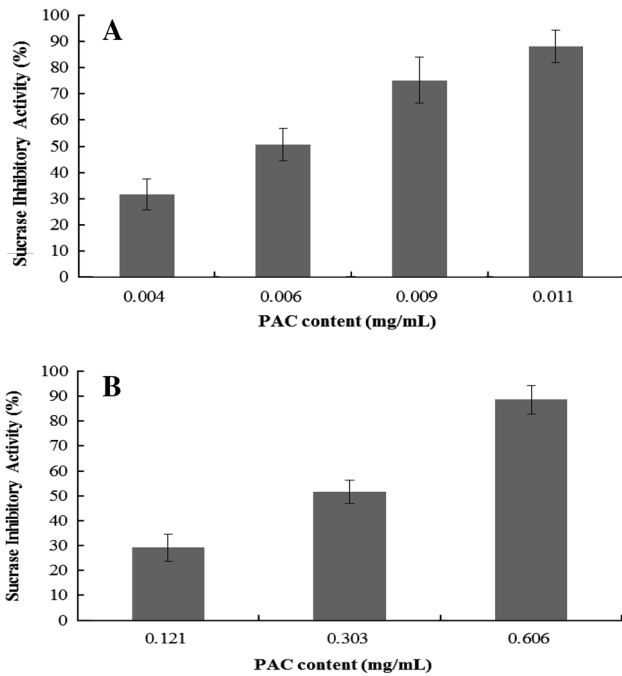

whether the observed bioactivity is PAC dependent, the inhibitory activities were expressed either based on total phenolic or PAC contents. Due to the low PAC contents observed in BAE-LMW fraction (Fig. 1b), the inhibitory activity of this fraction was not expressed based on PAC content. Our observations suggest that at the tested doses, all samples had significant rat alpha-glucosidase inhibitory activity (Figs. 2, 3). However, to assess the efficacy of the inhibition and understand whether the observed effect is PAC or low-molecular weight phenolic dependent, the IC_{50} values on both total phenolic basis and PAC basis were determined (Table 1). It was determined that the IC_{50} values for BAE were 0.390 mg/mL (TP basis) and 0.090 mg/mL (PAC basis) (Table 1). BAE-LMW fraction had higher inhibitory activity with IC_{50} value of 0.242 mg/mL (TP basis), while the BAE-PAC fraction had reduced inhibitory activity with observed IC_{50} values of 0.915 mg/mL (TP basis) and 0.299 mg/mL (PAC basis) (Table 1).

Our findings suggest that the observed rat alpha-glucosidase inhibitory activity is not PAC dependent, since the PAC basis IC_{50} value of BAE-PAC is higher when compared to the BAE (Table 1). The results also indicated that the observed inhibitory activity is dependent on lower molecular weight phenolics, based on the lower TP basis IC_{50} value of BAE-LWM fraction than that of BAE (Table 1). This is probably because non-bioactive higher MW phenolics have been removed and absent in BAE-LMW.
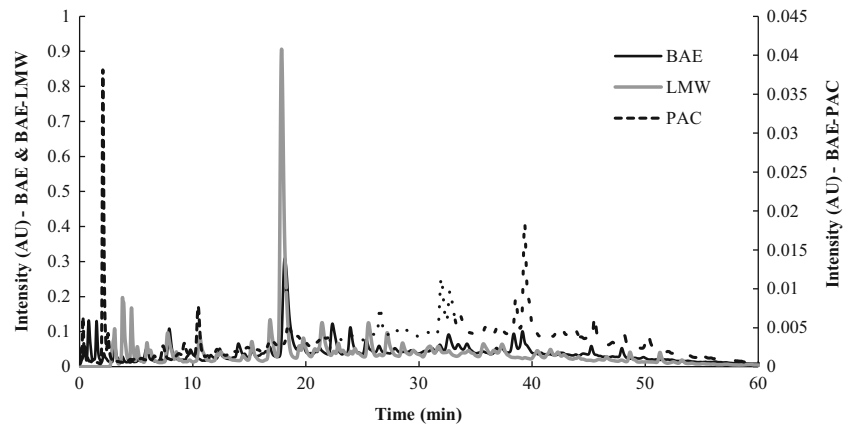
It is well documented that phenolic phytochemicals have alpha-glucosidase inhibitory activity that depends on the phenolic profile (Kwon et al. 2006, 2007; Apostolidis et al. 2012). A recent study reported that cinnamon extract has shown non-PAC-dependent rat alpha-glucosidase inhibitory activity (Kang et al. 2014). Our observations were consistent with cinnamon, showing the inhibitory activity of blueberry acetone extract on rat alpha-glucosidase, which is attributed to lower molecular weight phenolic phytochemicals with no
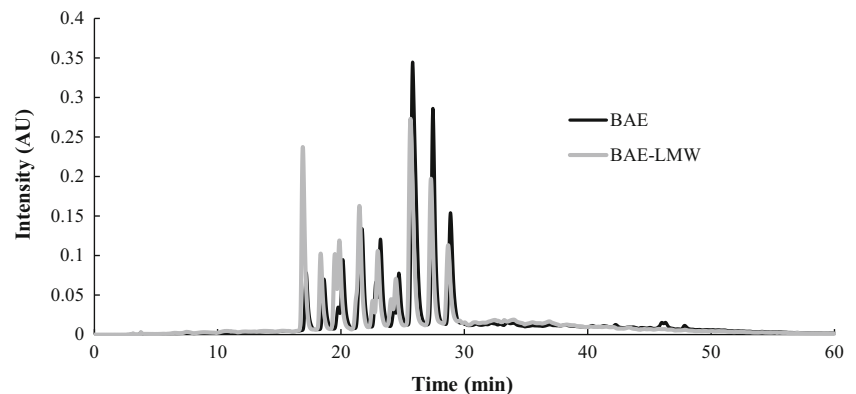
Fig. 7 Dose-dependent sucrase inhibitory activities of blueberry extracts on PAC content basis. a BAE, b BAE-PAC

PAC dependency. To further confirm this observation, the specific sucrase and maltase inhibitory activities of BAE, BAE-LMW, and BAE-PAC were also investigated.

Maltase inhibitory activity

The dose-dependent maltase inhibitory activities of BAE, BAE-LWM, and BAE-PAC were evaluated as outlined in materials and methods. At the tested doses, only BAE and BAE-LMW extracts showed significant inhibitory activity (Figs. 4, 5). The IC$_{50}$ values were evaluated for tested samples, on both total phenolics and PAC basis (Table 1). It was determined that the IC$_{50}$ values for BAE were 0.124 mg/mL (TP basis) and 0.026 mg/mL (PAC basis) (Table 1). As observed with alpha-glucosidase, BAE-LMW fraction had higher inhibitory activity with IC$_{50}$ value of 0.070 mg/mL (TP basis), while the BAE-PAC fraction had very low inhibitory activity resulting in IC$_{50}$ values of >1.853 mg/mL (TP basis) and >0.303 mg/mL (PAC basis) (Table 1).

These observations of maltase inhibitory activity in blueberry extracts confirm the findings from rat α-gluco-

Fig. 8 HPLC phenolic profiles of BAE, LMW, and PAC at 254 nm

Fig. 9 HPLC phenolic profiles of BAE and BAE-LMW at 520 nm

sidase inhibitory activity, since BAE-PAC fraction has no inhibitory activity at the tested doses. In addition, the BAE-LMW fraction had higher inhibitory activity on TP basis, when compared to that of BAE. These findings suggest that the observed maltase inhibitory activity of BAE is attributed to lower molecular weight phenolics.

Sucrase inhibitory activity

The dose-dependent maltase inhibitory activities of BAE, BAE-LWM, and BAE-PAC were evaluated as outlined in materials and methods. All evaluated samples showed significant inhibitory activity at the tested doses (Figs. 6, 7). The IC_{50} values for BAE were 0.027 mg/mL (TP basis) and 0.006 mg/mL (PAC basis) (Table 1). As observed with alpha-glucosidase, BAE-LMW fraction had higher inhibitory activity with an IC_{50} value of 0.016 mg/mL (TP basis), while the BAE-PAC fraction had reduced inhibitory activity with observed IC_{50} values of 0.889 mg/mL (TP basis) and 0.291 mg/mL (PAC basis) (Table 1).

These observations confirm our previous findings that the observed inhibitory activity of blueberry extract on carbohydrate-hydrolyzing enzymes depends on low-molecular weight phenolic compounds. It is also noteworthy that the observed sucrase inhibitory activities with BAE and BAE-LMW were significantly higher than those on maltase (Table 1). This suggests that the observed carbohydrate-hydrolyzing enzyme inhibitory activity of blueberry acetone extract is mainly due to sucrase inhibition.

HPLC phenolic profile

The phenolic profiles of BAE, BAE-LMW, and BAE-PAC at different wavelengths (254 and 520 nm) were determined as described in the materials and methods. Our results showed as expected different phenolic profiles between the evaluated samples (Figs. 8, 9). More specifically, we observed that the majority of phenolic compounds present in BAE eluted in the BAE-LMW fraction. In the BAE-LMW fraction, the major peak was eluted around 17 min and the vast majority of other compounds eluted between 20 and 30 min (Fig. 8). The compound eluted at 17 min had a λmax at 254 nm, suggesting that it is a phenolic acid, while the compounds eluted between 20 and 30 min had λmax at 520 nm, suggesting that they are anthocyanins (Fig. 9). In the case of BAE-PAC fraction, the observed compounds eluted around 26, 31, 32, 38, and 39 min (Fig. 8). All these compounds were absent in the BAE-LMW fraction but present in the BAE phenolic profile.

Based on our in vitro and HPLC observations, we can suggest that the bioactive components of blueberry acetone extract are considered to be either phenolic acids or anthocyanins, both of which are present in the BAE-LMW fraction. Further fractionation of the BAE-LMW is underway to separate and characterize phenolic acids and anthocyanins responsible for the observed effect.

In conclusion, our findings suggest that the inhibitory potential of blueberry extract against carbohydrate-hydrolyzing enzymes is shown to be attributed to low-molecular weight phenolics rather than PACs. Specifically phenolic acids and anthocyanins present in blueberries are considered to contribute to the bioactivity. Finally, we observed that blueberry extracts have shown higher inhibitory activity on sucrase, with much less on maltase. Our current findings provide a sound rationale for further fractionation, characterization, and evaluation of the low-molecular weight phenolic compounds present in blueberries, which will assist in better understanding the potential of blueberry bioactives for type 2 diabetes prevention. The ultimate goal of this work will be the development of the appropriate extraction and standardization procedures for the manufacture of a blueberry extract for type 2 diabetes prevention, to be used either in dietary supplements or food products.

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