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INHIBITORY OF α-AMYLASE AND α-GLUCOSIDASE BY RAW, BOILED, AND ROASTED POTATOES

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ABSTRACT

This study was to evaluate and compare the α-amylase and α-glucosidase inhibitory activity of extracts from common brownish potato during two cooking methods. The α-amylase inhibitory activity of the raw potatoes was high, but not when boiled or toasted. α-amylase inhibitory activity of water extract for raw potato was evaluated 15.5% at 0.25 mg/ml and 38.4% at 1.0 mg/ml. The values of the boiled potato evaluated 16.3% at 1.0 mg/ml and that of the roasted potato evaluated 19.9%. There was no significant difference in the inhibitory activity of the two enzymes between boiled and roasted potatoes ($p > 0.05$). The half maximal inhibitory concentration (IC_{50}) for α-amylase of raw potato was 170.8 ug/ml, whereas boiled potato was low, 209.7 ug/ml. α-glucosidase inhibition of water extract for raw potato evaluated at 0.25 mg/ml was 26.9% and that of 1.0 mg/ml was 51.5%. The values of α-glucosidase inhibition for boiled and roasted potatos evaluated at 1.0 mg/ml were 21.0% and 20.5%, respectively. The raw potatoes generally had slightly higher α-amylase and α-glucosidase inhibitory activity than boiled and roasted potatoes. Boiling and roasting treatments for potatoes showed reducing effects on the α-amylase and α-glucosidase inhibitory activity. The mode of inhibition of the raw potapo extracts against both α-amylase and α-glucosidase was confirmed by Lineweaver–Burk plots. Crude water extract competitively inhibited α-amylase and α-glucosidase.

KEYWORDS: α-amylase, α-glucosidase, inhibitory activity, Lineweaver–Burk plots, potato.

INTRODUCTION

The potato is a root vegetable native to the South Americas such as the Andes, a starchy tuber of the plant *Solanum tuberosum* L.[1] The major species grown worldwide is *Solanum tuberosum* (a tetraploid with 48 chromosomes), and modern varieties of this species are widely cultivated in the world. Today potatoes are staple foods in many parts of the world and an integral part of much of the world's food supply. Potato is rich in starch that it ranks as the world's fourth most important food crop, after maize, wheat and rice.[2] Starch is a carbohydrate consisting of lots of glucose branches. In plants, starch is their energy store. That's why many foods naturally contain it. The cells of the root tubers of the potato plant contain starch grains (leucoplasts). Starch derivatives are used in many recipes, for example in noodles, wine gums, cocktail nuts, potato chips, dog sausages, cream and instant soupssoups andes, in gluten-free recipes, in kosher foods for Passover, and in Asian cuisine.[3,4]

Starch digestibility is the proportion of starch that is digested under certain conditions. Many factors influence the digestibility of starches in foods such as carbohydrate contents of foods, nutritional composition of starch, method of cooking foods, and others.[5] Starch blockers inhibit the intestinal digestive enzyme, alpha (α)-amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) and delay slow carbohydrate absorption giving a body more time to handle all the starches in foods. In addition, information on the effect of cooking of potato on the activities of starch blockers that could naturally be present in it is also scarce in literature. α-amylase inhibitors appear helpful in the prevention and clinical treatment of metabolic syndromes such as type 2 diabetes mellitus and obesity.[6] A commercial starch blocker, containing the a-amylase inhibitor phaseolamin, was ineffective in reducing or retarding the total digestionand absorption of starch.[7] However, many studies have shown a positive association between the polyphenols in some plant-based foods with their inhibitory actions on these starch digestive enzymes.[8] These polyphenols being polar and heat labile could also be affected by different food cooking techniques, which in essence could affect the inhibitory actions of the plant based foods on these starch digestive enzymes. In addition, α-glucosidase inhibitor is particularly useful in individuals whose meals consist of high carbohydrate content. It works by competitively

inhibiting the enzyme α-glucosidaseat the brush border of the small intestines, thus delaying the digestion of complex carbohydrate and intestinal absorptionof glucose. There are a large number of natural products with α -glucosidase inhibitor action.[9] For example, there were evaluated the inhibitory effects of plant-based extracts (grape seed, green tea, and white tea) on α-amylase and α-glucosidase activity, glucosidases required for starch digestion.[10] The leaves of *Polyscias fruticosa*. showed inhibitory effects against porcine pancreas *α*-amylase and yeast *α*-glucosidase activities.[11] Other research has shown the culinary mushroom Maitake (*Grifola frondosa*) has a hypoglycemic effect.[12]

The objective of the present study was to provide an in-vitro study for the potential inhibitory activity of raw or post-cooking extracts of potatoes on α-amylase and α-glucosidase enzymes. It evaluated in inhibition of α-glucosidase and porcine pancreatic α-amylase activities by its crude aqueous and ethanolic extracts with raw, boiled, and roasted potatoes.

MATERIALS AND METHODS

Sample extract

Potatoes (1,200 g) divided into three groups: raw (400 g), boiled (400 g), and roasted (400 g). Potatoes (400 g) for the boiled group were added to 1,500 ml of water and boiled in a stainless steel. Potatoes (400g) for the roasted group were baked in an oven. Each potato of group was ground with distilled water or 80% ethanol and a grinding mixer. They were squeezed out with the muslin cloth. An aliquot of the mixture (200 mg sample/ml water or 80% ethanol) was further mixed with 100 mM Tris-HCl buffer (pH 7.4). The mixture of boiling group was further stirred with a magnetic bar at 100℃ for 5 minutes. The sample was treated with ultrasound at room temperature for 60 minutes. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature. 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 determination of α-amylase inhibitory activity was carried out by quantifying the reducing sugar (maltose equivalent) liberated under assay conditions by the method described Apostolidis and Lee[13] with some modification. The assay mixture containing 25 µl of 50 mM phosphate buffer pH 6.8, 2.5 µl extract and pre-incubated porcine α-amylase (0.25 U/ml) were incubated at 37℃ for 10 min. After pre incubation, 25 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 150 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-yclohexenylamino)-maltotriose) (Sigma Aldrich Chemical Co, USA) was used as reference standard (positive control). Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with vehicle. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. Experiments were performed in triplicate.

α-glucosidase inhibitory assay

The bioassay method of multiwell plate system was applied for α-glucosidase inhibitory activity assay as described by Deutschlander et al.[14] with some modification. Extracts and catechins were prepared as described above. The test compound and 2 mU of Yeast α-glucosidase (Cat. No: G 5003, Sigma Aldrich Chemical Co, USA) 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 sodiumacetate buffer (pH 5.6). Paranitrophenyl-α-D-glucopyranoside (pNPG) (Cat. No: N 1377, Sigma Aldrich Chemical Co, USA) was used as substrate. A total of 20 ul from each extractwere 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 assubstrate. 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, Califonia, USA) at the wavelength 410 nm. The reaction system without tea extracts was usedas 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

Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). All analysis was carried out at least in triplicate. The results were expressed as the mean±SD. Significance and confidence level were estimated at $p < 0.05$.

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.

The concentration of the extract that inhibits 50% of the enzyme activity (IC_{50}) was calculated. Extracts with high inhibitory activity were analyzed using a series of suitable extract concentrations. IC_{50} values were determined by plotting percent inhibition (Y axis) versus log10 extract concentration (X axis) and calculated by logarithmic regression analysis from the mean inhibitory values. Regression analysis by a doseresponse curve was plotted to determine the IC_{50} values.

Kinetics of inhibition against α-amylase and α-glucosidase

Inhibition modes of the crude potato extracts against α-amylase and α-glucosidase were determined according to the method described by Kim et al.[15] Briefly, fixed amounts of both α-amylase and α-glucosidase were incubated with increasing concentrations of their substrates (starch and PNPG, respectively) at 37°C for 20 min, in the absence or presence of water extracts (5 mg/ml). Reactions were terminated and absorption measurements carried out as a for ementioned. Amounts of products liberated (reducing sugars as maltose and p-nitrophenol, respectively) were determined from corresponding standard curves and converted to reaction rates according to the following formula.

$$\text{Reaction rate (v) } (mg.ml^{-1}.s^{-1}) = \frac{\text{Amount of product liberated } (mg.ml^{-1})}{1200 \text{ (s)}}$$

Inhibition types were then determined by Lineweaver–Burk plot (1/v versus 1/[S]) where [S] analysis of data is according to Michaelis–Menten kinetics.[16]

RESULTS

α-amylase inhibitory effects

In this study, the inhibitory effects of extracts against α-amylase were investigated. The percentage inhibition of three potato extracts showed a concentration-dependent reaction in percentage inhibition (Table 1). α-amylase inhibitory activity of water extract for raw potato was evaluated 15.5% at 0.25 mg/ml and 38.4% at 1.0 mg/ml. The α-amylase inhibitory activity of the raw potatoes was high, but not when boiled or toasted. α-amylase inhibitory activity of the boiled potato evaluated 16.3% at 1.0 mg/ml and that of the roasted potato evaluated 19.9%. There was no significant difference between boiled and roasted potatoes ($p > 0.05$). Although ethanol extracts were slightly higher in α-amylase inhibitory activity than those of water extracts, there was no significant difference between two extract groups ($p > 0.05$). Figure 1 was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for potatoes on 1.0 M. The values of water extracts for raw, boiled, and roasted potatoes were 44.8%, 18.8%, and 23.0%, respectively. The values for raw, boiled, and roasted states of ethanol extracts were 44.9%, 17.1%, and 24.5%, respectively.

An IC_{50} value is the concentration of the extract required to inhibit the activity of the enzyme by 50% of the free radicals present in the system. Table 3 showed the IC_{50} values of the extracts with different materials. The IC_{50} for α-amylase of raw potato was 170.8 ug/ml. Among analyzed extracts, raw potato was the highest α-amylase inhibition activety, whereas boiled potato was the most low, 209.7 ug/ml. Lower IC_{50} values mean greater amounts are needed that can be inhibited. The mode of inhibition of the crude potato extracts against α-amylase was confirmed by Lineweaver–Burk plots (Figure 3). Crude water extract competitively inhibited α-amylase.

α-glucosidase inhibitory effects

The results of the α-glucosidase inhibitory effects of extracts in comparison at 410 nm were shown in Table 2. It was observed that inhibition percentage values go on increasing with enhancements in concentration of potato extracts in the assay mixture. α-glucosidase inhibition of water extract for raw potato evaluated at 0.25 mg/ml was 26.9% and that of 1.0 mg/ml was 51.5%. The values of α-glucosidase inhibition for boiled and roasted potatoes evaluated at 1.0 mg/ml were 21.0% and 20.5%, 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 ($p<0.05$).

Figure 2 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for potato extracts on 1.0 M. The values of water extracts for raw, boiled, and roasted potatoes were 59.6%, 24.3%, and 23.7%, respectively. The values for raw, boiled, and roasted states of ethanol extracts were 62.6%, 26.7%, and 28.4%, respectively.

The IC_{50} for α-glucosidase of potatoes ranged from 331.5 to 487.2 ug/ml (Table 3). Among analyzed extracts, raw potato was the highest α-glucosidase inhibition activity (IC_{50} was 331.5 ug/ml).

The mode of inhibition of the crude potato extracts against α-glucosidase was confirmed by Lineweaver–Burk plots (Figure 4). Crude water extract competitively inhibited α-glucosidase.

Table 1. The degree of α-amylase inhibition (%) of aqueous and ethanol extracts of raw, boiled, and roast ed potatoes

Potato	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw tubers	0.25	15.47±3.21	14.86±4.37	0.028
	0.50	22.66±0.45	22.84±2.92	
	0.75	31.09±1.65	31.67±2.03	
	1.00	38.41±1.62	39.07±2.58	
Boiled tubers	0.25	5.10±1.29	6.32±1.59	0.532
	0.50	9.10±1.70	10.76±0.87	
	0.75	13.32±2.59	15.24±0.57	
	1.00	16.27±1.75	19.40±1.81	
Roasted tubers	0.25	5.68±1.82	6.86±2.81	0.380
	0.50	11.24±2.40	12.32±1.86	
	0.75	17.21±2.11	18.95±0.62	
	1.00	19.88±2.20	23.17±2.69	
F-test		5.989*	3.954*	
Data represented the mean ± SD from three replicates.				

Table 2. The degree of α-glucosidase inhibition (%) of aqueous and ethanol extracts of raw, boiled, and ro asted potatoes.

Potato	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw tubers	0.25	26.92±2.04	28.36±1.23	0.226
	0.50	37.08±2.89	38.38±1.94	
	0.75	45.73±1.73	47.43±2.74	
	1.00	51.46±2.28	54.01±1.91	
Boiled tubers	0.25	6.87±2.54	8.01±3.16	0.402
	0.50	12.28±2.35	14.77±2.68	
	0.75	18.45±1.95	20.18±2.73	
	1.00	21.01±2.02	23.03±2.52	
Roasted tubers	0.25	6.08±2.29	7.30±1.26	0.464
	0.50	12.13±2.15	13.75±0.92	
	0.75	16.47±1.85	18.41±0.92	
	1.00	20.45±1.43	24.54±1.16	
F-test		15.695**	13.375**	
Data represented the mean ± SD from three replicates.				

Table 3: The 50% inhibition (IC_{50}) of α-amylase and α-glucosidase of potatoes.

Sample	α-amylase	α-glucosidase
Raw tubers	170.8±3.5	331.5±15.3
Boiled tubers	209.7±2.8	425.8±19.5
Roasted tubers	202.6±3.1	487.2±16.9

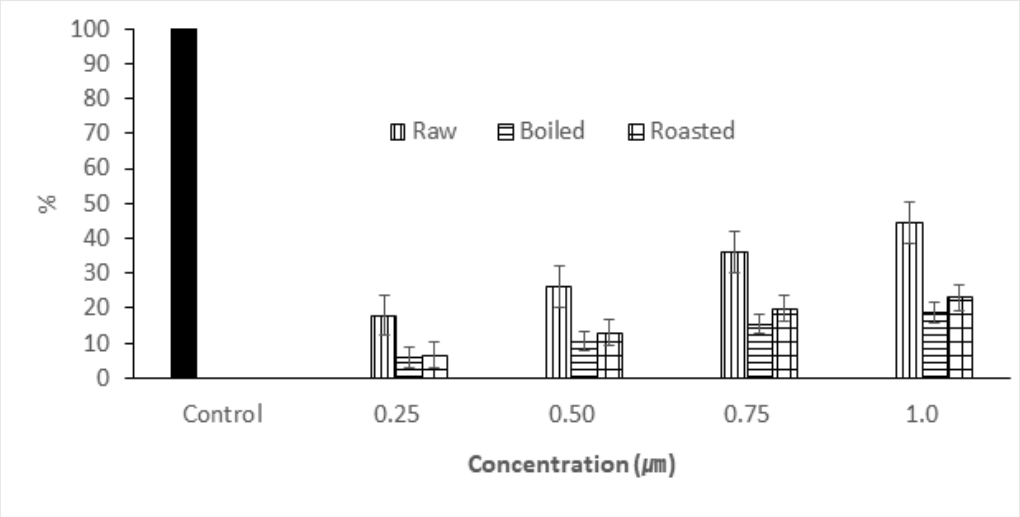

Figure 1. The rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for potatoes at different concentrations.

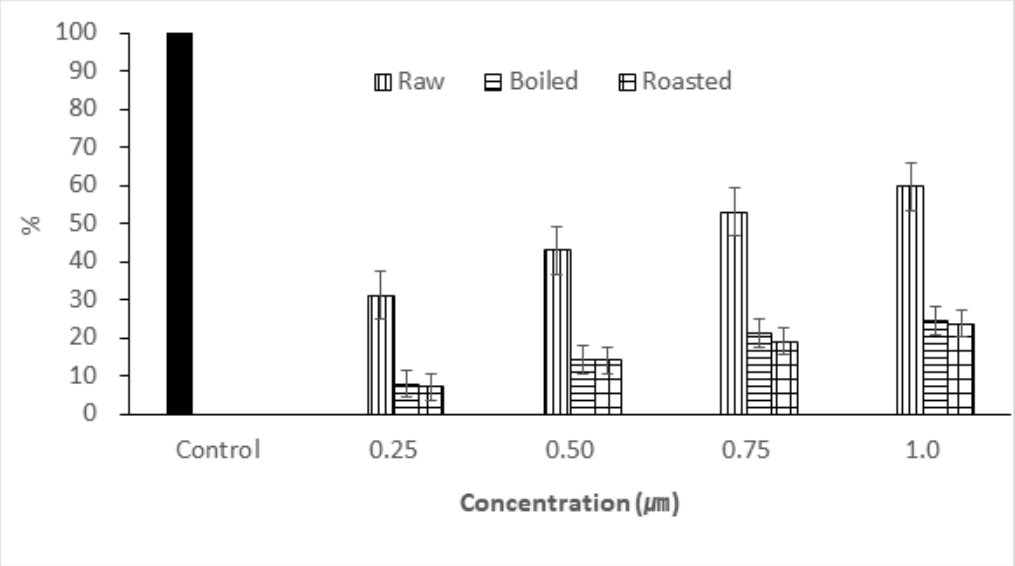

Figure 2. The rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for potatoes at different concentrations.

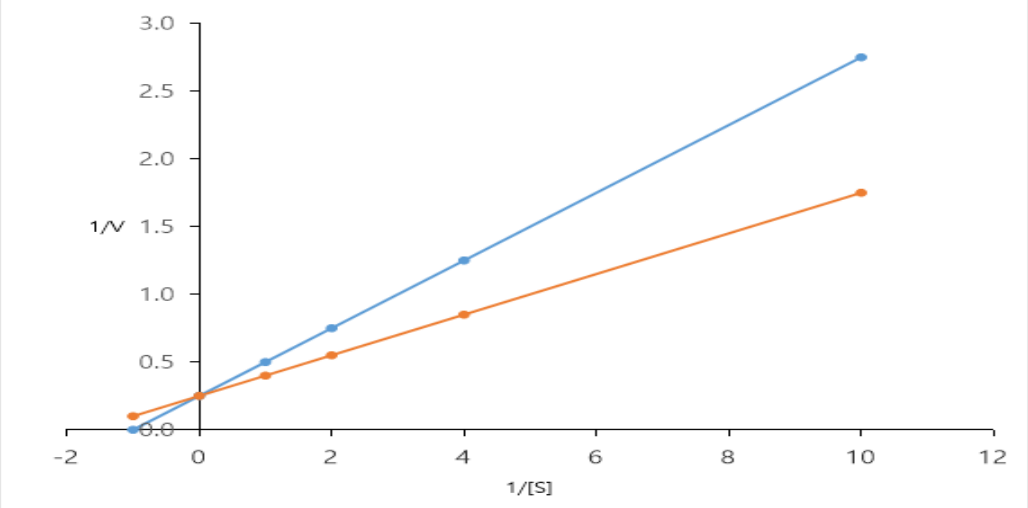

Figure 3. Lineweaver-Burk plot for the activity of α-amylase in the presence of concentration (1 ug/ml) of crude potato extract and inhibitor.

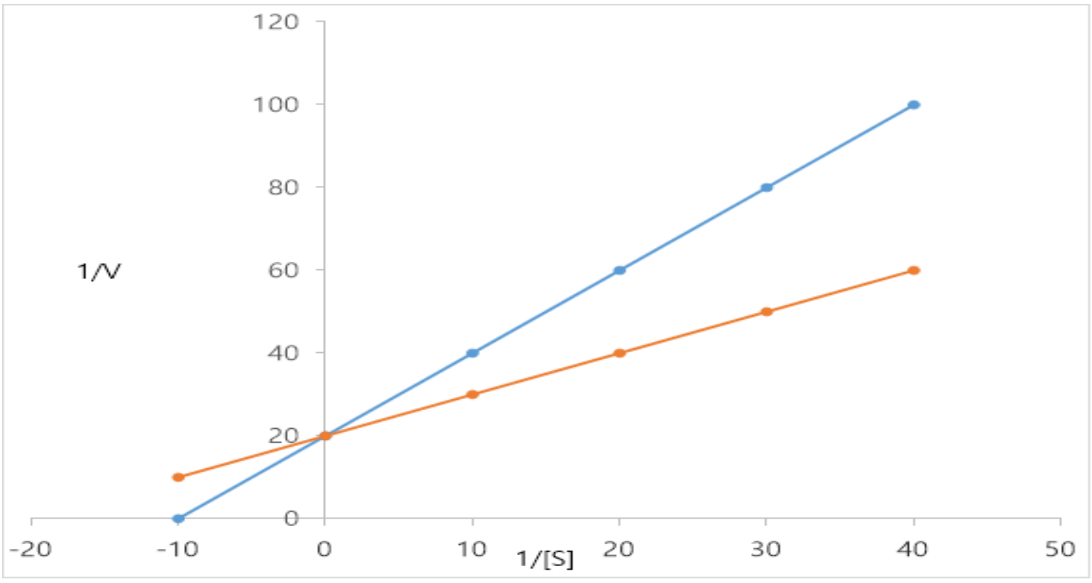

Figure 4. Lineweaver-Burk plot for the activity of α-glucosidase in the presence of concentration (1 ug/ml) of crude potato extract and inhibitor.

DISCUSSION

Eleazu et al.[17] investigated the starch digestibility, α-amylase and α-glucosidase inhibitory capacities of the flours from raw and processed (boiled and fried) tubers of three varieties (riyom, beebot and langaat) of three varieties of Livingstone potato (*Plectranthus esculenta*) using standard techniques. In their study, the IC_{50} values of the aqueous extracts of the flours ranged from 257.50 to 1515.94 µg/ml and 128.89 to 641.90 µg/ml for the α-amylase and α-glucosidase assays respectively. Frying of the *P. esculenta* tubers potentiates the activities of α-amylase and α-glucosidase enzymes compared with the raw or boiled forms. Several natural α-glucosidase and α-amylase inhibitors including a carbose, voglibose a nd miglitol are clinically used as a treatment, but their prices are high and clinical side effects occur[18,19] Natural products are still the most available source of these inhibitors.[20] Therefore, screening of α-amylase and glucosidase inhibitors in natural plants has received much attention. Therefore, in the present study, although potatoes are small in quantity, raw potatoes have an α-amylase and α-glucosidase inhibitory function by using the water or ethanolic extract of potatoes. Such enzyme inhibitory function was destroyed or reduced when boiled or roast heating was applied to potatoes. The effect study of cooking was showed the usefulness of boiled unpeeled potato, boiled potato peeled, and raw sweet potato as functional foods for people with type 2 diabetes.[15] However, some digestive enzymes such as alpha-amylase, trypsin, and lysozyme) were allowed to react with some simple phenolic and related compounds (caffeic acid, chlorogenic acid, ferulic acid, gallic acid, m-, o-, and p-dihydroxybenzenes, quinic acid, and p-benzoquinone).[21] Potatoes have very little such phenol. When potatoes are heated, the ingredients that cause such inhibitions will also be reduced, so the enzyme inhibits are not high.

The inhibition mode of the raw water and ethanol extracts of potatoes against both α-amylase and α-glucosidase was competitive inhibition by Lineweaver–Burk plots (Figures 3 and 4). These observations suggest that amylase is inhibited mostly by polar potato metabolites (probably pseudosaccharides) and α-glucosidase metabolites. Namely, α-amylase and α-glucosidase inhibitory capacity of potatoes were significantly lost during common cooking practices such as boiling and roasting. Crude *Sclerocarya birrea* stem bark methanolic extract competitively inhibited αamylase, whereas the hexane extract non-competitively inhibited α-glucosidase.[22] Amylase and glucoside involved in carbohydrate digestion and both enzymes perform competitive degradation in potatoes. Thus, understanding these enzyme functions could provide an understanding that could increase the effectiveness of the natural drug extract.

CONCLUSIONS

The α-amylase and α-glucosidase inhibitory capacity of the raw potatoes was high, but not when boiled or baked. Boiling and roasting treatments showed reducing effects on the digestive properties of common brownish yellow potato.

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