

Research Article

Alpha amylase inhibition and roasting time of local vegetables and herbs prepared for diabetes risk reducing chili paste

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Abstract

Food consumption behaviour of carbohydrates and sucrose rich food is one of the main causes of non-insulin dependent diabetes mellitus (diabetes type II). This has resulted in an increased number of diabetes patients in Thailand in the recent years. Potent inhibitors of mammalian alpha-amylase found in some vegetables and herbs have been known as effective antidiabetic treatment for diabetes. Therefore, this research was aimed to investigate the change of α -amylase inhibition in chili paste prepared from vegetables and herbs with different roasting time. Local vegetables and herbs which show high potential for diabetes risk reduction used in this study were *Gynura divaricata* DC. (Purple passion vine), *Coccinia grandis* Voigt (Ivy Gourd), *Thunbergia laurifolia* Linn. (Babbler's Bill Leaf.), *Piper retrofractum* Vahl (Long pepper), and *Cyperus rotundus* Linn. (Nut grass). 20% was added into the paste in the form of fresh (0 min) and roasted (5-15 min) materials. Other ingredients and preparation process correspondingly followed a production of a traditional roasted chili paste with dried fish. α -Amylase inhibition of the products were then determined.

It was found that all studied vegetables and herbs showed more than 80% alpha amylase inhibitory activity and this may contribute to enzyme inhibition effect of chili paste which showed high inhibition in most product preparations. In spite of the proportion of these vegetables and herbs being quite high, the products were mostly accepted by 30 untrained panelists. The correlation study of enzyme inhibition and roasting time was found to be second order polynomial regression type. Chili paste containing *Gynura divaricata* DC, *Piper retrofractum* Vahl, and *Cyperus rotundus* Linn. showed a decrease of α -amylase inhibitory activity by the longer time of roasting. In contrast, the enzyme inhibition of chili paste prepared from *Coccinia grandis* Voigt increased from 23.16% to 45.02% during 15 min roasting. Alpha amylase inhibition of chili paste prepared

with roasted *Thunbergia laurifolia* Linn. tended to decrease after 10 min roasting time. This would be studied further in model designs in order to apply in a decision support system for development of Thai food that could be applied to diabetes patients and consumed as healthy food for diabetes risk reduction.

Keywords: *Gynura divaricata*, *Thunbergia laurifolia*, *Coccinia grandis*, *Piper retrofractum*, *Cyperus rotundus*, Thailand.

Introduction

Non-insulin dependent diabetes mellitus (NIDDM or diabetes type II) is mainly caused by a high digestion rate of refined carbohydrates in the upper part of the small intestine and results in a rapid and high postprandial rise in blood glucose. The suppression of mammalian alpha-amylase enzyme in human digestive system would delay the degradation of starch and oligosaccharides to monosaccharides before they can be absorbed. This would decrease the absorption of glucose and consequently reduce postprandial blood glucose level [1]. Therefore, alpha-amylase inhibitors containing medicinal plants, including vegetables and herbs, have long been studied for diabetes therapeutic purpose since the number of diabetes patients increases worldwide. Several researches attempted to isolate active inhibitory compounds from foodstuffs in order to study and understand their functions for pharmaceutical application [2-10]. Local vegetables and herbs including *Gynura divaricata* DC., *Coccinia grandis* Voigt, *Thunbergia laurifolia* Linn., *Piper retrofractum* Vahl, and *Cyperus rotundus* Linn. were found to have high potential in treating and preventing diabetes, as well as various kinds of other illness and diseases [11-23]. They can be generally grown locally and have been commonly used as main ingredients for local meals. In addition, they have also been consumed as drinks, particularly in the form of tea, and Thai-traditional medicine. Chili paste is one of the most common and daily consumed dishes for Thai people. Therefore, addition of anti-diabetic vegetables or herbs into the paste might be an alternative for diabetes patients. However, heating process during the preparation of roasted vegetables and herbs before mixing with other ingredients for the production of the paste may affect the alpha-amylase inhibitory activity [24]. This study was thus aimed to investigate the effect of roasting time of local vegetables and herbs added into the paste on alpha-amylase inhibition change in chili paste. The correlation between roasting time factor and enzyme inhibition was drawn by regression equation. This would then benefit further studies in modelling of the change in enzyme inhibition of chili paste and to predict the change that might occur during the production process.

Materials and Methods

Local vegetables and herbs

Vegetables and herbs used in this study were leaves of *Gynura divaricata* DC. (or *G. procumbens*), *Coccinia grandis* Voigt, and *Thunbergia laurifolia* Linn., fruit of *Piper retrofractum* Vahl and *Cyperus rotundus* Linn.. They were locally collected from Phitsanuloke Province, Thailand. Vegetables and herbs were used in fresh form (0 min roasting time) and roasted type prepared by roasting using open pan at approximately 80°C for 5 to 15 minutes before mixing with other ingredients for chili paste production. Starch azure and porcine pancreatic alpha-amylase used in enzyme inhibition assay were purchased from Sigma Aldrich Japan Co., Tokyo, Japan. All reagents were of analytical grade.

Production of chili paste

Chili paste production was processed corresponding to a traditional process of roasted chili paste with dried fish. The ingredients were dried fish (15 g), roasted chili (15g), shallot (70g), garlic (70

g) and shrimp paste (15g). Tamarind sauce, fish sauce, and sweetener (Equal[®]) were added as seasoning ingredients. Fresh or roasted vegetables and herbs (20% by weight) were firstly mixed with chopped dried fish. Other ingredients were then added and well mixed. The seasoning ingredients were finally added to obtain the acceptable taste of the paste. The control sample was chili paste with no addition of vegetables and herbs.

Alpha-amylase inhibitory activity determination

Raw materials of local vegetables and herbs used in this study were dried in hot air oven at 45°C for 24 h, grounded, then extracted with 50% aqueous MeOH (10 ml/g dry wt.) for 24 h at room temperature. From one part of the extract the solvent was evaporated. The dried residue was redissolved in 50% aqueous DMSO (10 ml/g dry wt.) and subjected to alpha-amylase inhibitory activity [4]. Starch azure used as a substrate was suspended in 0.05 M Tris-HCl buffer (pH 6.9) containing 0.01 M CaCl₂ and boiled for 5 min. The test samples in 50% DMSO, and alpha-amylase (0.21 unit) were added into each assay. The reaction was carried out at 37°C for 10 min and stopped by acetic acid addition. The reaction mixture was then centrifuged and the absorbance of the supernatant was measured at 595 nm. The alpha-amylase inhibition was calculated as follows:

The alpha-amylase inhibitory activity (%)

$$= [(A_{c+} - A_{c-}) - (A_s - A_b)] / (A_{c+} - A_{c-}) \times 100$$

where A_{c+}, A_{c-}, A_s, and A_b are defined as absorbance of 100% enzyme activity (no inhibitor), 0% enzyme activity, test samples, and blank, respectively.

Chili paste products were extracted by homogenizing with 50% aqueous MeOH. The extracts were then filtrated and the solvent was evaporated. The dried sample was determined for alpha-amylase inhibition by following the same method as is used in raw material determination.

Proximate analysis and sensory evaluation

Vegetables and herbs materials and chili paste products were determined for contents of protein, fibre, moisture, ash, fat, and carbohydrate (AOAC methods). Chili paste products were tested for sensory acceptance by 30 untrained panelists using a 9 points hedonic scale (1 = disliked extremely, 9 = liked extremely). The products acceptance was based on colour, odor, taste, texture, and overall acceptance attributes.

Statistical analysis

The sensory results from the hedonic scale were analyzed using the analysis of variances (ANOVA). Correlation of roasting time and alpha- amylase inhibition was studied by regression analysis.

Results and Discussion

Alpha-amylase inhibition of local vegetables and herbs

To investigate the properties of vegetables and herbs used in this study on reduction of diabetes risk, they were determined for alpha-amylase inhibitory activity since this enzyme is known as one of the key enzyme in human digestive system to degrade starch to monosaccharides and cause the rise in blood glucose [25]. The results showed that all of studied samples had higher than 80% inhibitory activity against alpha-amylase (Figure 1). *T. laurifolia* showed the highest activity (99.05%) followed by *P. retrofractum* (95.20%), *G. divaricata* (91.58%), *C. rotundus* (84.23%) and *C. grandis* (81.13%), respectively.

T. laurifolia is a herbal medicine used to treat alcohol and drug addiction in Thai traditional medicine [11]. Its ethanol extract was found to have hepatoprotective activity against ethanol induced liver injury in both primary cultures of rat hepatocyte and rats. [12]. In addition, it was also found that a 15 days treatment of *T. laurifolia* extract decreased levels of blood glucose in diabetic rats [13]. This supports our result which found high amylase inhibition by this plant. Therefore, *T. laurifolia* would be possibly applied to anti-diabetic food product.

P. retrofractum or long pepper is originally of South East Asia and is mostly cultivated in Indonesia and Thailand. The used plant part is the fruit, the tiny berries, which merge to a single, rod-like structure which bears some resemblance to catkins (flowers of trees like hazelnut or willow). The sensory quality of *P. retrofractum* is hot and warm, with sweet overtones. It has been used in traditional medicines as stimulant, carminative, tonic and for postpartum women (fruit); antihypertensive and muscle relaxant (plant); and antifungal (stem). Its main constituent is piperine and is slightly higher in proportion than in black pepper (about 6%) [26]. This active compound derived from the fruit of *P. retrofractum* showed insecticidal activity against larvae of plants insects [23].

G. divaricata is found in various parts of Asia and widely used in Thailand and South East Asia as a traditional medicine. In Thailand, the aerial part of *G. divaricata* is used as a topical therapy for the treatment of inflammation, rheumatism and viral diseases of the skin [14]. The possible chemical constituents in the extracts and fractions were investigated and it was found that steroids might be one class of anti-inflammatory compounds in this plant. [15].

A herbal mixture with *C. rotundus* reduced stress-related physiological and psychological symptoms. [16]. It was also found that *C. rotundus* possess activity against contraction of the ileum, anti-pyretic and analgesic activities, anti-inflammatory and bactericidal properties. [17-20]

The ethanol extract of *C. grandis* showed significant triglyceride (TG) and cholesterol-lowering effects in dyslipidemic hamster model. The compound polyprenol isolated from leaves of *C. grandis* possess marked antidyslipidemic activity.[21]. *C. grandis* also showed the DPPH radical scavenging activity and significantly decreased the formation of oxygen radicals generated in rat peritoneal macrophages. [22]

From the studied results on biological functions of these plants it can be seen that there are no reports on anti-diabetic properties neither in vitro nor in vivo studies. However, our investigation revealed their high inhibition against alpha-amylase enzyme which corresponds to reports of their uses in traditional medicines for diabetes treatment. Therefore, addition of these plants into meals might possibly reduce the blood glucose level in diabetes patients.

Proximate analysis of raw material and chili paste

The studied vegetables and herbs showed high fibre content, particularly in *T. laurifolia* which showed the highest value followed by *G. divaricata*, *C. rotundus*, *C. grandis*, and *P. retrofractum*, respectively (Table 1). It can be seen that apart from their alpha-amylase inhibitory properties, the high fibre containing materials used for chili paste products would be beneficial to health. However, the fibre content determined in chili paste prepared from all herbs were low since only 20% (by weight) of vegetables and herbs were added into the paste (Table 2). This induced a concern on finding the optimal contents of herbs in the paste to obtain higher fibre content with the acceptable sensory qualities for consumers.

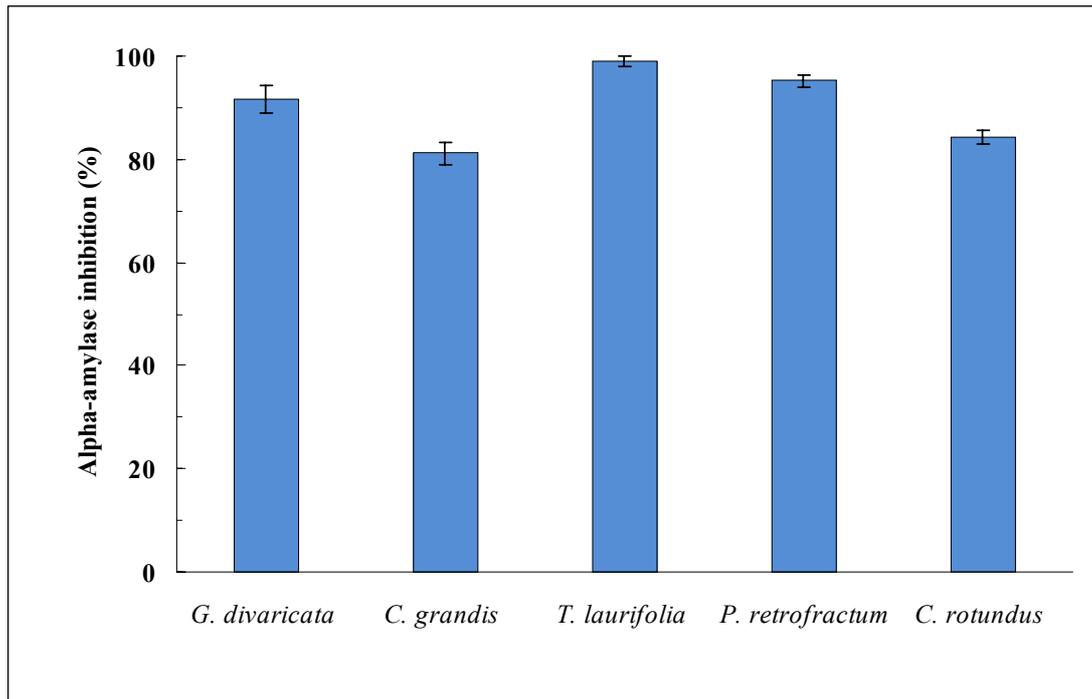


Figure 1. Alpha-amylase inhibitory activity of local vegetables and herbs extracted with 50% aqueous methanol.

The extract of 1 g (dry wt. basis) is reacted with 0.21 unit of alpha amylase enzyme.

Table 1. Proximate analysis of local vegetables and herbs (% dry weight basis).

Vegetables and herbs	Fibre (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)
<i>G. divaricata</i>	16.53 ± 0.59	22.02 ± 0.13	17.67 ± 0.07	5.99 ± 0.13	37.78 ± 0.50
<i>C. grandis</i>	12.47 ± 0.06	20.28 ± 0.70	31.63 ± 0.37	5.82 ± 0.33	29.81 ± 0.94
<i>T. laurifolia</i>	16.82 ± 0.74	18.79 ± 0.54	16.70 ± 0.30	1.68 ± 0.04	46.01 ± 0.13
<i>P. retrofractum</i>	9.73 ± 0.47	7.37 ± 0.57	15.26 ± 0.06	7.48 ± 0.28	60.16 ± 0.87
<i>C. rotundus</i>	14.26 ± 0.64	4.97 ± 0.06	9.04 ± 0.32	1.36 ± 0.14	70.36 ± 0.02

Table 2. Proximate analysis of chili paste containing local vegetables and herbs.

Vegetables and herbs	Moisture (%)	Fibre (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)
<i>G. divaricata</i>	63.61 ± 0.17	2.66 ± 0.35	4.82 ± 0.13	12.38 ± 0.19	0.29 ± 0.00	16.23 ± 0.40
<i>C. grandis</i>	55.42 ± 0.12	2.10 ± 0.10	4.95 ± 0.25	13.21 ± 0.17	0.91 ± 0.12	23.41 ± 0.43
<i>T. laurifolia</i>	62.12 ± 0.20	2.04 ± 0.21	4.75 ± 0.21	12.51 ± 0.23	0.22 ± 0.05	18.37 ± 0.55
<i>P. retrofractum</i>	57.88 ± 0.52	2.88 ± 0.21	4.97 ± 0.12	13.55 ± 0.40	1.13 ± 0.17	19.59 ± 0.38
<i>C. rotundus</i>	57.62 ± 0.11	2.46 ± 0.08	4.94 ± 0.28	13.01 ± 0.21	2.48 ± 0.05	19.49 ± 0.49

The effect of roasting time on alpha-amylase inhibitory activity of chili paste

In the preparation process of Chili paste production, fresh samples of vegetables and herbs were roasted using open pan with high temperature (about 80°C) for 0 to 15 min before mixing with all other ingredients and seasoning materials. This was based on the hypothesis that heating might affect the release of active compounds for enzyme inhibition into the product [24]. The results showed that alpha-amylase inhibition of the paste prepared from *G. divaricata* continuously decreased during prolonged roasting time (Figure 2.). Its enzyme inhibitory activity reduced from

74% to 54% after 15 min roasting. This probably due to the structural characteristic of *C. divaricata* leaves that is easily destroyed by high temperature and results in inactivated inhibitory function of active compounds. Contradictory result was found in *C. grandis* added paste in which the enzyme inhibition increased quite sharply from fresh material (23%) to 10 min roasted sample (45%) and there was less change in enzyme inhibition at 15 min roasting (Figure 3). In this case, heating might caused an increase in alpha-amylase enzyme inhibition. *T. laurifolia* showed quite similar result to *C. grandis* in which the enzyme inhibition increased during 10 min roasting (from 35% to 59%) and tended to decrease after that (Figure 4.). This may be explained by the fact that in certain heating time the inhibitory function of potent compounds would be expressed. Similar results were found in a study of alpha-glucosidase inhibition in mulberry leaf tea [27]. There were a slight change of enzyme inhibition found in *P. retrofractum* and *C. rotundus* during 10 min roasting time although at 15 min these activities decreased to 35% in both products (Figures 5 and 6). This can be explained as mentioned earlier in case of *T. laurifolia*.

The correlation between alpha-amylase inhibition by chili paste containing vegetables and herbs and roasting time was found to be second order polynomial regression and R-square values were nearly 1. These results suggested that a modeling study of inhibition changes during production process by controlling the major key factor, like roasting time, could be drawn. Thus, it would be useful for decision support system to help developing products for anti-diabetic purpose.

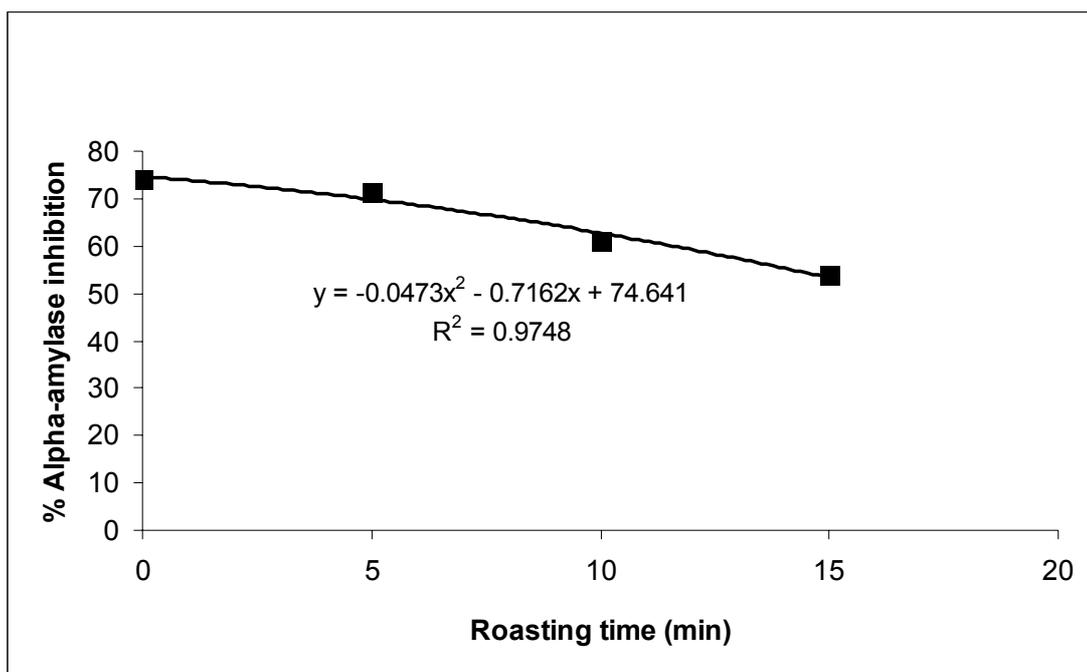


Figure 2. Alpha-amylase inhibition of chili paste containing *Gynura divaricata* DC with different roasting time.

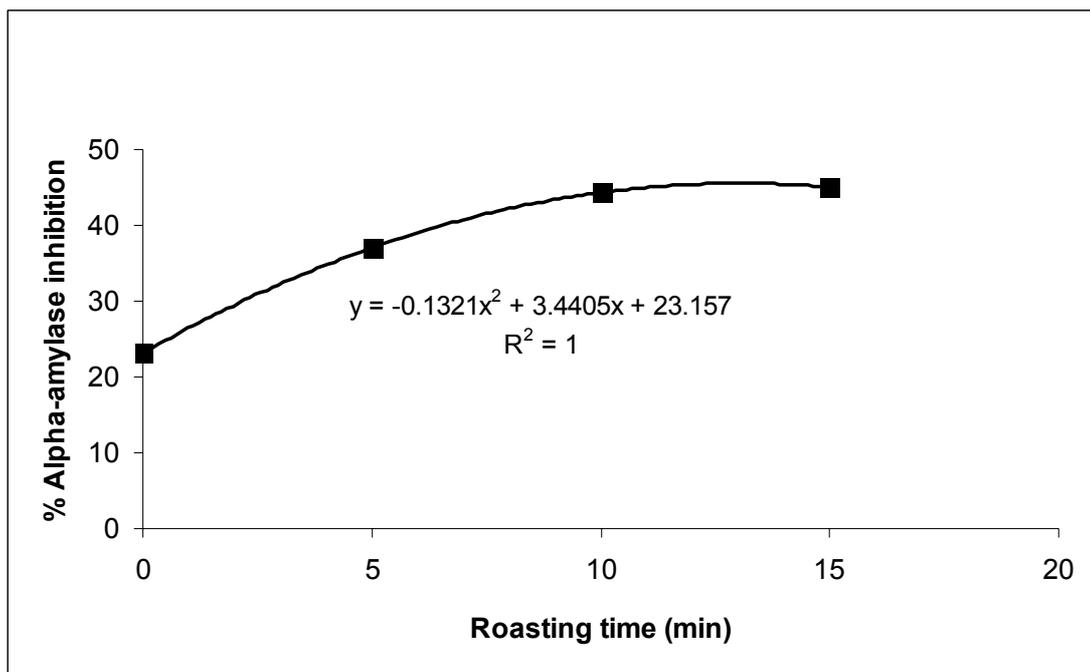


Figure 3. Alpha-amylase inhibition of chili paste containing *Coccinia grandis* Voigt with different roasting time.

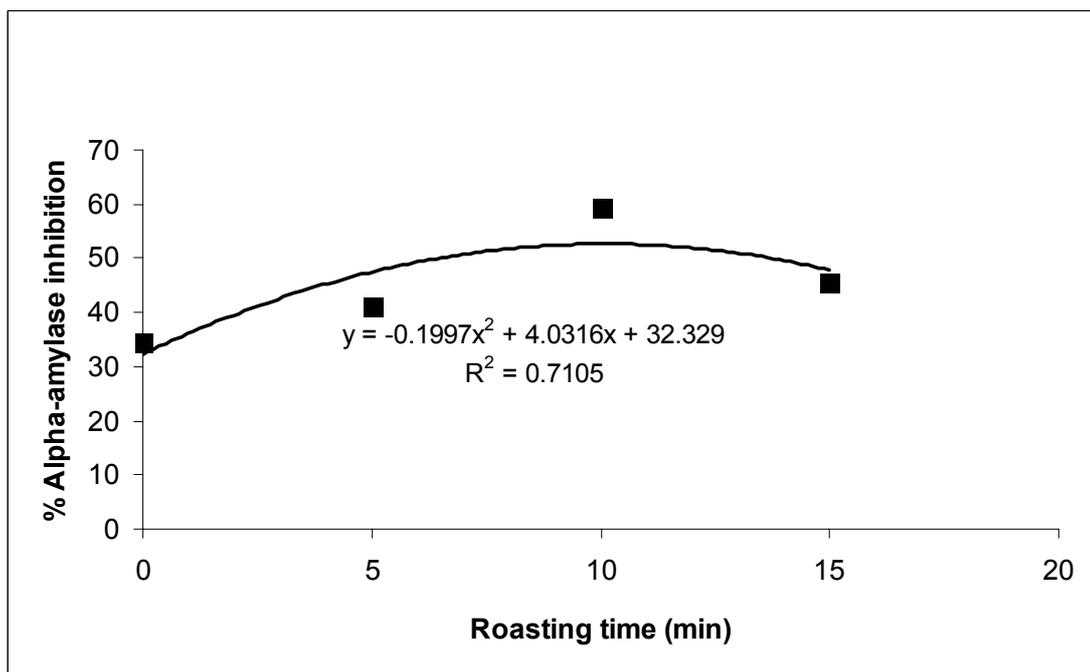


Figure 4. Alpha-amylase inhibition of chili paste containing *Thunbergia laurifolia* Linn with different roasting time.

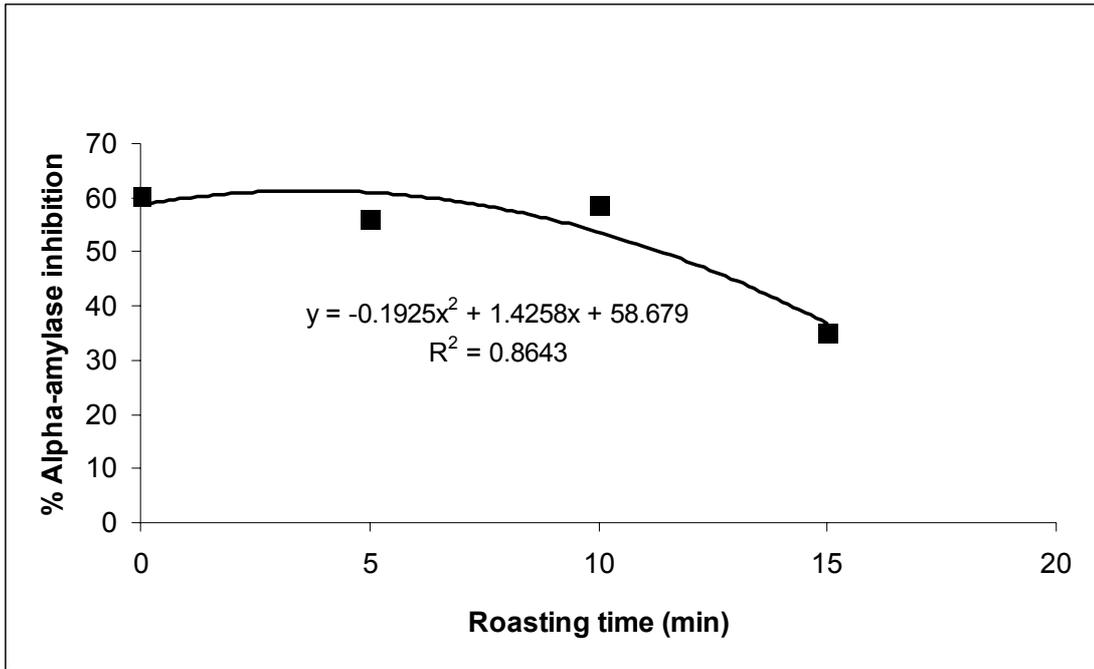


Figure 5. Alpha-amylase inhibition of chili paste containing *Piper retrofractum* Vahl with different roasting time.

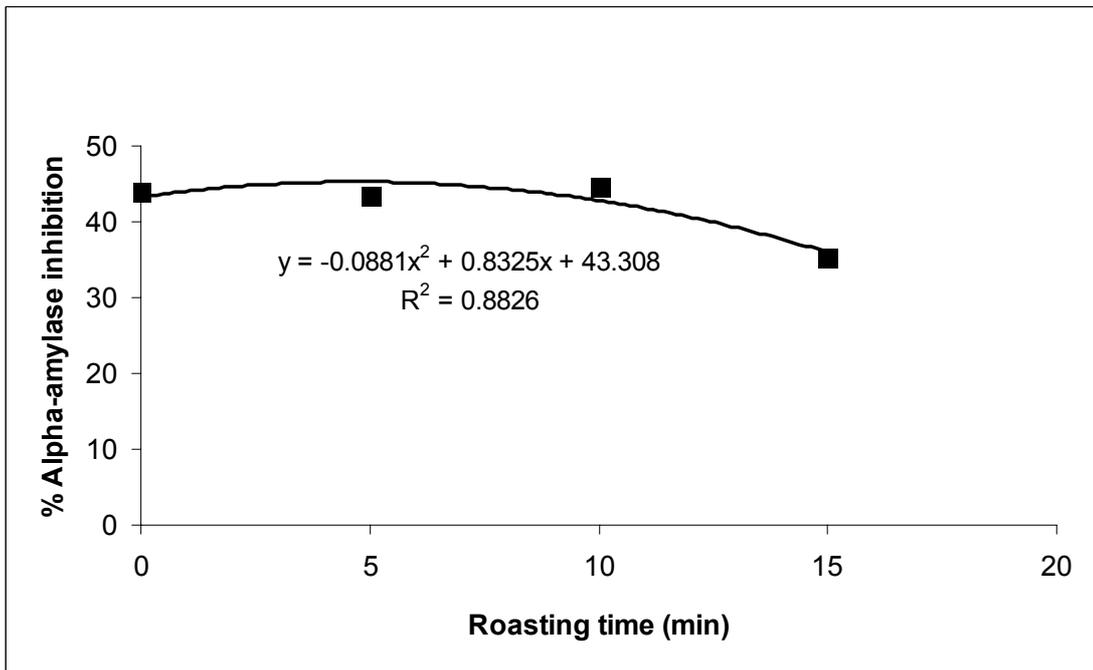


Figure 6. Alpha-amylase inhibition of chili paste containing *Cyperus rotundus* Linn with different roasting time.

Sensory evaluation of chili paste product

Local vegetables and herbs of 20% (by weight) were added into the paste since the preliminary study on sensory acceptance of the paste revealed unacceptable preference on the paste with more than 20% herbs addition. The sensory evaluation study was performed on chili pastes included 5

min roasted vegetables and herbs due to that they obtained the highest scores for overall acceptance (data not shown). The results shown in Table 3 found that most of the sensory attributes of all herbs added pastes were about the level of “like slightly”. The overall acceptance score of *G. divaricata* and *T. laurifolia* added pastes were the highest (like slightly) followed by *C. grandis*, *P. retrofractum* and *C. rotundus* which were accepted by the level of “neither like nor dislike”. The preference in terms of colour, pastes containing *G. divaricata* and *P. retrofractum* obtained the highest acceptance score tested by the panelists. In addition, *G. divaricata* and *T. laurifolia* added pastes showed the most acceptable odor. The results also showed that the panelists disliked slightly the paste incorporating *C. rotundus*. This is probably due to the strong odor of *C. rotundus* itself. It can also be seen from the results that *T. laurifolia* added paste showed the highest score of taste attributes, followed by pastes containing *C. grandis*, *C. rotundus*, and *P. retrofractum*, respectively.

In addition it was found that the acceptance level of texture were about “like slightly” and similar in all pastes samples. It can be seen from the result that sensory acceptance score of the control sample (without herbs addition) was about one point higher (like very much) than the pastes that contained herbs. Thus it can be assumed that the addition of herbs into chili paste slightly lowered panelists preferences from paste produced by the standard formula and traditional process.

Table 3. Sensory acceptance of chili paste products containing local vegetables and herbs.

Vegetables and herbs	Sensory attributes				Overall acceptance
	Colour	Odor	Taste	Texture	
Control	7.96	7.32	7.18	6.82	7.48
<i>G. divaricata</i>	6.80	6.80	6.15	6.10	6.60
<i>C. grandis</i>	5.50	5.95	5.9	5.90	5.70
<i>T. laurifolia</i>	5.90	6.25	9.15	6.05	6.25
<i>P. retrofractum</i>	6.55	5.65	5.15	6.05	5.60
<i>C. rotundus</i>	5.05	4.90	5.25	6.05	5.20

Conclusion

Local vegetables and herbs used in this study showed high inhibitory effect against alpha-amylase enzymes. Therefore, it would be possible to consume products incorporating these plants for effective anti-diabetic purposes. This notion is supported by the results of this study which found the enzyme inhibitory activity of chili paste product prepared incorporating those herbs. However, the enzyme inhibition could be changed by the production process, particularly roasting time of herbs before addition into the paste. It could be concluded that 10 min roasting time of herbs provided the highest inhibitory activity for most of the pastes. Nevertheless, roasting the herbs for 5 min resulted in the highest acceptance scores tested by the panelists and pastes prepared from *G. divaricata* and *T. laurifolia* showed the most likeness in terms of overall acceptance. In addition, the relationship between changes of enzyme inhibition and roasting time suggested that further study on modelling design should be done in order to develop the decision support system for the production of anti-diabetic food products.

Acknowledgement

The authors would like to thank the National Research Council of Thailand for research funding.

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