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Research Article
Inhibition Activity of α-amylase by Crude Acidic Water Extract from Fresh Purple Sweet Potato (*Ipomoea batatas* L.) and its Modified Flours

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Abstract

Background and Objective: Crude acidic water extracts contains phenolic compounds including anthocyanin, a very important constituent in purple sweet potato. The aim of this study was to analyzed the *in vitro* inhibition of α-amylase activity in purple sweet potato. **Materials and Methods:** Four treatments; fresh purple sweet potato, original purple sweet potato flour, partially gelatinized purple sweet potato flour and resistant starch-rich purple sweet potato flour were set up in a Complete Randomized Block Design (CBRD) replicated 4 times. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) were used to examine the effect of the treatments as well as the difference among treatments. **Results:** The results showed that crude extract of purple sweet potato and modified purple sweet potato flours significantly affected the inhibition of α-amylase enzyme activity. The inhibition activity by resistant starch rich-purple sweet potato flour (TP) was 41.98%, partially gelatinized purple sweet potato flour (TG) was 32.59%, original purple sweet potato flour (TU) was 30.72% and fresh purple sweet potato (US) was 23.13%. **Conclusion:** The results proved that crude water extract from purple sweet potato and its original flour as well as modified flours had significant role in inhibiting α-amylase enzyme activity.

Key words: α-amylase, anthocyanin, crude extract, enzyme activity, resistant starch rich- purple sweet potato flour

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the agriculture product categorized as a potential benefit to health is purple sweet potato (*Ipomoea batatas* L). Initially sweet potatoes commonly found were only white and yellow flesh. Since the development of 2 purple sweet potato varieties from Japan, namely Ayamurasaki and Yamagawamurasaki, the cultivation and utilization of purple sweet potato has increased partly because of its potential health benefit. Nurdjanah *et al.*[1] reported that crude water extract anthocyanin of purple sweet potato and its products showed inhibition to glucosidase enzyme activity. The inhibition of α-glucosidase is reported to be helpful to maintain blood glucose level for people suffering from diabetes mellitus.

Purple color on purple sweet potato is caused by the anthocyanin content found in the skin and flesh of purple sweet potato. Anthocyanin compound in local purple sweet potatoes ranges from 0-210 mg/100 g fresh weight[2,3]. The anthocyanin pigment contained in purple sweet potato is a mono or diacetyl form of peonidin and cyanidin which acts as antimutagenic, anticarcinogenic, prevents liver dysfunction, anti-hypertensive and anti-hyperglycemic so that it can be used as functional food for diagnosed people with diabetes mellitus[4]. Matsui *et al.*[5] also stated that anthocyanin from purple sweet potato showed inhibitory effect on maltase enzyme in intestinal membrane.

Purple sweet potato is one of the perishable product and therefore, the anthocyanin content often undergoes degradation before consumed or utilized. One way to prevent anthocyanin lost in purple sweet potato is processing it into flour as well as modified flour. In addition to its ability to maintain anthocyanin content, modified purple sweet potato flour in the form of resistant starch-rich purple sweet potato flour has the potential to be used as functional food. Nurdjanah *et al.*[1] reported that anthocyanin crude extract from physically modified purple sweet potato flour had higher ability to inhibit α-glucosidase enzyme compared to those of purple sweet potato in the form of fresh or flour.

Enzyme α-amylase has similar roles and mechanism in the digestive system for breaking down starch into simple sugars. However, the information on inhibition capacity of crude water extract of purple sweet potato and its modified flours on α-amylase has not been revealed. This study aims to analyze the ability of crude acidic water extract of purple sweet potato and modified purple sweet potato flour to inhibit the activity of the α-amylase enzyme.

MATERIALS AND METHODS

Study area: This experiment was conducted at Agriculture Product Processing Laboratory, Chemistry and Biochemistry Laboratory, University of Lampung, Indonesia from April-November, 2018.

Materials and equipment: The main material used in this study was purple sweet potato with distinctive characteristics, which was brownish from outer skin color and very dark purple flesh color and was sent directly from Padang, West Sumatra, Indonesia. Other materials used for analysis were fungal α-amylase, soluble starch, dinitro salicylate (DNS), NaOH, aquadest, phenol, PP indicator, sodium metabisulfite, phosphate buffer and glucose. The equipment used in this study included aluminum foil, filter cloth, grinders, glassware, hummer mill, hot plate, oven blower, 80 mesh sieve, vortex, M054-E093J electronic balance (Shimadzu), incubator, micro pipette, centrifuge, refrigerator and Genesis 10S UV-Vis spectrophotometers.

Preparation of original purple sweet potato flour: The original purple sweet potato flour was prepared according to Nurdjanah *et al.*[3]. First, purple sweet potatoes are sorted and washed until they were clean, then drained. Then, the purple sweet potatoes were peeled and sliced with a thickness of 1 mm. The sliced purple sweet potatoes were then weighed as much as 200 g and dried in an oven at 60°C for 24 h. After being cooled at 25°C for 15 min (room temperature), dried purple sweet potato chip was ground using a hummer mill (Ramesia, FCT-Z300, Fomac Food Processing Machines, Indonesia).

Preparation of partially gelatinized purple sweet potato flour: The partially gelatinized purple sweet potato flour was prepared by washing and peeling fresh purple sweet potatoes, then sliced with a thickness of 1 mm and then weighed as much as 200 g followed by a heating process using a rotary cooker (KirinKBO 200RA low watt, which was slightly modified in the temperature control and rotating blade) at temperature of 90°C for 25 min and then cooled at 25°C for 15 min (room temperature). The samples then were dried using an oven at 60°C for 24 h and cooled at room temperature. Then the dried purple sweet potato chips were ground using a hummer mill[1,3].

Preparation of resistant starch-rich purple sweet potato flour: The preparation of resistant starch-rich purple sweet potatoes were carried out using the method developed by Nurdjanah *et al.*[1]. Purple sweet potatoes were washed thoroughly, drained, peeled and sliced with a thickness of 1 mm, then weighed up to 200 g followed by heating in a rotary cooker at 90°C for 25 min. As the heating process was finished, it was continued by cooling at 25°C for 15 min (room temperature), then the samples were stored in a refrigerator with temperature of 5°C for 48 h, then the samples were dried using an oven at 60°C for 24 h. After the samples were dried, then they were ground using a hummer mill.

Preparation of sample's extracts: Extraction of samples (fresh purple sweet potato, original purple sweet potato flour, partially gelatinized purple sweet potato flour and resistant starch-rich purple sweet potato flour) were carried out using a method developed by Rodriguez-Saona and Wrolstad[6]. Each sample was weighed up to 25 g. Furthermore, 0.2% of citric acid solution up to 250 mL was added. The pH of the solvent before and after the addition to the sample was measured using a pH-meter to determine the change in pH in each sample. Then the sample's extracts were agitated for 24 h using shaker in dark room at 25°C (room temperature). The extract was then filtered using a Buchner funnel Capacity 30 mL (Sigma-Aldrich).

Total phenolic and anthocyanin analysis: The Folin-ciocalteu reagent assay as described by Singleton and Rossi[7] was employed to quantify the total phenolic compound of crude water extract samples. Total anthocyanin content was quantified using the colorimetric pH-differential method developed by Lee *et al.*[8].

Inhibition of α-amylase assay: Inhibition of α-amylase enzyme was determined using the method of Kidane *et al.*[9] with slight modification. A 50 µL sample, crude water extract of fresh sweet potato or its flours was pipetted into a test tube and 25 µL of 20 mM phosphate buffer pH 6.9, containing α-amylase from *Aspergillus oryzae* powder, ≥30 units mg^{-1} protein (Merck, CAS Number: 9001-19-8) at a concentration of 0.5 mg mL^{-1}, kept at 25°C for 10 min. After that, 25 µL of 0.5% soluble starch (Sigma-Aldrich) solution in 20 mM phosphate buffer, pH 6.9 was added. The mixtures were re-stored at 25°C for 10 min. The reaction was terminated with 50 µL of 96 mM 3, 5-dinitrosalicylic acid (DNS) (Sigma Aldrich Chemical Co, USA) color reagent. The screw capped test tubes were then boiled in a boiling water bath for 5 min and cooled to room temperature. Absorbance of the sample (Abs Sample) was read at 540 nm using UV-Vis Genesys 10S UV-VIS Spectrophotometer (Thermo Scientific Catalog No. 840-209700). Another test tube was prepared up using the same procedure but without addition of extracted sample (Abs Control). Inhibition (%) was calculated using equation[9]:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Samples}}}{\text{Abs}_{\text{Control}}} \times 100$$

Statistical analysis: The non-factorial experiment was set up in a Complete Randomized Block Design (CRBD) replicated 4 times. The treatments were fresh purple sweet potato (US), original purple sweet potato flour (TU), partially gelatinized purple sweet potato flour (TG) and resistant starch-rich purple sweet potato flour (TP). The homogeneity and the additivity of the data were analyzed using Bartlett and Tukey tests. Homogeneous data were then subjected to analysis of variance (ANOVA) using SPSS 16.0 software to determine whether there was an effect of treatments. Data were analyzed further using Duncan Multiple Range Test (DMRT) to find out the differences among treatments at $p<0.05$.

RESULTS

Total phenol: Total phenol compound contained in crude acidic water extract of purple sweet potato and modified flour products ranged from 737.09-2218.64 mg/100 g dry weight. The results showed that the treatments of the modified technique in producing purple sweet potato flour had a very significant effect on the total phenol of fresh purple sweet potato and modified purple sweet potato flours. Figure 1 shows that the total phenol of fresh purple sweet potato (US) was the highest, then followed by that of in the original purple sweet potato flour (TU), then that of in the resistant starch-rich purple sweet potato flour (TP) which was not significantly different from partially gelatinized purple sweet potato flour (TG), but significantly different from original purple sweet potato flour (TU) and fresh purple sweet potato (US).

Total anthocyanin: Total anthocyanin in fresh purple sweet potato and its flours ranged from 117.98-237.26 mg/100 g dry weight. The results showed that the modified techniques of purple sweet potato flour significantly affected the total anthocyanin. Figure 2 shows the highest content of total anthocyanin was found in fresh purple sweet potato (US), followed by that of in the original purple sweet potato flour (TU), then that of in the resistant starch-rich purple sweet potato flour (TP) which was not significantly different from that of in the partially gelatinized purple sweet potato flour (TG) but significantly different from original purple sweet potato flour (TU) and fresh purple sweet potato (US).

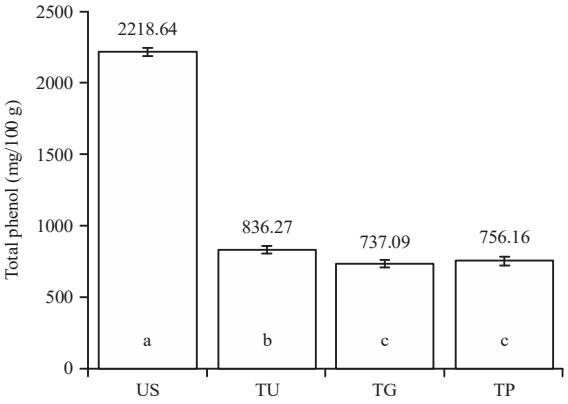

Fig. 1: Total phenol of crude acidic water extract from fresh purple sweet potato (*Ipomoea batatas* L.) and its modified flours (mg/100 g)

Same letters shown at different bars are not significantly different at p<0.05, US: Fresh purple sweet potato, TU: Original purple sweet potato flour, TG: Partially gelatinized purple sweet potato flour, TP: Resistant starch-rich purple sweet potato flour

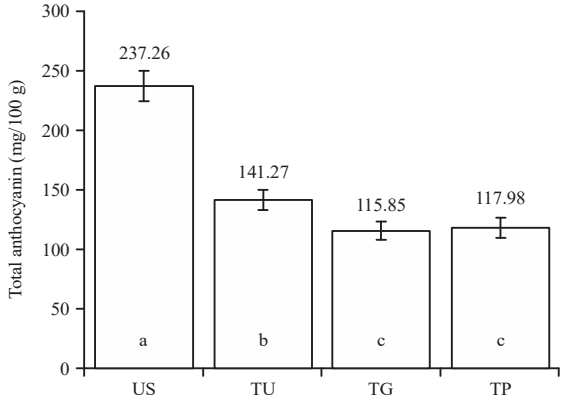

Fig. 2: Total anthocyanin of crude acidic water extract from fresh purple sweet potato (*Ipomoea batatas* L.) and its modified flours (mg/100 g)

Same letters shown at different bars are not significantly different at p<0.05, US: Fresh purple sweet potato, TU: Original purple sweet potato flour, TG: Partially gelatinized purple sweet potato flour, TP: Resistant starch-rich purple sweet potato flour

Inhibition of α-amylase enzyme activity: The inhibition of α-amylase enzyme activity in each treatment was presented in Fig. 3. The inhibition of α-amylase enzyme activity of resistant starch-rich purple sweet potato flour (TP) was 41.98%, partially gelatinized purple sweet potato flour (TG) was 32.59%, original purple sweet potato flour (TU) was 30.72% and fresh purple sweet potato (US) was 23.13%. The results showed that the difference in modification technique of purple sweet potato flour had a very significant effect on the inhibition of α-amylase enzyme activity. The inhibition of

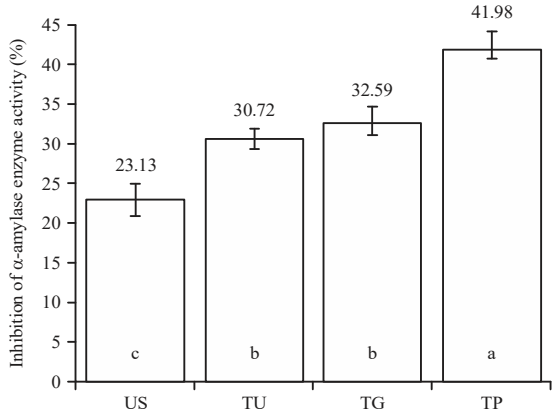

Fig. 3: Inhibition of α-amylase enzyme activity of crude acidic water extract from fresh purple sweet potato (*Ipomoea batatas* L.) and its modified flours (mg/100 g)

Same letters shown at different bars are not significantly different at p<0.05, US: Fresh purple sweet potato, TU: Original purple sweet potato flour, TG: Partially gelatinized purple sweet potato flour, TP: Resistant starch-rich purple sweet potato flour

resistant starch-rich purple sweet potato (TP) was significantly different from those of all other treatments, while inhibition of partially gelatinized purple sweet potato flour (TG) was not significantly different from that of purple sweet potato flour (TU), however, the inhibition of both partially gelatinized purple sweet potato flour (TG) and original purple sweet potato flour (TU) were significantly different from that of fresh purple sweet potato (US).

DISCUSSION

Differences in total phenols in each treatment were due to different process condition applied to each treatment. Fresh purple sweet potato showed the highest total phenol compared to those of in modified purple sweet potato flours because there was no drying, heating and cooling process applied on fresh purple sweet potato (US). The original purple sweet potato flour (TU) has higher total phenol compared to partially gelatinized purple sweet potato flour (TG) and resistant starch-rich purple sweet potato flour (TP). This was due to the total phenol in the original purple sweet potato flour did not expose to high heating process (90°C) for 25 min followed by low temperature cooling process (5°C) for 48 h which may contribute to the phenol compound degradation.

The results of this study were in line with Nurdjanah *et al.*[3] who reported that purple sweet potato flour produced a lower total phenol than fresh purple sweet potato. Partially gelatinized purple sweet potato flour (TG) and resistant starch-rich purple sweet potato flour (TP) had the lowest total

phenol due to high heating process (90°C) for 25 min and continued with drying in an oven with the temperature of 60°C for 24 h. The total phenol found in resistant-rich purple sweet potato flour (TP) was not significantly different from that of partially gelatinized purple sweet potato flour (TG). This was due to the fact that both of rich-resistant starch purple sweet potato flour (TP) and partially gelatinized purple sweet potato flour were exposed to high temperature heating process (90°C) for 25 min before the drying process occurred.

Phenol compounds undergo oxidation easily and are very sensitive to heat treatment. Therefore, drying process (60°C) could reduce the total phenolic compounds. Liyana-Pathirana and Shahidi[10] stated that there was a relationship between temperature and phenolic compounds, the content of phenolic compounds will decrease in conjunction with the increase of high temperatures, this was due to the process of decomposition of phenolic compounds to simple compounds. Vatai *et al.*[11] stated that the content of phenolic compounds was very sensitive, unstable and very susceptible to degradation, this was due to high temperature as the main factor.

The partially gelatinized purple sweet potato flour (TG) and resistant starch-rich purple sweet potato flour (TP) both had the lowest of total phenol and also total anthocyanin, which were significantly different from those of the original purple sweet potato flour (TU) and fresh purple sweet potato (US). The decrease in total anthocyanin compounds in modified purple sweet potato flour was caused by the different modification techniques, causing the instability of anthocyanin compounds in purple sweet potatoes during the treatment process. Total phenol levels and total anthocyanin levels of fresh sweet potato and modified purple sweet potato flour showed similar results (Fig. 1, 2). This was probably because all types of antioxidants including anthocyanin are derivatives of phenol compounds that have the exactly same structure composed of six carbon chains or the benzene ring. Huang *et al.*[12], Liaudanskas *et al.*[13] and Aryal *et al.*[14] also reported that there was strong positive correlation between total phenol and antioxidant properties of fruits and vegetables.

According to Jackman and Smith[15], there were several factors which affects the color stability of anthocyanin including enzymatic activities and processing. Nurdjanah *et al.*[3] reported that discoloration in purple sweet potato flour was caused by anthocyanin degradation due to peroxidase enzyme activity during the processing of purple sweet potato. Peroxidase enzyme (POX) will oxidize the flavonoid compounds in purple sweet potato into brown melanoidin compounds and decrease the level of anthocyanin compounds contained in modified purple sweet potato flour.

In addition to the enzyme activity, the decrease of anthocyanin content in modified purple sweet potato flour probably could be caused by the high temperature process of modified purple sweet potato flour such as; heating process at high temperature (90°C) for 25 min and drying process at 60°C for 24 h. Jackman and Smith[15] reported that an increasing temperature in the food process caused the structure of anthocyanin degrade rapidly through the stages of hydrolysis on anthocyanin glycosidic bonds. The hydrolysis processes of anthocyanin glycosidic bonds will produce unstable aglycones and lead to the opening of the aglycone rings to form colorless carbinol and chalcone groups. The chalcone then will be degraded to form a simpler and colorless compound, named carboxylic acid.

The result showed although containing the lower amount of total phenolic compound and total anthocyanin, the extract from resistant starch-rich purple sweet potato flour (TP) had the highest potential in inhibiting α-amylase enzyme activity by 41.98%. The result indicated that the ability crude acidic water extract from purple sweet potato to inhibit α-amylase enzyme was not directly proportional to total phenol or total anthocyanin contents. It seems that resistant starch-rich purple sweet potato flour, processed through retrogradation had a major role in preserving phenol and anthocyanin structure that lead to increasing ability to inhibit α-amylase activity.

Inhibition of α-amylase enzyme activity was probably caused by the anthocyanin compounds in purple sweet potato which occurred in a competitive and non-competitive ways. Akkarachiyasit *et al.*[16] reported that fruits flavonoids which also function as natural colorant have the capability of α-amylase inhibition in the way of non-competitive type. In this type, an inhibitor can bind to the complex of enzyme-substrate with an equal binding activity or bind to the enzyme active site with equal binding activity[17].

Homoki *et al.*[18] reported that inhibition type of all anthocyanins extract of different Hungarian sour cherry varieties on α-amylase activity showed competitive type. Strelow *et al.*[19] stated anthocyanin could bind to the active side of the enzymes very well but could still occupy another part of the enzymes if a certain substrate had filled the active side of the enzymes. A substrate could experience two inhibitions in its mechanism, it was the combination of competitive inhibition and non-competitive inhibition which was often referred to mixed inhibition.

Judging from the inhibition of α-amylase enzyme activity on the treatment of rich-resistant starch purple sweet potato flour (TP), it was thought not only phenolic compounds which played a role in inhibiting the α-amylase enzyme but also the retrogradation process which occurred on the treatment of rich-resistant starch purple sweet potato flour (TP). The retrogradation process was thought to protect the anthocyanin molecular structures from broken down, so although the total anthocyanin levels in the treatment of resistant starch-rich purple sweet potato flour (TP) was low, but it also had the ability to inhibit α-amylase enzyme activity better than the treatment of partially gelatinized purple sweet potato flour (TG) and original purple sweet potato flour (TU).

CONCLUSION

Crude acidic water extract from different forms of purple sweet potato showed different ability to inhibit α-amylase activity. The crude acidic water extract of resistant starch-rich purple sweet potato flour (TP), although contained lower phenolic compounds and total anthocyanin, exposed the highest inhibitor effect on α-amylase activity which was 41.98%. These result open possibility to develop purple sweet potato as functional food.

SIGNIFICANCE STATEMENT

This study reveals that physical modification of purple sweet potato flour reduced the total phenol including anthocyanin content. However it was found that acidified water extract from modified flour (resistant starch-rich) proved to increase inhibitory effect on α-amylase. This finding opens possibility to promote the production and utilization of resistant starch-rich purple sweet potato flour for main raw material of food products designated for curing diabetes mellitus.

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