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Original research article

Inhibitory activities of vegetable juices on digestive enzymes for starch and sugar digestions

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ABSTRACT

Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes. Besides drug therapy, lifestyle modification, particularly a healthy diet, is important. Vegetable-based diet consumption is highly recommended; thus, the present study aimed to investigate and identify types of vegetables possessing potential for management of blood glucose levels. Nineteen vegetables were selected to prepare freeze-dried vegetable juices. α-Glucosidase inhibitory activity was tested using natural substrates (maltose and sucrose) in comparison with a synthetic glucose derivative (*p*NPG). α-Amylase was also tested. The enzyme inhibitory activities of heated vegetable samples were also monitored to investigate the influence of cooking conditions. The results indicated that among all tested samples holy basil, sweet basil, and okra possessed high potential as α-glucosidase inhibitors in which 50% inhibitory concentration (IC_{50}) of all three samples were lower than that of acarbose regardless of type of substrates. Juices from holy basil, sweet basil, and okra exhibited non-competitive α-glucosidase inhibition whereas acarbose showed competitive inhibition. Boiling differently affected α-glucosidase inhibitory activities of each vegetable but it seems not to have much effect on holy basil, sweet basil, and okra activity. In conclusion, consumption of these vegetables may reduce carbohydrate digestion, delay glucose absorption, and reduce postprandial blood glucose, thus being of benefit not only for diabetic patients but also for people with a high risk for developing diabetes, as well as generally healthy people.

Keywords: diabetes, α-glucosidase, sugar digestion, digestive enzyme, vegetables

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https://li01.tci-thaijo.org/index.php/JBAP

1. Introduction

Diabetes is a non-communicable disease caused by the inability to regulate plasma glucose level. Failure to properly control plasma glucose can lead to other important health problems such as heart disease, retinopathy, and kidney disease, which causes premature death.[1] Diabetes is divided into 2 major types; type 1 diabetes, and type 2 diabetes. Type 2 diabetes is usually found in adults who are overweight, obese, and those who have poor eating habits. According to World Health Organization, the number of diabetes rose from 108 million in 1980 to 422 million in 2014, with the number of patients continually increasing, especially those with Type 2 diabetes.[2]

The 2022 American Diabetes Association (ADA) recommends continuous glucose monitoring (CGM) and the goals of treatment for patients with diabetes are HbA1C < 7% and plasma glucose in the range of 70-180 mg/dL.[3] One of the therapeutic targets is to inhibit enzymes such as α-amylase and α-glucosidase which therefore reduces carbohydrate digestion and delays the absorption of glucose into the bloodstream.[4] Besides drug therapy, a good diet plan, and exercising are also the main keys to prevent high blood sugar in diabetic patients.[2] A systematic analysis for the Global Burden of Disease Study 2016 reported that 1) increasing dietary intake with high consumption of red meat, starchy food, sugar, and low consumption of green leaves, leads to the increasing risks of health problems such as obesity, diabetes, premature death from an unhealthy diet, 2) changing a dietary pattern by increasing the vegetable, whole grain, and fruit intake such as a vegan diet or a plant-based diet will be beneficial.[5,6] Lifestyle modification, particularly healthy eating behaviour, is important not only for diabetic patients but also for people with high risks for developing diabetes, as well as general healthy people.

As an alternative source of anti-diabetic agents, natural products, particularly of plant origin, have been intensively studied and reviewed for their beneficial potentials.[7,8] Many of these studied have focused on medicinal plants and characterization of biologically active compounds aiming to investigate new findings in phytotherapy for diabetes. In addition, some studies were focused on the potential of food-based plants and reported the association between vegetables or plant-based diet and the decreased risks of developing chronic diseases such as diabetes, stroke, and metabolic syndromes.[9-11] Although consumption of a diet with a high portion of vegetables is generally considered healthy, such healthy behavior often has poor adherence. To achieve such a specific purpose as to reduce risk of diabetes development, specific vegetable recommendation should be developed. Thus, the present study aimed to investigate and identify types of vegetables possessing potential for management of blood glucose levels.

The present study determined the inhibitory activity of freeze-dried vegetable juices on carbohydrate digestion by measuring the interference of digestive enzymes including α-amylase and α-glucosidase. Natural substrates (maltose and sucrose) were tested in comparison with a synthetic glucose derivative (*p*NPG) for α-glucosidase inhibitory activity. The enzyme inhibitory activities of heated-vegetable samples were also monitored to investigate the influence of cooking conditions.

2. Materials and Methods

2.1 Chemicals and reagents

Saccharomyces cerevisiae α-glucosidase (catalog number G5003), *p*-nitrophenyl α-D-glucopyranoside (*p*NPG), gallic acid, quercetin, porcine pancreatic α-amylase, starch, dinitrosalicylic acid (DNS), acarbose, 4-nitrophenol, Folin-Ciocalteu reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA). Maltose and sucrose were obtained from Ajax Finechem (Taren Point, NSW, Australia). Glucose liquicolor complete test kit was purchased from Human GmbH (Wiesbaden, Germany).

2.2 Vegetable sample preparation

Vegetables (Table 1) used in the study were collected from local markets in Phitsanulok, Thailand. Vegetable samples were washed, weighed, chopped into small pieces, blended with water (1:1), and passed through filter cloth. The juices were lyophilized before and after heating at 90 °C for 10 min to prepare fresh and boiled juice samples, respectively. All lyophilized samples were stored at -20 °C until use.

2.3 Total phenolic content determination

Lyophilized vegetables were suspended in distilled water and supernatants were collected after centrifugation at 3,000 rpm for 10 min. Supernatant (20 µL) was mixed with Folin-Ciocalteu reagent (100 µL) and 15 g/L sodium carbonate (80 µL), incubated at 50 ºC for 5 min, cooled at room temperature for 30 min, and the absorbance at 750 nm measured. Gallic acid (GA) was used as a standard phenolic compound. Total phenolic content was calculated and expressed as milligram gallic acid equivalent (GAE).

2.4 Total flavonoid content determination

Lyophilized vegetables were suspended in distilled water and supernatants were collected after centrifugation at 3,000 rpm for 10 min. Supernatants (20 µL) were mixed with 95% ethanol (60 µL), 4% $AlCl_3$ (10 µL), 0.4 M CH_3COOK (10 µL), and distilled water (100 µL) in a 96-well plate. The mixtures were incubated at room temperature for 40 min before measuring the absorbance at 415 nm. Quercetin was used as a standard. Total flavonoid content was calculated and expressed as milligram quercetin equivalent (QE).

2.5 α-Glucosidase inhibitory activity and kinetic methods

The α-glucosidase activity was determined by measuring the release of *p*-nitrophenol from *p*NPG. Briefly, 500 mU/mL of α-glucosidase was prepared in phosphate-buffer saline (PBS) at pH 6.8. The lyophilized vegetables (50 µL) were mixed with α-glucosidase (50 µL), incubated in 96-well plates at 37 °C for 10 min, and 0.4 mM *p*NPG solution (100 µL) was added. After incubation at 37 °C for 30 min, the absorbance was measured at 405 nm using a microplate reader. Acarbose, an α-glucosidase inhibitor, was used as a positive control.

Two kinetic methods, the Lineweaver-Burk plot and the Dixon plot, were used in this study. The reactions were conducted with 50 to 500 µg/mL of lyophilized vegetables, 0.05-0.25 mM *p*NPG, and 125 mU/ml α–glucosidase solution at 37 ºC. The initial rate of reaction (v) was calculated and plotted in a double reciprocal plot of enzyme kinetics, and the inhibition pattern was evaluated by Lineweaver-Burk plot (1/v versus 1/[S]) which was obtained in the presence of various compound concentrations. Similarly, a Dixon plot for α-glucosidase inhibition was taken in the presence of various concentrations of the *p*NPG. The inhibition constants (Ki) were determined by interpreting the Dixon plots, in which the value of the x-axis represents Ki.[12-14]

2.6 Sucrase and maltase inhibitory activity

Maltase and sucrase inhibitory activity of lyophilized vegetables were determined by using maltose and sucrose as a substrate, respectively. The α-glucosidase (2 U/mL, 50 µL) was mixed with lyophilized vegetables in water (50 µL) in the presence of maltose or sucrose (50 mM, 100 µL). The liberated glucose was measured by the glucose oxidase method using Glucose liquicolor complete test kit. The absorbance readings were taken at 510 nm. Acarbose was used as a positive control.

2.7 α-Amylase inhibitory activity

The α-amylase activity was determined by 3, 5 dinitrosalicylic acid (DNS) colorimetry. Briefly, the lyophilized vegetables (5 mg/mL) in PBS (pH 7.4) were mixed with porcine pancreatic α-amylase (1.5 unit/mL, 100 µL) at 37 °C for 5 min, and incubated with starch (12.5 mg/mL, 100 µL) for 10 min. The mixture was sampled to react with 1% DNS (100 µL), heated at 100°C for 5 min, cooled to ambient temperature, diluted with water (1 mL), and the absorbance at 540 nm was measured. Acarbose was used as positive control.

2.8 Statistical analysis

All data represent the mean ± standard deviation (SD) of at least three separate experiments. Half maximal inhibitory concentrations (IC_{50}) of samples against α-glucosidase and α-amylase activity were calculated using the Prism program (GraphPad Software Inc). The data analysis of variance with means separated by least significant difference using SPSS statistics 17.0. Comparisons between two groups used student's *t*-test and differences were considered significant when $p \leq 0.05$.

3. Results

3.1 Quantitative phytochemical analysis

Among 19 vegetable juices, climbing wattle and okra contained high levels of total phenolic contents of approximately ~30 mg GAE/g lyophilized powder (Table 1). Flavonoids could not be detected from most vegetable juices. Only juices prepared from fingerroot, onion, lemon grass, okra and eggplant showed detectable levels of flavonoids (Table 1).

3.2 Inhibitory activities of vegetables on sugars and starch digestion

To investigate the inhibitory activity on sugar digestion of the vegetable samples, synthetic substrate (*p*NPG) and natural disaccharides (maltose and sucrose) were used in an α-glucosidase activity assay. Based on *p*NPG hydrolysis, holy basil, sweet basil, onion, and okra (at 1 mg/mL) strongly inhibited α-glucosidase activity with > 80% inhibition (Table 2). Among these four, only holy basil, sweet basil, and okra effectively inhibited maltose and sucrose digestion, whereas the inhibitory activity of onion was apparently reduced (20-30% inhibition). In contrast to onion, daikon's inhibitory activity was obviously increased when using natural substrates (Table 2). Most vegetables exhibited low inhibitory activity against amylase or starch digestion. These data suggest that certain vegetables possess potential to reduce sugar digestion and consequently might delay glucose absorption without effecting starch digestion. Holy basil, sweet basil, and okra were then selected for further experiments.

3.3 α–Glucosidase enzyme kinetics

To investigate the mode of α-glucosidase inhibition, a Lineweaver-Burk plot was used, and data indicated that juices from holy basil, sweet basil, and okra exhibited non-competitive α-glucosidase inhibition whereas acarbose showed competitive inhibition (Fig. 1, Table 3). Among the inhibition constants (*Ki*) of three vegetables, *Ki* of holy basil was lower than acarbose indicating higher potency and biding affinity to α-glucosidase (Table 3).

3.4 Half inhibitory activity (IC_{50}) on α-glucosidase of selected vegetables

The IC_{50} of holy basil, sweet basil, and okra on α-glucosidase were determined using both synthetic and natural substrates in comparison with acarbose. The results showed that the IC_{50} of all three samples were lower than that of acarbose regardless of type of substrates (Table 4). When using sucrose and maltose as substrates, the IC_{50} of each vegetable was increased compared to *p*NPG (Table 4). These data suggest the potential of holy basil, sweet basil, and okra as α-glucosidase inhibitors.

Table 1. Total phenolic and flavonoid contents in vegetables used in this study.

Common name	Botanical name	Part	Phenolic content (mg GAE/g lyophilized powder)	Flavonoid content (mg QE/g lyophilized powder)
Onion (Hom-yai)*	*Alium cepa* L.	Bulb	12.82±1.45	8.13±4.37
Red onion (Hom-dang)*	*Allium ascalonicum* L.	Bulb	6.86±0.79	ND
Ginger (King)*	*Zingiber officinale* Roscoe	Root	2.64±0.02	ND
Daikon (Chi-tao)*	*Raphanus sativus* L.	Root	18.52±0.95	ND
Fragrant pandan (Tuey-hom)*	*Pandanus amaryllifolius* Roxb.	Leaf	7.81±0.56	ND
Okra (Kra-jeab-keaw)*	*Abelmoschus esculentus* L. Moench	Fruit	28.91±0.59	2.40±2.43
Turkey berry (Ma-kue-poung)*	*Solanum torvum* Swartz	Fruit	7.48±0.22	ND
Garlic (Kra-tiam)*	*Allium sativum* L.	Bulb	9.40±0.71	ND
Cabbage (Ka-lum-plee)*	*Brassica oleracea L. var. capitata* L.	Fruit	8.46±0.22	ND
Eggplant (Ma-kue-meong)*	*Solanum melongena* L.	Fruit	7.48±0.68	0.36±0.48
Cucumber (Tang-gua)*	*Cucumis Sativus* L.	Fruit	4.42±0.22	ND
Piper sarmentosum (Cha-plu)*	*Piper sarmentosum* Roxb.	Leaf	16.46±0.72	ND
Climbing wattle (Cha-om)*	*Acacia pennata* L.	Leaf	33.78±2.41	ND
Holy basil (Ka-praw)*	*Ocimum sanctum* L.	Leaf	12.76±0.88	ND
Chinese cabbage (Pak-kad-kaow)*	*Brassica pekinensis*	Leaf	6.87±0.64	ND
Fingerroot (Kra-chay)*	*Boesenbergia rotunda* L. Mansf.	Root	16.73±1.50	11.79±1.16
Sweet basil (Ho-ra-pa)*	*Ocimum basilicum* L.	Leaf	7.92±0.61	ND
Lemon grass (Ta-krite)*	*Cymbopogon citratus* DC. Stapf	Stem	7.38±0.33	4.69±1.78
Yardlong bean (Tou-fak-yao)*	*Vigna unguiculata subsp. sesquipedalis* L. Verdc.	Fruit	9.27±0.71	ND

Note: GAE = Gallic acid equivalent
QE = Quercetin equivalent
ND = not detected
Values are mean ± SD (n = 3).
* Thai name

Table 2. Inhibitory activities of vegetables on sugars and starch digestion.

Vegetables	Digestion inhibition (%)			
	***p*NPG**[a]	**Maltose**[a]	**Sucrose**[a]	**Starch**[b]
Onion	91.89±9.75	30.19±7.11	19.29±8.49	17.68±13.83
Red onion	6.75±5.68	18.21±7.89	13.83±5.48	NI
Ginger	4.97±4.82	18.13±3.06	19.56±3.03	NI
Daikon	7.56±4.11	68.24±7.71	50.09±13.77	2.02±2.83
Fragrant pandan	19.68±12.17	1.62±4.24	NI	NI
Okra	86.41±23.34	101.61±9.78	100.72±5.45	10.52±7.96
Turkey berry	14.40±4.89	11.16±4.03	12.38±3.59	NI
Garlic	12.41±5.67	3.38±3.92	1.00±5.50	NI
Cabbage	8.00±2.06	19.31±7.31	8.25±13.71	3.13±11.01
Eggplant	11.25±2.12	11.91±2.96	7.96±6.58	3.82±5.38
Cucumber	4.89±2.14	2.77±7.83	NI	3.28±1.52
Piper sarmentosum	6.69±2.07	NI	NI	5.25±3.99
Climbing wattle	NI	2.13±1.45	NI	NI
Holy Basil	97.72±0.36	97.53±0.83	93.53±0.29	0.41±5.77
Chinese cabbage	1.52±2.82	6.87±2.16	NI	1.49±7.13
Fingerroot	28.14±1.57	22.21±10.88	16.59±3.43	NI
Sweet basil	97.38±0.72	95.55±1.42	83.28±1.32	2.98±3.29
Lemon grass	19.24±1.75	10.36±6.66	14.71±7.76	NI
Yardlong bean	7.52±2.00	12.11±3.55	9.55±3.36	4.23±6.42

Note: [a] α-glucosidase inhibition, tested at 1 mg/mL of lyophilized samples
[b] α-amylase inhibition, tested at 5 mg/mL of lyophilized samples
NI = no inhibition
Values are mean ± SD (n = 3).

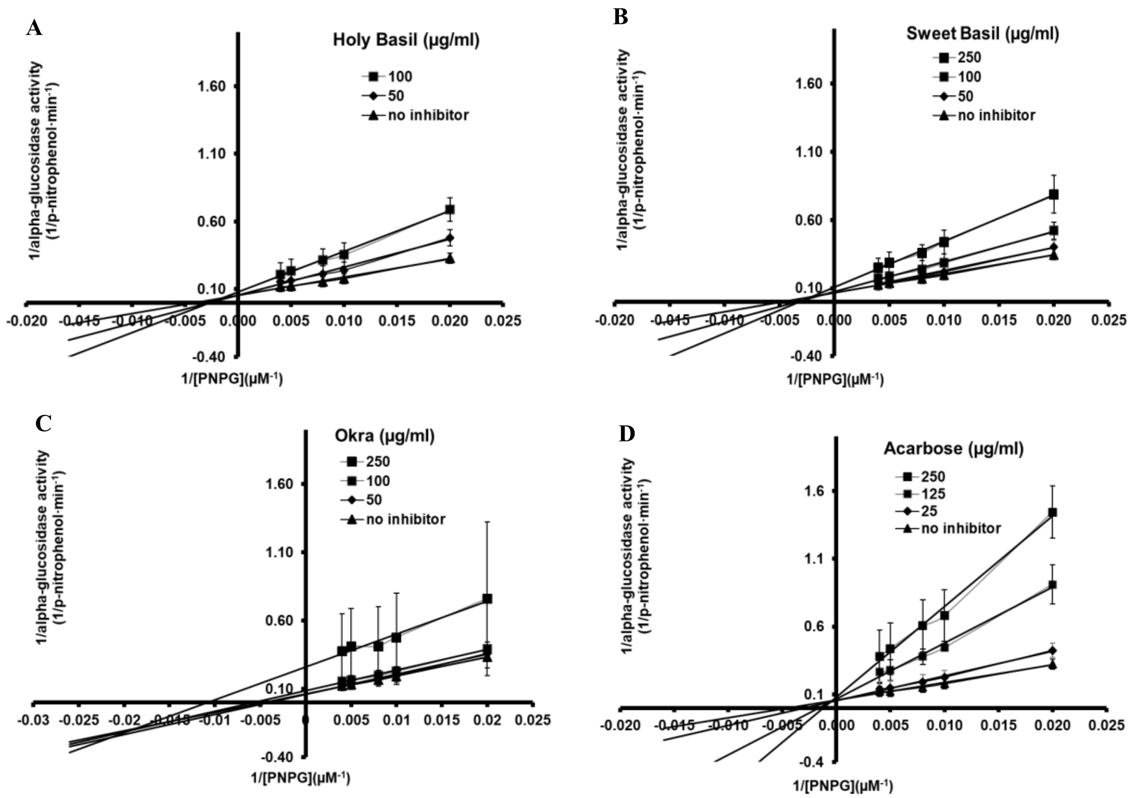

Fig. 1. Enzyme kinetics of α–glucosidase in the presence of holy basil (A), sweet basil (B), okra (C), or acarbose (D). The enzyme activity was tested using *p*NPG as a substrate. Data are displayed as double reciprocal Lineweaver-Burk plots.

Table 3 Mode of inhibition of selected vegetables on α-glucosidase activity.

Vegetables	*Ki* (μg/mL)	Mode of inhibition
Holy basil	25.66 ± 1.19	Non-competitive
Sweet basil	162.98 ± 4.47	Non-competitive
Okra	113.81 ± 3.57	Non-competitive
Acarbose	36.14 ± 1.58	Competitive

Note: Values are expressed as mean ± SD (n = 3).

3.5 The effect of heating on α-glucosidase inhibitory activity of vegetable samples

To examine whether heat affects α-glucosidase inhibitory activity of tested vegetables, samples were boiled prior to testing. The result showed that boiled samples of holy basil, sweet basil, and okra could effectively inhibited sugar digestion, although there are statistically significant changes in some IC_{50} values (Table 4).

In addition, the effect of heat on the vegetable samples was tested using both synthetic and natural substrates as shown in Fig. 2-4. The results indicated that boiling differently affected α-glucosidase inhibitory activities of each vegetable. Although some heated vegetables led to an increase in α-glucosidase activity, the activity of most vegetables were reduced after boiling. Taken together, the effectiveness of sugar digestion inhibition might not be destroyed after cooking the vegetable by boiling.

Table 4. α-Glucosidase inhibitory activity of fresh and boiled holy basil, sweet basil, and okra.

Substrates		IC_{50} (μg/mL)			
		Holy basil	**Sweet basil**	**Okra**	**Acarbose**
***p*NPG**	**Fresh**	48.57±15.45	47.67±20.39	92.86±14.52*	722.3±1.48
	Boiled	65.81±21.57	82.31±29.63	182.48±24.32	
Maltose	**Fresh**	111.70±17.17	95.49±9.39*	165.90±37.38	841.6±3.09
	Boiled	140.20±10.25	115.25±11.46	175.33±43.01	
Sucrose	**Fresh**	102.35±10.17	93.55±4.41*	279.90±59.16	584.3±3.12
	Boiled	130.90±17.54	123.60±10.57	149.10±20.23	

Note: Values are mean ± SD (n = 3 - 4),
* $p \leq 0.05$; fresh versus boiled vegetables

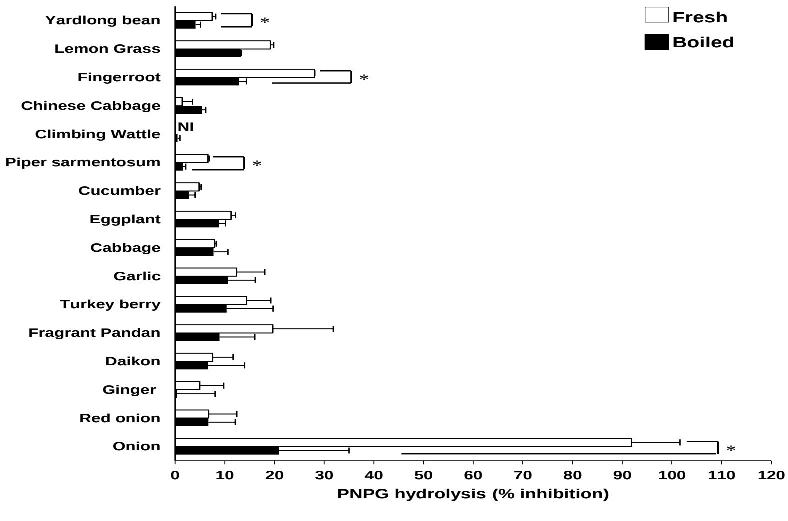

Fig. 2. Inhibitory activity of fresh and boiled vegetables on *p*NPG hydrolysis. All vegetable samples were tested at 1 mg/mL of lyophilized powder. Values are mean ± SD (n = 3). * $p \leq 0.05$

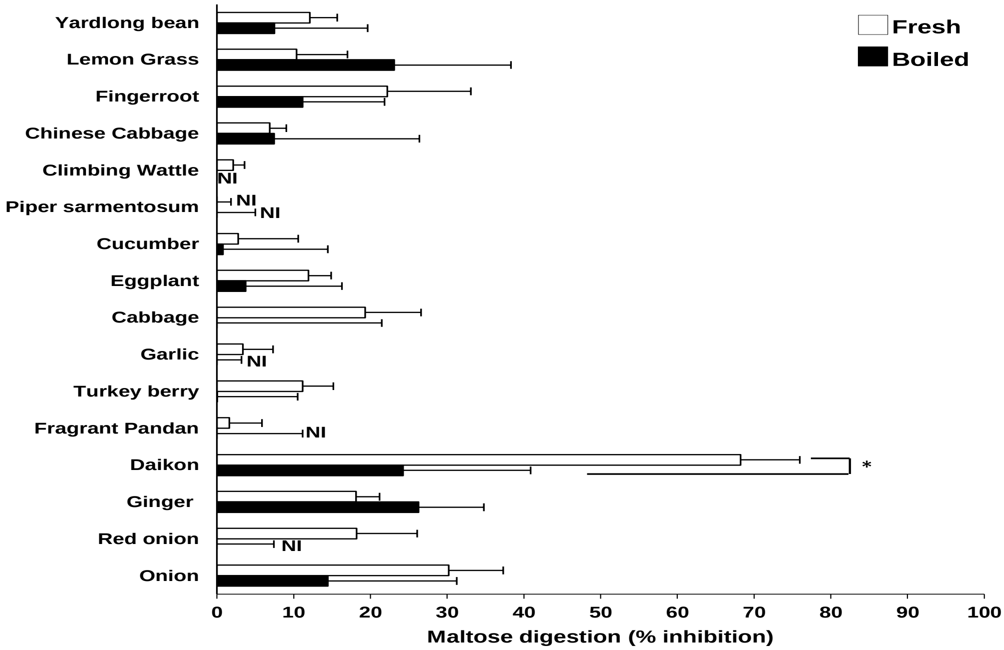

Fig. 3. Inhibitory activity of fresh and boiled vegetables on maltose digestion. All vegetable samples were tested at 1 mg/mL of lyophilized powders. Values are mean ± SD (n = 4). * $p \leq 0.05$

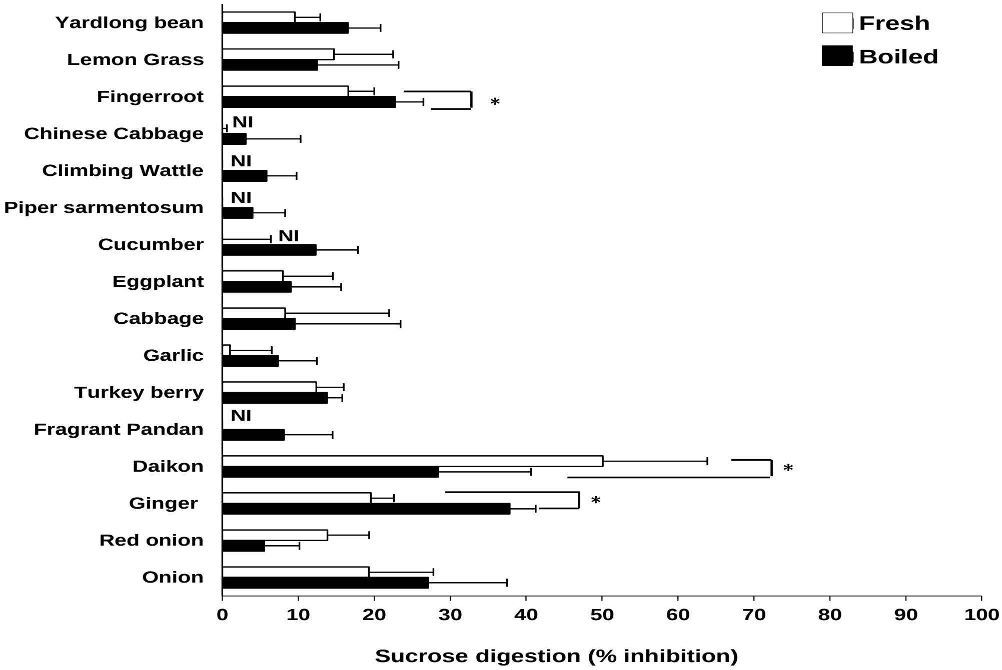

Fig. 4. Inhibitory activity of fresh and boiled vegetables on sucrose digestion. All vegetable samples were tested at 1 mg/mL lyophilized powders. Values are mean ± SD (n = 4). * $p \leq 0.05$

4. Discussion

Consuming a diet with high portion of vegetables is generally considered healthy. Food-based plants were thought to associate with the decreased risks of developing metabolic syndromes such as diabetes. The goal of the present study was to identify types of vegetables having the potential to control blood glucose levels through the interference of sugar and starch digestion. Among 19 vegetables tested, holy basil, sweet basil, and okra demonstrated high potential as α-glucosidase inhibitors. These three vegetables non-competitively inhibited α-glucosidase, and heat (by boiling) did not affect their actions.

Okra has been claimed to have health benefits such as antioxidant, antidiabetic, and antihyperlipidaemic effects.[15,16] According to previous studies, okra fruits showed antidiabetic effect in alloxan-induced diabetic rats in which the mechanism of action to reduce blood glucose and HbA1c was possibly through stimulation of glycogen synthesis and delay of intestinal glucose absorption.[17] α-Glucosidase inhibitory activity of okra was demonstrated from various preparations such as seeds,[18,19] mucilage,[20,21] and peel.[19] Our findings displayed that components in okra juice non-competitively inhibited α-glucosidase and slightly inhibited α-amylase activity. Among all tested vegetables, okra showed high content of phenolic and flavonoid compounds. Phenolic compound might play a role in α-glucosidase inhibitory activity since there is a demonstrated correlation between α-glucosidase inhibition and total phenolic content in aqueous extracts of herbs.[22] Dietary flavonoid-rich fruit and vegetable consumption was shown to improve diabetes-related biomarkers in adults with diabetes.[23]

Sweet basil and holy basil are the two important species of the Lamiaceae family extensively used for traditional medicines due to their anti-diabetic activity.[24] According to our study, both sweet and holy basil effectively inhibited α-glucosidase activity and slightly inhibited α-amylase activity. In previous studies, sweet basil exhibited a certain degree of α-amylase inhibitory activity but with less potency compared to its α-glucosidase inhibitory activity.[25,26] Differences in observation of α-amylase inhibition of sweet basil could be due to the differences in the enzymatic assay procedure and sample preparation. However, most evidence points to sweet basil dominantly inhibiting α-glucosidase over α-amylase. Inhibition of α-glucosidase and α-amylase retards the rate of carbohydrate digestion and consequently reduces postprandial hyperglycemia. In alloxan induced diabetic rats, sweet basil extract (100-200 mg/kg for 4 weeks) reduced blood glucose levels and promoted glucose tolerance.[26] Experiments on streptozotocin (STZ) induced diabetic rats found that both sweet basil leaves and seeds extracts can lower blood sugar levels.[27,28] Fixed oil extracted from holy basil leaves also showed antidiabetic effects in rats.[29]

Cooking encompasses a variety of processes, such as boiling, frying, steaming, etc. that could differently affect the chemical and physical properties of vegetables. Most evidence has reported changes in phytochemical contents of foods but not changes in biochemical activities. A review article reported that among different cooking procedures, steaming is better for preserving yield of phenolic compounds than other cooking methods.[30] Temperature slightly affected the phenolic content in the vegetables prepared by blanching (boiling in water or steam for a short time).[31] In our study, α-glucosidase inhibitory activity of vegetables was variously affected after boiling: increases as well as decreases in activity were observed. According to the α-glucosidase inhibitory activity of okra, sweet basil, and holy basil, boiling might not have a big impact on their carbohydrate digestion inhibitions. The effect of other cooking methods requires further study.

5. Conclusion

Most tested vegetables differently demonstrated α-glucosidase and α-amylase inhibitory activities. Holy basil, sweet basil, and okra showed high potency at inhibiting α-glucosidase enzyme. Boiling seemed not to have much effect on their activity. Therefore, consumption of these vegetables may reduce carbohydrate digestion and delay glucose absorption resulting in reduced postprandial blood glucose.

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Conflicts of Interest

The authors declare no conflict of interest.

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