The Investigation on α-glucosidase Inhibitory from Legume Extracts

Abstract

Type 2 diabetes (T2DM) is a public health problem, which increased rapidly, especially in developing country. The cause of T2DM is suggested as irregular in carbohydrate metabolism. This study aimed to investigate the α-glucosidase inhibitory activities and determine the effect of cooking process using different types of legumes, including mung bean, soybean, black bean, red kidney bean and peanut. All samples (1 g) were extracted with 70% (v/v) aqueous ethanol (20 mL) and determined the α-glucosidase inhibitory activity using p-nitrophenol-α-D-glucopyranoside (0.5 mM). As results, the raw legumes exhibited higher α-glucosidase inhibitory activities than cooked legumes (p<0.05), suggesting that anti-α-glucosidase agents from legumes might be destroyed during cooking process. Raw peanut exhibited the highest inhibitory activity, followed by soybean, red kidney bean, white bean, black bean and mung bean, respectively. On the other hand, cooked red kidney bean exhibited the highest inhibitory activity, followed by white bean, mung bean, peanut, soy bean and black bean, respectively. The information received from this study could promote health benefit of legumes regarding the control of T2DM through α-glucosidase inhibitory pathway.

Keywords: diabetes, α-glucosidase inhibition, legume extract
Introduction

Prevalence of diabetes in adults was estimated to increase up to 5.4% worldwide in year 2025 (King et al., 1998). Main causes of diabetes are associated with irregular insulin action, insulin secretion and endogenous glucose output (Weyer et al., 1999). Pharmacological intervention for diabetic treatment involves inhibition of enzymes that control carbohydrate degradation (Inzucchi, 2002). The enzyme, α-glucosidase, can hydrolyzed carbohydrate into disaccharide and monosaccharide; therefore, inhibition of this enzyme would prevent carbohydrate digestion and glucose absorption. These, in turn, could retard and deduce the level of postprandial hyperglycemia after the meal as well as decrease triglyceride synthesis in adipose tissue, liver and intestinal wall (Puls et al., 1977).

Recently, legumes have been reported as excellent sources of diverse phytochemicals with potential efficacy for prevention or treatment of various diseases. Previous studies have suggested that high antioxidant activities and total phenolic compounds (TPCs) were found in pigmented legumes such as black and red beans (Amarowicz and Pegg, 2008). Besides, cooking process could as well affect phytochemicals and antioxidant activity (Chipurura et al., 2010). Since α-glucosidase can be inactivated by several phytochemicals such as flavonoids and phenolics, the compounds that are rich in legumes, it is highly possible that legumes can provide negative effect towards α-glucosidase activity. Nevertheless, the information regarding control of diabetes through α-glucosidase inhibitory activity of legumes is limited. Therefore, the aim of this study was to determine the α-glucosidase inhibitory activities from legumes regarding the effects of legume types (colors) and heat treatment by cooking process.

Materials and Methods

All legumes including peanut, mung bean, black bean, red kidney bean, white bean and soybean were purchased from local market under the brand names, Khaothong (Thai Food Industry (1964) Co., Ltd, Bangkok, Thailand) and Raitip (Thai cereals world (1957) Co., Ltd, Bangkok, Thailand). Cooked legumes were prepared by boiling legumes (1:2 w/v) at 96-98°C for 20 minutes before freeze-drying. Cooked and raw legumes were then ground into fine powder by a cyclotex sample mill (series 1903 with 200 50/60 Hz from FOSS, Höganäs, Sweden) until the particle size was approx. 80 sieve (0.18 mm). The moisture content was calculated prepared by boiling legumes (1:2 w/v) at 96-98°C for 20 minutes before freeze-drying. Cooked and raw legumes were then ground into fine powder by a cyclotex sample mill (series 1903 with 200 50/60 Hz from FOSS, Höganäs, Sweden) until the particle size was approx. 80 sieve (0.18 mm). The moisture content was calculated using AOAC method (AOAC, 2000) and was found to be in a range of 5-8%. Legume powder (1 g) was extracted with 70% (v/v) aqueous ethanol (20 mL) for 2 hours at 30°C with shaking at 100 rpm. The extractant was filtered through Whatman No.1 filter paper and stored at 4 °C for further analysis.

The determination of α-glucosidase inhibitory activity was performed according to the method of You et al. (2011). The enzyme assay consisted of Saccharomyces cerevisiae α-glucosidase (0.1 U/mL), p-nitrophenol-α-D-glucopyranoside (pNPG, 0.5 mM) and legume extract (12.5 mg/mL) in 50 mM potassium phosphate buffer (KPB, pH 7.0). The reaction was monitored at a wavelength of 405 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT) and a Gen5 data analysis software. The results were calculated as a percentage of inhibition using the equation; %inhibition = 100 x (1-((B-b)/(A-a))), where A was the initial velocity of the reaction mixture using the enzyme with substrate, a was the initial velocity of the reaction mixture using the substrate only, B was the initial velocity of the reaction mixture using the enzyme with substrate and legume extracts, and b was initial velocity of the reaction mixture using substrate and legume extracts. All data were expressed as mean of triplicate experiments (n=3) ± standard deviation (SD). One way analysis of variance (ANOVA) and Tukey’s multiple comparison tests were performed to determine the significant differences at p<0.05. The significant difference in the effect of cooking was determined by independent t-test group with p<0.05. All statistical analyses were carried out using SPSS statistical analysis (version 16 for Windows, SPSS Inc., Chicago, USA).

Results

As results, raw legumes exhibited higher percentage of inhibition than cooked legumes (Figure 1). Among all raw legumes, peanut exhibited the highest α-glucosidase inhibitory activity (60.63% inhibition),
followed by soybean, red kidney bean, white bean, black bean and mung bean, respectively. However, in cooked legumes, red kidney bean exhibited the highest α-glucosidase inhibitory activity (37.28% inhibition), followed by white bean, mung bean, peanut, soybean and black bean, respectively.

Discussion

Peanut has been reported to contain many phytochemicals such as trans-resveratrol, phytosterols, isoflavones, genistein and daidzein (Isanga and Zhang, 2007). A predominant polyphenol found in peanut is p-coumaric acid, which is accounting for 40–68% of the total phenolics present (Seo and Morr, 1985; Mattila and Hellstrom, 2007; Phan-Thien et al., 2014). Previous research also suggested that p-coumaric acid content was correlated with potential inhibition against α-glucosidase with an IC₅₀ (inhibition constant at 50% inhibition) of 0.004 mM (Wongsara et al., 2012). Comparing to the IC₅₀ of acarbose (0.42 mM), an anti-diabetic agent. Under the same studied condition, p-coumaric acid is considered as a very effective α-glucosidase agent. Besides, resveratrol (3,5,4‘-trihydroxy-trans-stilbene) that is abundantly found in peanut (84 μg/100 g) exhibited IC₅₀ <0.1 mM against α-glucosidase (Chen and Blumberg 2008; Kerem et al., 2006). Thus, high anti-α-glucosidase activity in peanut might be a result of abundant p-coumaric acid and resveratrol contents.

Red kidney bean was previously reported to be able to decrease rate of carbohydrate digestion through inhibition of α-amylase by a particular protein that acts as α-amylase inhibitor. It is thought that this inhibitor may reduce calorie intake by possibly preventing the complex carbohydrate digestion, thereby promoting weight loss. However, the inhibition of α-glucosidase that functions similarly to α-amylase in term of carbohydrate digestion has not been investigated yet. Red kidney bean has been reported to contain five major anthocyanins, including cyanidin-3,5-diglucoside, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside and pelargonidin-3-glucoside with delphinidin-3-glucoside being the most abundantly found (Choung et al., 2003). It was previously found that cyanidin-3,5-diglucoside, delphinidin-3-glucoside and some acylated anthocyanin derivatives could inhibit α-glucosidase (Matsui et al., 2001; You et al., 2011; Rojo et al., 2012). Thus, α-glucosidase inhibitory activity in red kidney bean might be come from these bioactive compounds.

Besides, raw legumes exhibited higher inhibitory activity than cooked legumes in all cases. It was possible that phenolics contained seed coat of legumes are destroyed during heat treatment (Vadivel et al., 2012). For example, the decrease in anti-α-glucosidase activity in peanut might be due to stability of its bioactive compounds, p-coumaric acid and resveratrol. The former was undergone thermodegradation, in which it was continuing destroyed under 75°C, while the later was found to be heat stable. Likewise, anthocyanins in red kidney bean have been reported to be sensitive toward heat treatment (Xu and Chang, 2009). It was also previously reported that boiling and soaking processes could cause decreased anthocyanin contents and total phenolics by oxidative degradation, release of free phenolic acids from conjugate forms, and formation of complex structure of phenolic substances (Xu and Chang, 2009).

Summary

Raw peanut and cooked red kidney bean exhibited the highest α-glucosidase inhibitory activity among raw and cooked legumes, respectively. This information provide supportive evidence of the fundamental knowledge to promote the usage of legumes as the excellent choices of healthy food for health conscious individual regarding control of T2DM through α-glucosidase inhibition.

Literature cited


**Figure 1** The α-glucosidase inhibitory activities (presented as percentage of inhibition) of raw and cooked legumes including peanut, soybean, red kidney bean, white bean, black bean and mung bean (12.5 mg/mL).