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Research Article

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

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Abstract

Consumption behaviour of carbohydrate and sucrose rich food is one of main causes of non-insulin dependent diabetes mellitus (diabetes type II). This results in an increase in the number of diabetes patients in Thailand in 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 alpha 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., *Coccinia grandis* Voigt, *Thunbergia laurifolia* Linn.., *Piper retrofractum* Vahl and *Cyperus rotundus* Linn.. They were added into the paste to make up 20% in the form of fresh (0 min) and roasted (5-15 min) materials. Other ingredients and preparation processes correspondingly followed the production of a traditional roasted chili paste with dried fish. Alpha amylase inhibition of the products were then determined. The result found that all of the studied vegetables and herbs showed more than 80% alpha amylase inhibitory activity and this may contribute to the enzyme inhibition effect of chili paste, resulting in this high level of inhibition. Despite the added portion 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 alpha amylase

inhibitory activity the longer the time of roasting. This was in contrast to enzyme inhibition of chili paste prepared from *Coccinia grandis* Voigt which increased from 23.16% to 45.02% during 15 min roasting. Alpha amylase inhibition of chili paste added with roasted *Thunbergia laurifolia* Linn. tended to decrease after 10 min roasting time. This will be further studied to develop design models for application 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: diabetes, alpha amylase inhibition, local vegetables, herbs, Chili paste

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. Suppression of the mammalian alpha-amylase enzyme in the 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 found in medicinal plants, including vegetables and herbs, have long been studied for diabetes therapeutic purposes since the number of diabetes patients continues to increase worldwide. Much research work has focused on attempting to isolate active inhibitory compounds from food products 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 cure and prevention of diabetes, apart from their use in the treatment of various kinds of illness and disease [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, the addition of anti-diabetic vegetables or herbs into the paste might be an alternative for diabetes patients. However, the heating process during the preparation of roasted vegetables and herbs before mixing with other ingredients for the production of the paste may affect alpha-amylase inhibitory activity [24]. This study was thus aimed at investigating 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 be of benefit for further study in modelling of the change in enzyme inhibition of Chili paste and to predict the changes that might be affected by 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 the processed type prepared by roasting using an 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 analytical grade from commercial sources.

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), shallots (70g), garlic (70g) and shrimp paste (15g). Tamarind sauce, fish sauce and sweetener (Equal® was used in this study) 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, ground and 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 re-dissolved 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 $CaCl_2$ 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, 0% enzyme activity, test samples, and blank, respectively.

Chili paste products were homogenized with 50% aqueous MeOH for 24 h and alpha-amylase inhibition was determined followed the same method used in raw material determination.

Proximate analysis and sensory evaluation

Vegetable and herb materials and chili paste products were examined 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 9 point hedonic scales (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 examined for alpha-amylase inhibitory activity since this enzyme is known as one of the key enzymes in the human digestive system to degrade starch to

monosaccharides and cause the rise in blood glucose [25]. The results found that all of the studied samples showed higher than 80% inhibitory activity against alpha-amylase (Figure 1.). *T. laurifolia* showed the highest activity (100%) and 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 the 15-day-treatment of *T. laurifolia* extract decreased levels of blood glucose in diabetic rats [13]. This supports the results of this research in which high amylase inhibition was determined in this plant. Therefore, *T. laurifolia* would have high potential for application as an anti-diabetic food product.

P. retrofractum or long pepper is originally from 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 medicine as a stimulant, carminative, tonic and for postpartum women (fruit); antihypertensive and relaxes muscles (plant); antifungal (stem); and colic (root). The main constituent is piperine and content is slightly higher than in black pepper (about 6%) [26]. This active compound derived from the fruit of *P. retrofractum* also showed insecticidal activity against larvae of plants insects [23].

G. divaricata is found in various parts of Asia and is 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 antinflammatory compounds in this plant. [15].

The herbal mixture with *C. rotundus* reduces 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 a dyslipidemic hamster model. The compound polyprenol isolated from leaves of *C. grandis* possesses 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, nor *in vitro* or *in vivo* studies. However, our investigation revealed their high inhibition against alpha-amylase enzymes and corresponds to reports of use in traditional medicine for diabetes treatment. Therefore, addition of these plants into meals might possibly reduce the blood glucose level in diabetes patients.

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

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 alpha-amylase inhibition properties, the materials with high fibre content 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 suggested a need to find the optimal contents of herbs in the paste to obtain higher fibre content while retaining the acceptable sensory attributes for consumers.

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

Vegetables and herbs	Fibre (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)
G. divaricata	16.53	22.02	17.67	5.99	37.78
C. grandis	12.47	20.28	31.63	5.82	29.81
T. laurifolia	16.82	18.79	16.70	1.68	46.01
P. retrofractum	9.73	7.37	15.26	7.48	60.16
C. rotundus	14.26	4.97	9.04	1.36	70.36

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

Vegetables and herbs	Moisture (%)	Fibre (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)
G. divaricata	63.61	2.66	4.82	12.38	0.29	16.23
C. grandis	55.42	2.10	4.95	13.21	0.91	23.41
T. laurifolia	62.12	2.04	4.75	12.51	0.22	18.37
P. retrofractum	57.88	2.88	4.97	13.55	1.13	19.59
C. rotundus	57.62	2.46	4.94	13.01	2.48	19.49

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 an 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 to the products [24]. The results showed that alpha-amylse 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 is probably due to the structural characteristics of C. *divaricata* leaves which are easily destroyed by high temperature and this results in inactivated inhibition function of active compounds. The contradictory result was found in *C. grandis* added paste by 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 cause an increase in alpha-amylase enzyme inhibition. *T. laurifolia* showed quite similar results 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 inhibition function of potent compounds would be expressed. Similar results were found in a study of alpha-glucosidase inhibition in mulberry leaves tea [27]. There was slight change of enzyme inhibition found in *P. retrofractum* and *C. rotundus* during 10 min roasting time and at 15 min they decreased to 35% in both products (Figures 5 and 6). This can be explained as mentioned earlier in case of *T. laurifolia*.

The correlation study of alpha-amylase inhibition and roasting time revealed that Chili paste containing vegetables and herbs second order polynomial regression and R-square values were nearly to 1. From the results it is suggested that a modelling study of inhibition changes during production process by controling the major key factors like roasting time could be drawn. Thus it would be then useful for decision support system to help developing the product for anti-diabetic purposes.

Figure 2. Alpha-amylase inhibition of Chili paste containing *G. divaricata* 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 paste since the preliminary study on sensory acceptance of paste revealed unacceptable preference on the paste with more than 20% herbs addition. The sensory evaluation study was performed on Chili pastes added with 5 min roasted vegetables and herbs due to their obtaining 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 highest (like slightly), followed by *C. grandis*, *P. retrofractum* and *C. rotundus,* which were accepted by the level of neither like nor dislike. In terms of colour, pastes containing *G. divaricata* and *P. retrofractum* obtained the highest acceptance score tested by the panelists, while *G. divaricata* and *T. laurifolia* showed the most acceptable odor. The results also showed that the panelists disliked slightly the paste added with *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 for taste attributes, followed by pastes containing *C. grandis, C. rotundus and P. retrofractum*, respectively.

It was found that the acceptance level of texture were about "like slightly" and similar in all paste samples. It can be seen from the results that the sensory acceptance score of the control sample (without herbs addition) was about one point upper (like very much) from the pastes containing herbs. From this it can be assumed that the standard formula and traditional process of produced Chili paste may not reach the high score of acceptance by the panelists.

Table 3. **The sensory acceptance of Chili paste products containing local vegetables and herbs.**

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

Conclusion

Local vegetables and herbs used in this study showed high inhibitory effects against alpha-amylase enzymes. Therefore, there is potential for consuming their derived products for effective anti-diabetic purposes. This was supported by the results of this study which found the enzyme inhibitory activity of Chili paste product prepared by 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 would provide the highest inhibition activity for most of the pastes. Nevertheless, roasting the herbs for 5 min resulted in highest acceptance scores tested by the panelists and pastes prepared from *G. divaricata* and *T. laurifolia* showed the highest acceptance in terms of overall liking. 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.

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