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A-AMYLASE AND A-GLUCOSIDASE INHIBITORY ACTIVITY OF CHOCOLATE PRODUCTS ASSESSED BY INGREDIENTS

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ABSTRACT

Chocolate or cocoa is a food made from roasted and ground cacao seedkernels that is available as a liquid, solid, or paste, either on its own or as a flavoring agentin other foods. To make our chocolate products, each material (dried flour, dried rice powder, potato, sweet potato) was put in a previous purchased chocolate productand made a new modified chocolate product. This study evaluated the α-amylase and α-glucosidase inhibitory activity in the aqueous and ethanoic extracts of chocolate products assessed by ingredients. α-amylase inhibitory activities of water extract for the sweet potato, potato, rice, and flour were evaluated 43.5%, 41.1%, 38.5%, and 41.2% at 1.0 mg/ml, respectively. The α-amylase inhibitory activity of the sweet potato was the highest among four chocolate products assessed by nutrients ingredients. However, there was no significant difference among three groups ($p>0.05$). α-glucosidase inhibition of water extract for potato chocolate evaluated 36.2% for water extract at 1.0 mg/ml and 38.3% at same concentration. The values of α-glucosidase inhibition for rice and flour water extracts evaluated were 31.9% and 38.2% at 1.0 mg/ml, respectively. Although sweet potato chocolate extracts were slightly higher in α-amylase inhibitory activity among four of chocolate products assessed by nutrients ingredients, there was no significant difference ($p>0.05$).

KEYWORDS: α-amylase, α-glucosidase, Flour, Potato, Sweet potato, Rice.

INTRODUCTION

Cacao, (*Theobroma cacao* L.), also called cocoa, tropical evergreen tree (family Malvaceae) grown tropical regions of Africa and South America for its edible seeds, whose scientific name means "food of the gods" in Greek. The cacao tree, native to the Amazon rainforest, was first domesticated 5,300 years ago in South America before being introduced to Central America by the Olmecs. Now, cocoa is the food of common people with its tasty, nutritious, and valuable products.

Chocolate/cocoa has been known for its good taste and proposed health effects for centuries. Chocolate is a product widely consumed by all generations. It is rich in fat, proteins, carbohydrates, polyphenols and other bioactive compounds.[1] Chocolate is a range of foods derived from cocoa (cacao), mixed with fat (e.g., cocoa butter) and finely powdered sugar to produce a solid confectionery.[2]

Although many biologically or physiologically active compounds such as phenol have been reported in cocoa, many physicians tended to warn patients about the potential health hazards of consuming large amounts of chocolate.[3] Nowadays, chocolate used to be criticized for its fat content and its consumption was a sin rather than a remedy, associated with acne, caries, obesity, high blood pressure, coronary artery disease, diabetes, and atherosclerosis.[4] On the one hand, many opposing results have been reported. For example, in an animal model of atherosclerosis, cocoa powder at a human dose equivalent of two dark chocolate bars per day significantly inhibited atherosclerosis, lowered cholesterol, low-density lipoprotein, and triglycerides, raised high-density lipoprotein, and protected the lower density lipoproteins from oxidation.[5]

Many food ingredients are used when manufacturing chocolate products, which contain excessive high sugars and fat components, resulting in diabetes and hyperlipidemia. Although each chocolate product may have different ingredients, chocolates are high in caloric value (about 500 kcal/100 g) due to the presence of high saturated fat (about 30–40% in weight) and sucrose (40-50% in weight).[6] Thus, increasing awareness of overweight, obesity, diabetes, and its association with the high calorific value of chocolates is reflected in the consumer preference of low/no sugar and low/fat-free chocolates.[7]

While the use of sugar or sucrose prevails in traditional chocolate industry, numerous nutritive and non-nutritive

sweeteners offer new opportunities for the manufacturer. Although several alternative sweeteners have been invented, safety issues continue to be raised. Consequently, edible carbohydrates with lower energy contents have been developed which are suitable for inclusion as bulking agents in chocolate manufacture.[8-9]

The purpose of this study was evaluated the α-amylase and α-glucosidase inhibitory activity in the aqueous and ethanoic extracts of chocolate products assessed by ingredients.

MATERIALS AND METHODS

Preparation of sample

Chocolate products commonly purchased in Korea were purchased and used in this experiment. It was contained roasted nibs of cocoa (45 g), cocoa butter (20 g), vegetable oil (40 g), powdered sugar (100 g), milk powder (50 g), almond powder (5 g), and peanut powder (5 g). 140 g chocolate products were prepared for this experiment. 35 g dried flour, 35 g dried rice powder, Potatoes (35 g) and sweet potatoes (35 g) of the plants were also prepared. Potatoes and sweet potatoes were cut into small pieces and ground them into powder. To make our chocolate products, each material (dried flour, dried rice powder, potato, sweet potato) was put in a previous purchased chocolate product and made a new modified chocolate product. Namely, we made a new chocolate (flour-chocolate) with 35 g chocolate ingredients and 35 g flour. In the same way, we made three new chocolates, rice-chocolate, potato-chocolate, and sweet potato-chocolate. It was used in this experiment.

Newly reproduced chocolate products (70 g) were added to 500 ml of distilled water or 80% ethanol and a grinding mixer. The mixture was stirred with a magnetic bar at 60℃ for 30 minutes. An aliquot of the mixture was further mixed with 100 mM Tris-HCl buffer (pH 7.4). The sample was treated with the ultrasonic bath (5510, Branson, USA) at room temperature for 60 minutes. They were squeezed out with the muslin cloth. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

α-amylase inhibitory assay

The α-amylase inhibitory assay was performed by slight modification in the method described Apostolidis and Lee.[10] α-amylase inhibitory activity was carried out by quantifying the reducing sugar (maltose equivalent) liberated under assay conditions. The starch solution (1% w/v) was obtained by boiling and stirring 1 g of potato starch in 100 mL of sodium phosphate buffer for 30 min. The enzyme, porcine α-amylase (EC 3.2.1.1) (Sigma Aldrich Chemical Co, Steinheim, Germany) hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose as the major form of amylase in humans and other mammals)solution (50 unit/1 mL) was prepared by mixing 0.01 g of α-amylase in 10 mL of sodium phosphate buffer (pH 6.9) containing 0.0006 mM sodium chloride.

50 µl of each extraction and 150 µl of starch solution as well as 10 µl of enzyme were mixed in a 96 well plate and incubated at 37°C for 20 min. After pre-incubation, 20 µl of 0.5% starch solution was added. The reaction mixtures were then incubated at 37℃ for 10 min. The reaction was terminated with the addition of 20 µl of sodium hydroxide and 20 µl of 90 mM 3,5-dinitrosalicylic acid (DNS) reagent and placed in boiling water bath for 10 minutes. The extract was then cooled to room temperature until use. Absorbance (A) was measured at 540 nm. Acarbose (4,6-Dideoxy-4-([1S]-[1,4,6/5]-4,5,6-trihydroxy-3-hydroxymethyl-2-clohexenylamino)-maltotriose) (Sigma Aldrich Chemical Co.) was used as reference standard (positive control).

Blank samples were used to correct the absorption of the mixture, in which the enzyme was replaced with buffer solution. The inhibitor, different extracts, and fractions of water or solvent (control) were added to different well of 96 well microplate.

α-glucosidase inhibitory assay

The α-glucosidase inhibitory activity assay was applied for as described by Deutschlander et al.[11] with some modification. Extracts were prepared as described α-amylase above. The control inhibitor (Acarbose), different extracts of distill water or ethanol were prepared. The test compound and 2 mU of Yeast α-glucosidase (Cat. No: G 5003, Sigma Aldrich Chemical Co, Steinheim, Germany) was dissolved at a concentration of 0.1 U/ml in 100 mM sodium acetate buffer (pH 5.6). Enzyme source was prepared bovine serum albumin 2000 mg/ml and sodium azide 200 mg/ml in 100 mM sodium acetate buffer (pH 5.6). Paranitrophenyl-α-D-glucopyranoside (pNPG) (Cat. No: N1377, Sigma Aldrich Chemical Co, USA) was used as substrate. A total of 20 ul from each extract were diluted to 97 µl in 0.1 M sodium acetate buffer (pH 5.6) and pre-incubated in 96-wellplates at 37℃ for 10 min. The reaction was initiated by adding 3 µl of 3 mM pNPG as a substrate. The plate was incubated for an additional 10 min at 60℃, followed by addition of 100 µl 1 M NaOH to stop the reaction. All test compounds were prepared in DMSO as described above. The final concentrations of extracts and catechins were between 0.03-10 µg/mL and 5–1000 µM, respectively. The final concentration of α-glucosidase was 20 mU/mL. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, California, USA) at the wavelength 410 nm. The reaction system without tea extracts was used as control and system without α-glucosidase was used as blank for correcting the background absorbance. Acarbose was used as reference standard (positive control). Acarbose, known as BAY g 5421, is an α-glucosidase inhibitor that prevents absorption of sucrose

and maltose. All samples were prepared in triplicate.

Inhibitory analysis

All assays were performed in triplicate. Statistical analyses were suggested as means ± SD. Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). Significant differences *p* value was $p < 0.05$.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

Inhibition (%) = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

RESULTS

α-amylase inhibitory effects

The α-amylase inhibitory activity in chocolate products assessed by nutrients ingredients was obtained by setting up the equations. The percentage inhibition of four carbohydrate materials within chocolate products showed a concentration-dependent reaction in percentage inhibition (Table 1). α-amylase inhibitory activity of water extract for the sweet potato was evaluated 19.4% at 0.25 mg/ml and 43.5% at 1.0 mg/ml. α-amylase inhibitory activity of water extract for the potato was evaluated 19.0% at 0.25 mg/ml and 41.1% at 1.0 mg/ml. α-amylase inhibitory activity of water extract for the rice and flour were evaluated 38.5% at 1.0 mg/ml and 41.2% at the same concentration, respectively. The α-amylase inhibitory activity of the sweet potato was the highest among four chocolate products assessed by nutrients ingredients. However, there was no significant difference among three groups ($p>0.05$).

Figure 1 was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for water extract of four chocolate products assessed by nutrients ingredients on four different concentrations. The values of water extract for sweet potato, potato, rice, and flour were 79.6%, 76.2%, 71.0%, and 76.0%, respectively.

α-amylase inhibitory activities of ethanol extract for sweet potato, potato, rice, and flour were evaluated 46.2%, 42.3%, 36.0%, and 38.6% at 1.0 mg/ml, respectively. The α-amylase inhibitory activity of the ethanol sweet potato extract was also the highest among four chocolate products assessed by nutrients ingredients. Although ethanol extracts were slightly higher in α-amylase inhibitory activity than those of water extracts, there was no significant difference between two extract solvent groups ($p>0.05$). Figure 2 was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for ethanol extract of four chocolate products assessed by nutrients ingredients on four different concentrations. The values of water extract for sweet potato, potato, rice, and flour were 77.9%, 71.4%, 69.0%, and 65.1%, respectively.

α-glucosidase inhibitory effects

Alpha-glucosidase inhibitory potentials of four chocolate products assessed by nutrients ingredients on four different concentrations were determined (Table 2).

It was observed that inhibition percentage values go on increasing with enhancements in concentration of four chocolate product extracts in the assay mixture. There was non-significant difference among all the sweet potato water and ethanol extracts at the lowest (11.0% and 12.2% at 0.25 mg/mL) and highest (43.2% and 45.6% at 1.0 mg/mL) concentrations.

α-glucosidase inhibition of water extract for potato chocolate evaluated 36.2% for water extract at 1.0 mg/ml and 38.3% at same concentration. The values of α-glucosidase inhibition for rice and flour water extracts evaluated were 31.9% and 38.2% at 1.0 mg/ml, respectively. The values of α-glucosidase inhibition for rice and flour ethanol extracts evaluated were 48.3% and 46.4% at 1.0 mg/ml, respectively. The all values of α-glucosidase inhibitory for ethanol extracts were higher than those of water extracts. However, they are not showed a statistically significant difference ($p<0.05$). Although sweet potato chocolate extracts were slightly higher in α-amylase inhibitory activity among four of chocolate products assessed by nutrients ingredients, there was no significant difference ($p>0.05$).

Figure 3 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for three water extracts on four different concentrations. The relative values of water extract for sweet potato, potato, rice, and flour chocolate products were 73.0%, 66.7%, 0.49.7%, and 59.5%, respectively.

Figure 4 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for three ethanol extracts on four different concentrations. The values for sweet potato, potato, rice, and flour chocolate products were 70.7%, 66.1%, 71.5%, and 68.5%, respectively.

Table 1: The degree of α-amylase inhibition (%) of Aqueous and Ethanol extracts of chocolate products assessed by nutrients ingredients.

Ingredient	Concentration (mM)	Solvent		*t*-test
		Water	Ethanol	
Sweet potato	0.25	19.42±2.13	23.11±1.17	0.433

	0.50	29.71±2.84	32.55±3.65	
	0.75	37.64±3.33	40.96±3.72	
	1.0	43.51±3.62	46.24±3.57	
Potato	0.25	18.97±2.67	21.29±1.73	0.250
	0.50	28.78±3.67	30.33±1.93	
	0.75	35.50±1.45	37.03±2.47	
	1.0	41.15±0.88	42.34±2.56	
Rice	0.25	11.10±3.81	15.92±2.86	0.613
	0.50	22.26±4.99	25.75±2.74	
	0.75	27.01±5.25	36.02±1.50	
	1.0	38.51±4.01	40.62±1.69	
Flour	0.25	13.47±1.57	16.67±3.76	0.101
	0.50	24.75±1.07	25.39±2.80	
	0.75	31.43±2.63	33.26±0.81	
	1.0	41.23±0.70	38.61±2.21	
F-test		0.762	0.665	

Data represented the mean ± SD from three replicates.

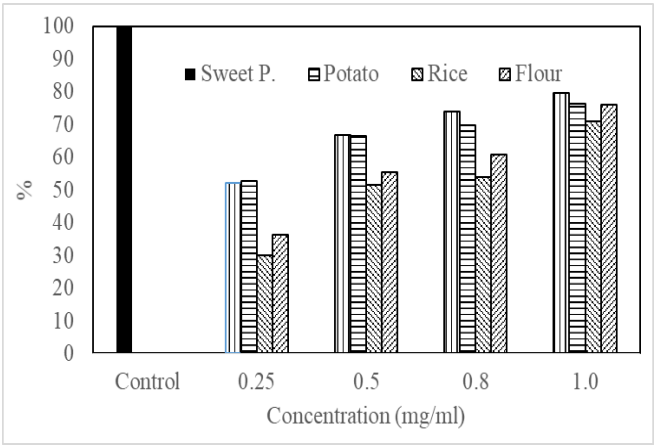

Figure 1: The rate of α-amylase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for water extractions of chocolate products assessed by nutrients ingredients at different concentrations.

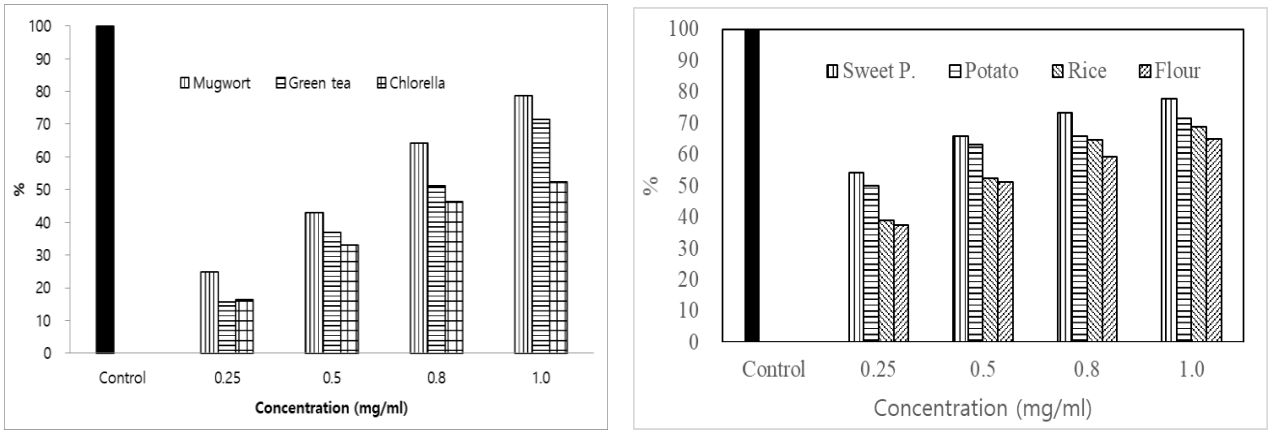

Figure 2: The rate of α-amylase inhibitory of Acarbose (Positive control) and relative inhibitory rate for ethanol extractions of chocolate products assessed by nutrients ingredients at different concentrations.

Table 2: The degree of α-glucosidase inhibition (%) of aqueous and ethanol extracts of chocolate products assessed by nutrients ingredients.

Ingredient	Concentration (mM)	Solvent		*t*-test
		Water	Ethanol	
Sweet potato	0.25	11.02±3.18	12.22±2.53	0.200
	0.50	25.21±5.85	24.49±2.41	
	0.75	36.24±2.62	38.51±2.58	
	1.0	43.15±3.86	45.61±1.64	
Potato	0.25	7.33±1.84	9.26±2.28	0.242
	0.50	18.91±2.07	20.21±1.82	
	0.75	28.19±1.94	31.45±3.61	
	1.0	36.17±2.71	38.42±1.85	
Rice	0.25	7.07±1.84	15.19±4.51	1.654
	0.50	14.83±2.46	32.66±1.64	
	0.75	23.95±3.49	40.51±0.96	
	1.0	31.87±5.94	48.25±0.90	
Flour	0.25	8.07±2.47	13.65±2.81	0.889
	0.50	19.04±3.65	28.66±3.55	
	0.75	28.88±2.40	39.76±2.28	
	1.0	38.15±1.51	46.35±2.61	
F-test		0.808	0.984	

Data represented the mean ± SD from three replicates.

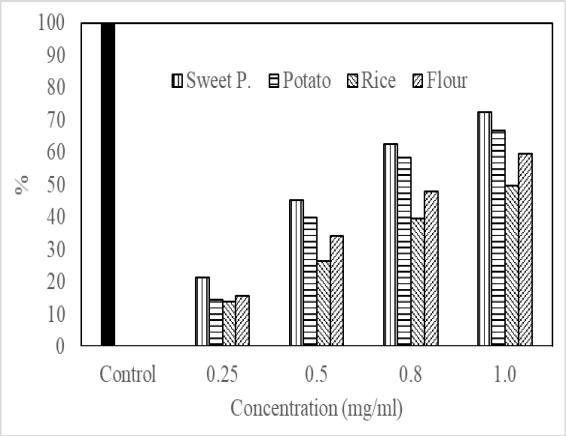

Figure 3: The rate of α-glucosidase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for water extractions of chocolate products assessed by nutrients ingredients at different concentrations.

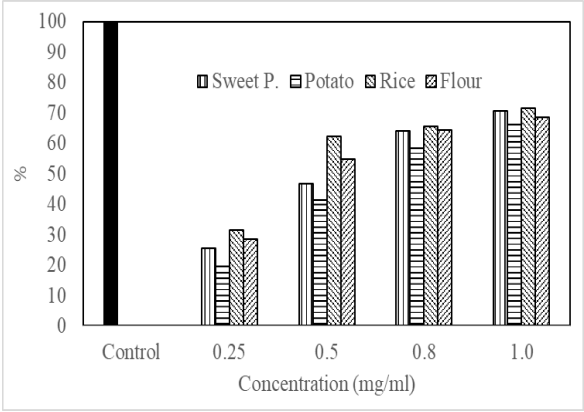

Figure 4: The rate of α-glucosidase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for ethanol extractions of chocolate products assessed by nutrients ingredients at different concentrations.

DISCUSSION

Alpha-amylase is an enzyme (EC 3.2.1.1) that hydrates α bonds o large, α-linked polysaccharides and found in the pancreatic juice and saliva.[12] On the other hand, α-Glucosidase (EC 3.2.1.20) hydrolyzes terminal non-reducing (1→4)-linked α-glucose residues to release a single α-glucose molecule. α-glucosidase is an enzyme found in the mucosal brush border of the small intestine.[13] Effective means of lowering the levels of postprandial hyperglycemia have been offered by α-amylase and α- glucosidase inhibitors by delaying the breakdown of ingested carbohydrates in the small intestine thereby reducing the postprandial blood glucose excursion.[14-15] Several inhibitors of α-amylase and α-glucosidase have been isolated from medicinal plants to serve as an alternative drug with increased potency and less adverse effects than existing synthetic drugs.[16] *Senna surattensis* inhibited carbohydrate digestive enzymes and increased the peripheral uptake of glucose.[17]

Red rice (*Oryza punctate*) methanolic extract possess strong inhibitory activity on α-amylase and α-glucosidase compared with acarbose ($P < 0.01$).[18] γ-aminobutyric acid and ferulic acid isolated from wheat (*Triticum aestivum*) sprouts were found to exert more profound inhibition effects against α-amylase and α-glucosidase.[19] Frying of the livingstone potato (*P lectranthus esculenta*) tubers potentiates the activities of α-amylase and α-glucosidase enzymes compared with the raw or boiled forms.[20]. The sweet potato used for bread production had a peak inhibitory effect on α-glucosidase than α–amylase.[21].

Chocolate is classified as a high calorie food due to the high fat and sugar contents because Chocolate is made to suit the taste of consumers. Cocoa butter, vegetable oil, powder sugar, milk powder, almond powder, and peanut powder used in chocolate production are food materials that produce a lot of calories. According to Codex Alimentarius Commission[22] dark chocolate is a confectionery product containing not less than 35 % of total dry cocoa solids, 18 % of cocoa butter and 14 % of dry non-fat cocoa solids with addition of any sugar, the most common sweetener used in chocolate is sucrose, which causes chocolate to contain a large amounts of calories.[23]

Halving these ingredients used in chocolate production and replacing them with rice, flour, potatoes, and sweet potatoes instead can reduce calorie production, reducing obesity and diabetes. The consumption of foods rich in hydrolyzing enzyme inhibitors is recommended for diet therapy of diabetes.[24] Cereal-derived phenolic compounds, peptides, non-starch polysaccharides, and lipids have been shown to inhibit α-amylase and α-glucosidase activities. However, there are some inhibiting effects of carbohydrate-degrading enzymes within rice, flour, potatoes, and sweet potatoes, but these food ingredients themselves contain carbohydrates, so excessive intake should be avoided. Even if chocolate materials replace these ingredients, they didn't have high α-amylase and α-glucosidase inhibition (Tables 1 and 2), this study suggests that there is some evidence for whole cereals or potato intake to be beneficial in intakes of less calories through inhibiting α-amylase and α-glucosidase activities.

CONCLUSIONS

When making chocolate, reducing high-calorie ingredients and using ingredients such as rice, flour, potatoes, and sweet potatoes can reduce calorie intake by partially inhibiting digestive enzyme activity.

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