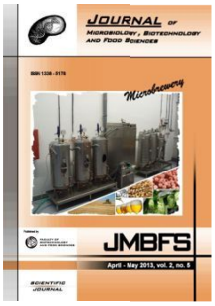

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PARTIAL HYDROLYSIS OF PURPLE SWEET POTATO FLOUR BY AMYLASE FROM *Saccharomycopsis fibuligera* AND ITS APPLICATION FOR COMPOSITE BREADMAKING

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ABSTRACT

Purple sweet potato is one of underutilized carbohydrate sources in Indonesia, whilst known as good source of carbohydrate and can act as functional food due to its anthocyanine and dietary fiber contents. Therefore in the present study, we try to modify the sweet potato flour by partial hydrolysis using amylase produced by *Saccharomycospsis fibuligera* R64 and apply the partially hydrolyzed flour for composite breadmaking. The amylase was produced using batch method and partially purified by the addition of ammonium sulfate followed by gel filtration chromatography on Sephadex G25 using fast performance liquid chromatography system. The enzyme was then used to hydrolyze the purple sweet potato flour. Characterizations of the partially hydrolyzed flour comprise reduction in amylose-iodine complex, SEM and XRD. Partially hydrolyzed flour was then used as composite flour for bread, with ratio of wheat to partially hydrolyzed purple sweet potato flour was 70 : 30. The produced bread was then analyzed for its texture, organoleptic test and visualization of the bread crumb using TEM. The results of the present study indicate that the enzyme partially hydrolyzed the sweet potato flour. Even though the quality of the composite bread is not as good as wheat bread, partial hydrolysis seems to improve the texture and appearance of the composite bread, as indicated by better swelling volume and firmness of the composite bread using partially hydrolyzed purple sweet potato flour.

Keywords: Purple sweet potato, amylase, partial hydrolysis, composite bread

INTRODUCTION

Purple sweet potato is one of carbohydrate source which can be considered as functional food due to its natural content of dietary fiber **(Huang *et al.*, 1999)** and anthocyanin that can act as antioxidant, anticarcinogenic, antihypertensive and antimutagenic **(Terahara *et al.*, 1999; Oki *et al.*, 2002; Kano *et al.*, 2005)**. However, its application in various food products is still limited. Therefore there are many efforts has been conducted to develop various sweet potato based food product **(Collado *et al.*, 2001; Singh *et al.*, 2004; Ahmed & Ramaswamy, 2006)**.

Bread is usually made from wheat flour because the presence of two specific proteins, gliadin and glutenin, that form protein network known as gluten which can seize carbon dioxide produced during fermentation of bread dough, which consequently make the bread swell **(Goesaert *et al.*, 2005)**. Unlike wheat, sweet potato does not have network forming protein. Instead, it has different type of protein, sporamin, that has been confirmed to have trypsin inhibitory activity **(Shewry, 2003)**.

Enzyme application in breadmaking has been widely known to improve the quality of bread **(Gerrard *et al.*, 1998; León *et al.*, 2002; Caballero *et al.*, 2007)**. Amylases are one of the enzymes that are used to improve physicochemical properties of bread **(Goesaert *et al.*, 2009; Fadhlillah, 2011)**. However, as far as we aware, none of the published work used enzyme pretreated flour for making composite bread. Our lab has been working with amylase from locally isolated food-borne yeast (*Saccharomycopsis fibuligera* R64), which known to produce both α-amylase and glucoamylase and it was also found that the α-amylase belongs to mesophilic enzyme **(Soemitro, 1996)**. The α-amylase produced by this yeast was found to have raw starch degrading activity without adsorption mechanism, while the glucoamylase was found to adsorb onto starch granule **(Hasan *et al.*, 2008)**.

The objective of the present study was to investigate the effect of partial hydrolysis using partially purified amylase produced by *S. fibuligera* R64 and apply the partially hydrolyzed flour for composite bread making.

MATERIAL AND METHODS

Production and partial purification of amylase

S. fibuligera cells were maintained on agar slant containing sucrose (6% w/v) and bacto agar (1.5% w/v) in bean sprout broth (10% w/v). Starter culture was developed by aseptically inoculated one yeast colony to 50 mL media containing sago starch (1% w/v) and yeast extract (1% w/v) for 48 hours at room temperature with 180 rpm shake speed. The starter culture was then transferred into a 500 mL fermentation media which has the same composition as starter culture media. The fermentation conducted for 72 hours at room temperature with 180 rpm shake speed. After 72 hours, the media was centrifuged at 4000×*g* to separate it from the cells. The supernatant contains enzyme which then partially purified by addition of ammonium sulfate (60-100% saturation), followed by gel filtration chromatography on Sephadex G-25 matrix (2 × 20 cm) using ÄKTAprime plus fast performance liquid chromatography system with 1 mL/min flow rate. The presence of protein and salt was detected using UV detector at 280 nm and conductivity meter attached to the system, respectively. Fractions with high absorbance at 280 nm were pooled and used for partial flour hydrolysis. Activity of the enzyme was monitored as described elsewhere **(Hasan *et al.*, 2008)**.

Partial hydrolysis of sweet potato flour

Purple sweet potato used in this study was *Ipomoea batatas* var. Ayamurasaki. The potato was peeled, washed and thin cut followed by sun dried. The dried chip was then grinded to obtain the sweet potato flour. Native purple sweet potato flour was refereed as NF. Hydrolysis was conducted in 20% w/v suspension of sweet potato flour in 50 mM phosphate-citrate buffer pH 5.8. Partially purified enzyme was added to achieve 50 unit/g flour. The hydrolysis was performed at two different temperatures, i.e. the optimum temperature of enzyme activity (50°C) and room temperature for 12 hours. The flour was then separated by centrifugation at 4000×*g* and the reducing sugar content of the supernatant was determined using alkaline ferricyanide assay **(Walker & Harmon, 1996)**. The resulting flour was referred as HTr and HTo for partially

hydrolyzed purple sweet potato flour hydrolyzed at room temperature and 50°C, respectively. The partially hydrolyzed flour was then washed twice using distilled water and used for composite breadmaking. Samples of the partially hydrolyzed flour was analyzed for residual amylose by measuring amylose-iodine complex at 600 nm, granule morphology by scanning electron microscopy (SEM), and changes in crystallinity by X-Ray Diffraction.

Breadmaking and analysis of bread

The following formula was used for breadmaking: wheat flour with 13-14% protein content (300 g), sugar (17 g), table salt (5.6 g), instant bakery yeast (3.4 g), skim milk powder (11.3 g), unsalted margarine (11.3 g), and cold water (180 g). As for composite bread, the wheat flour was substituted by mixture of wheat flour (210 g) and either native or partially hydrolyzed sweet potato flour (90 g).

The ingredients without water and white margarine were mixed for 1 minute followed by addition of water into the mixture, mixed for another 10 minutes, and followed by addition of unsalted margarine. The mixture was kneaded until proof dough formed. The dough was then put into an incubator at 37°C for 20 minutes. The dough was re-kneaded for another two times, and followed by 15 and 60 minutes of incubation at 37°C, respectively. After the last incubation, the loaves were baked at 200°C for 30 minutes and the breads were then cooled for 2-4 hours to room temperature. The swelling volume of bread was measured by rapeseed displacement method. The bread produced in this experiment was refereed as WR, CB, CBHr and CBHo for wheat bread, composite bread made of wheat flour and native purple sweet potato flour, composite bread made of wheat flour and purple sweet potato flour partially hydrolyzed at room temperature and composite bread made of wheat flour and purple sweet potato flour partially hydrolyzed at 50°C, respectively. Analyses of bread comprise the morphology of crumb by transmission electron microscope (TEM), organoleptic test by hedonic test, and firmness of crumb by texture analyzer.

Statistical Analysis

When available, data were presented as means of measurements of several experiments as indicated on the legend. Differences between means were checked using one-way ANOVA followed by Fisher's LSD post hoc test when statistically significant differences found. All statistical calculations were performed using MINITAB 15.

RESULTS AND DISCUSSION

Production and partial purification of amylase

Supernatant of fermentation media contains α-amylase and glucoamylase. Due to their similar properties, it is difficult to separate them with simple method **(Soemitro, 1996)**. Therefore, we decided to partially purify the amylase enzyme using ammonium sulfate precipitation followed by desalting on Sephadex G25 matrix. We used 60-100% saturation of ammonium sulfate as this condition gave the highest amylase activity (data not shown). The result of the desalting step is presented on Figure 1. It was obvious that the protein was nicely separated from the salt, and amylase assay confirmed that the amylase was present in the protein peak. The pooled protein peak has ~2800 unit/mL unit activity.

Production and partial hydrolysis of purple sweet potato flour

From 10 kg of fresh purple sweet potato 3.25 kg of flour was obtained. The flour was then partially hydrolyzed using the partially purified enzyme. The hydrolysis process was confirmed by reduction of amylose-iodine complex absorbance of the hydrolyzed flour and increase of reducing sugar content of the hydrolysate as presented on Table 1. The reduction of amylose-iodine complex absorbance indicated that amylose molecules in starch granules of the flour were attacked by amylase to form shorter glucooligosaccharide which cannot form complex with iodine. Consequently, the intensity of amylose-iodine complex decreased. This was also confirmed by the increase of reducing sugar content of the hydrolysate, because glucooligosaccharide produced as the hydrolysis product increase the amount of reducing sugar. It was also observed that hydrolysis at optimum temperature of the enzyme activity (50°C) even promote the hydrolysis process as indicated by lower reduction in amylose-iodine complex intensity and higher reducing sugar content.

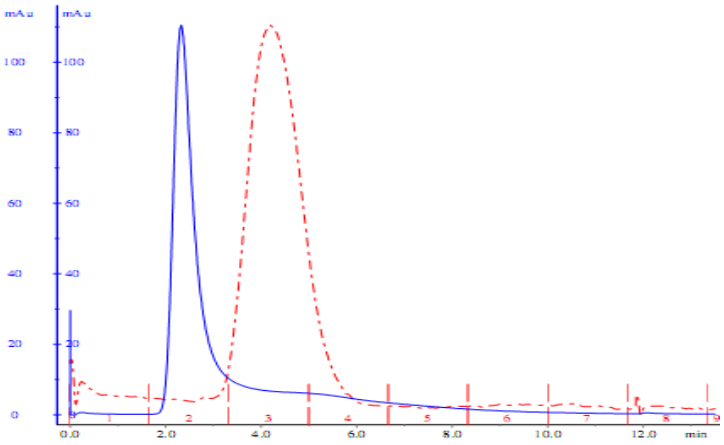

Figure 1 Gel filtration chromatography of 60-100% saturation of ammonium sulfate precipitate (solubilized in 50 mM phosphate citrate buffer pH 5.8) on Sephadex G25 matrix. Blue line represents absorbance at 280 nm, while red dashed line represent conductivity

Table 1 Amylose-iodine complex intensity of partially hydrolyzed sweet potato flour and reducing sugar of hydrolysis supernatant. Data are means of two measurements followed by SD. Means in same row with different letter are significantly different at α = 0.05 followed by Fisher's LSD post hoc test

	Sample		
	NF	After Hydrolysis	
		HTr	HTo
A_{600nm} (1% w/v flour)	0.240 ± 0.023^{a}	0.198 ± 0.001^{a}	0.132 ± 0.003^{b}
Reducing sugar (μg/mL)	2900 ± 38^{a}	3246 ± 82^{b}	3563 ± 41^{c}

XRD was used to examine qualitative crystallinity of flour. The result of XRD analysis is presented on Figure 2. It was observed that some sharp peaks at NF were weakening following hydrolysis by amylase. Sharp peak at 2θ of ~15, ~17, and ~23° was observed for native purple sweet potato flour. These peaks were weakening in HTr and even clearer in HTo. The most drastic change was observed for ~23°, where it was almost disappear in HTo. This data indicated that the enzyme attack crystalline portion of the starch granules, consequently reduce its crystallinity. Other study indicate that enzymatic hydrolysis tend to increase crystallinity **(Shariffa *et al.*, 2009; Uthumporn *et al.*, 2010)**, but similar result to the present study was also observed in β-amylase treated maize starch **(Ao *et al.*, 2007)**. Further study is required to confirm this finding.

SEM scanning (Figure 3) revealed no obvious evidence that the enzyme deform the surface of the starch granules. This might be because the enzyme is mildly active toward the starch granules due to its non adsorbing nature **(Hasan *et al.*, 2008)**. Similar result is also found by **Hayashida *et al.* (1988)** who found that a non adsorbing α-amylase require up to 5 days to disrupt the surface of the starch granules. Adsorbing properties of an enzyme seems to assists the enzyme activity to disrupt the starch granules by anchoring itself to the starch granules and hence ease the disruption process. The adsorbing properties of an amylase enzyme is usually facilitated by the presence of starch binding domain **(Machovič & Janeček, 2006)**, which does not exist in the enzyme used in the present experiment **(Hasan *et al.*, 2008)**.

Properties of composite bread

Compared to wheat bread, the composite bread has lower swelling volume as can be seen on Table 2. This result is mainly because the absence of gluten forming protein in sweet potato flour, which required for seizing carbon dioxide formed during dough fermentation **(Goesaert *et al.*, 2005)**. Even though gluten forming protein present in wheat flour used for composite dough making, the presence of sweet potato flour interfere the formation of gluten network. However, partial hydrolysis of the flour seems to improve bread swelling compared to native sweet potato flour in composite bread product. This phenomena might be caused by the presence of simple sugar, such as glucooligisaccharide, left from the hydrolysis process and become readily available sugars during dough fermentation process, and thus promote the formation of carbon dioxide.

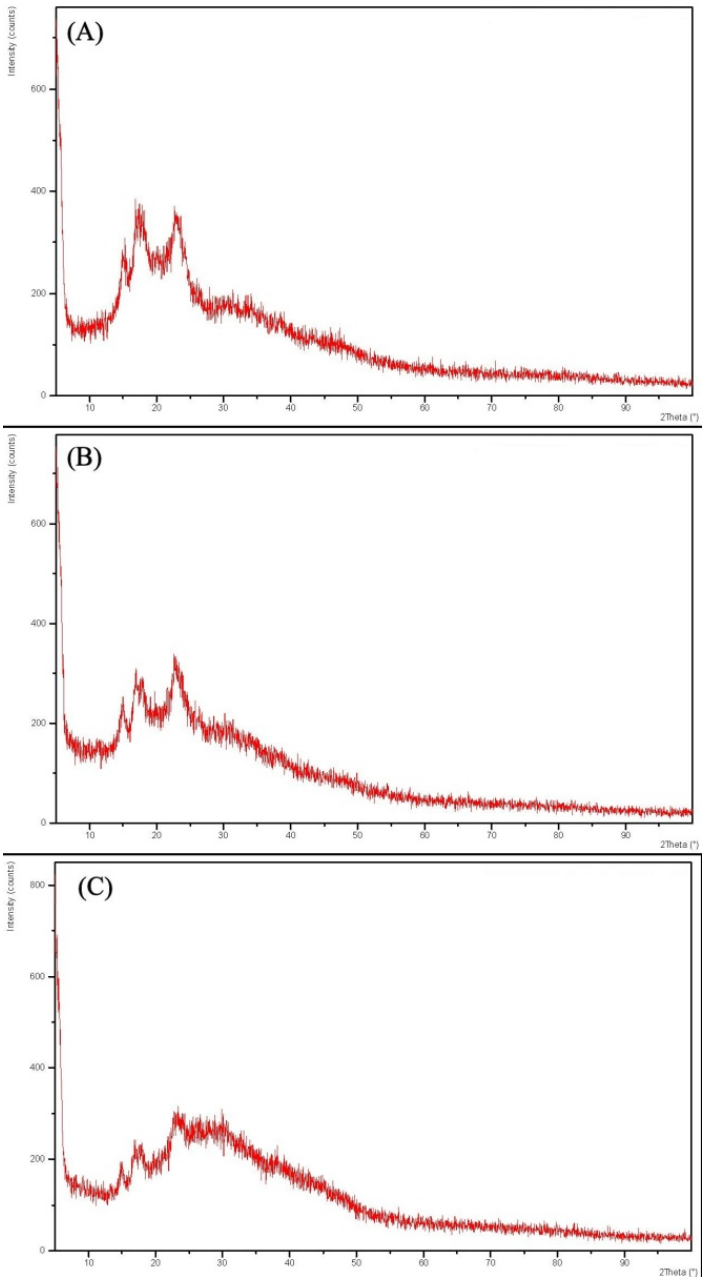

Figure 2 XRD pattern of (A) NF (B) HTr and (C) HTo. All hydrolysis performed in 50 mM phosphate-citrate buffer pH 5.8 for 12 hours

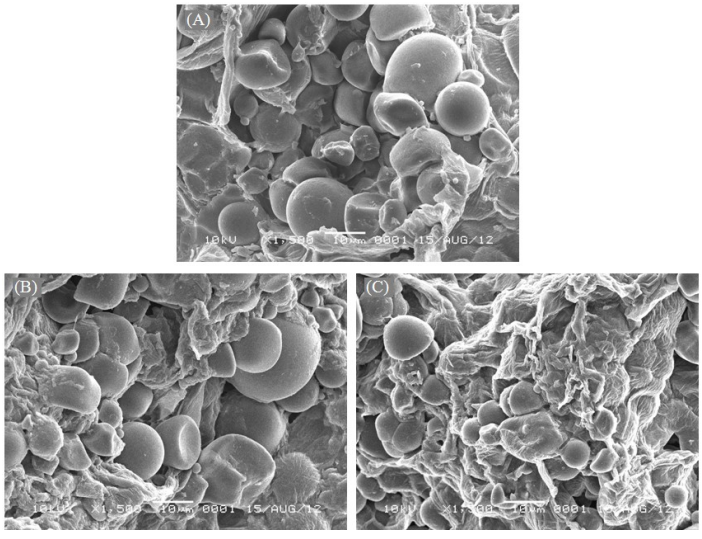

Figure 3 Scanning electron micrograph of (A) NF, (B) HTr and (C) HTo

Table 2 Swelling volume of wheat bread and sweet potato composite bread. Ratio of wheat flour to sweet potato flour for all composite bread were 70 : 30

Sample	Dough volume (mL)	Bread volume (mL)	Height (cm)	Volume increment (%)
WB	475	1525	8.8	221.05
CB	425	800	5.0	88.23
CBHr	365	790	6.0	116.44
CBHo	400	865	6.6	116.25

Due to limited instrument, unlike the flour which was examined using SEM, we examine the microstructure of the starch granule of bread using TEM (Figure 4). It was found that CBHr and CBHo seems to have stickier microstructure compared to CB or WB. This might be caused by the presence of glucooligosaccharides as product of partial hydrolysis, which is known can be used for gelling agent **(Angioloni & Collar, 2009)** and observed as sticky material in bread crumbs.

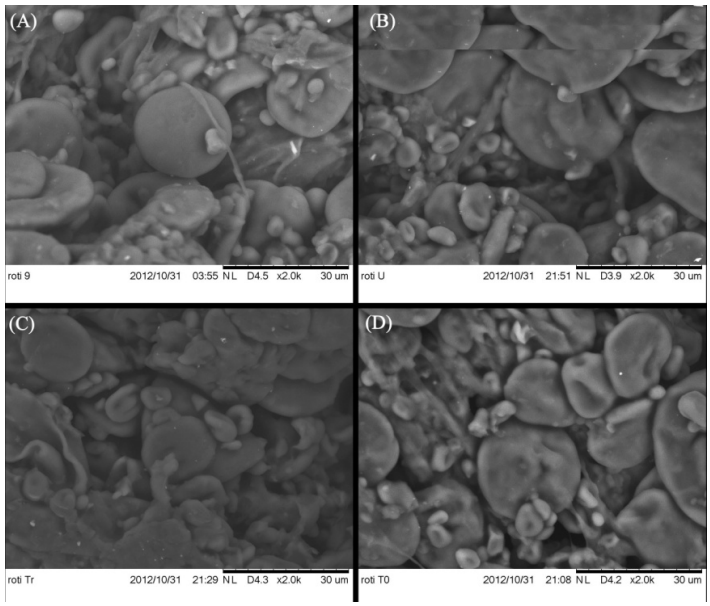

Figure 4 Transmission electron micrograph of (A) WB, (B) CB, (C) CBHr, and (D) CBHo. Magnification 2000 ×

Texture analyzer was used to measure the firmness of the bread crumb. The results of texture analysis are presented on Table 3. It was found that the softest bread was WB, followed by CBHr, CBHo and CB. This indicate that partial hydrolysis of the sweet potato flour improve the quality of composite bread by soften the end product. Statistical analysis even showed that WB and CBHr were not statistically different, which means that both of the samples have similar level of hardness. However, as mentioned earlier, the swelling volume of the CBHr is lower than WB, and hence will affect the preference of consumer as will be described in the organoleptic test section.

Table 3 Firmness of bread crumb made in this study. Values presented are means of two measurement followed by standard deviation. Means in same column with different letter are significantly different at $\alpha = 0.05$ followed by Fisher's LSD post hoc test

Sample	Firmness (g)
WB	743.7 ± 70.0^{a}
CB	5221.1 ± 473.6^{c}
CBHr	967.2 ± 39.8^{a}
CBHo	2980.4 ± 613.3^{b}

Consumer acceptance is one of the most important factors in development of new product. It is affected by various factors such as color, texture, taste, crumbness, flavor, and bread swelling volume. Therefore, we examine consumer acceptance to our samples, by asking trained panelist acceptance level on crumb appearance, pore, softness, tenderness, aroma and flavor, and the data are presented on Table 4. From the results presented on Table 3, WB was the most accepted bread by the panelist for all parameters tested. This means that the panelist prefer WB over the other bread. However, data of pore and aroma indicate that composite bread using partially hydrolyzed flour were more preferred than CB. This finding indicates that partial hydrolysis of sweet potato

flour can alter consumer's preference over composite bread that using native sweet potato flour, even though it is less preferred over the WB. Further improvement is still required to achieve better product, so that it can equal to WB.

Table 4 Results of organoleptic test by hedonic test. Data presented are means of ten data followed by standard deviation. Means in same column with different letter are significantly different at $\alpha = 0.05$ followed by Fisher's LSD post hoc test

Sample	Crumb appearance	Pore	Softness	Tenderness	Aroma	Flavor
WB	2.09±0.18[b]	2.18±0.16[c]	2.14±0.18[b]	2.21±0.16[b]	2.18±0.20[c]	2.09±0.22[b]
CB	1.33±0.17[a]	1.36±0.23[a]	1.33±0.17[a]	1.39±0.24[a]	1.46±0.23[a]	1.49±0.26[a]
CBHr	1.29±0.15[a]	1.67±0.14[b]	1.29±0.15[a]	1.46±0.23[a]	1.62±0.25[a,b]	1.62±0.30[a]
CBHo	1.36±0.23[a]	1.29±0.15[a]	1.36±0.18[a]	1.46±0.27[a]	1.67±0.14[b]	2.09±0.22[a]

CONCLUSION

Partial hydrolysis of purple sweet potato flour was successfully conducted using amylase from *S. fibuligera* R64 as confirmed by reduction of amylose content of the pretreated flour and increase of reducing sugar in hydrolysate solution. Even though there were no strong indications of starch granule disruption based on SEM result, XRD results indicate changes in crystallinity of partially hydrolyzed flour compared to the native flour.

Partially hydrolyzed flour gave better performance when used for composite breadmaking compared to native purple sweet potato flour, even though the resulting product was still less preferred and have poorer properties compared to wheat bread.

Further investigation is required to improve the quality of the composite bread. Among possible treatment that can improve the quality of the composite bread is treatment or addition of enzyme that can assist the formation of protein network, which in turn can seize carbon dioxide produced during fermentation and increase swelling volume of the composite bread.

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