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Annealled purple yam (*Dioscorea alata* var. *purpurea*) flour improved gelatinisation profile, but increased glycemic index of substituted bread

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Abstract

Yam starch and flour has been explored for its functional properties including textural, antioxidative, hypoglycemic, and hypolipidemic characteristics. Our previous study showed that subtitution of 20% purple yam flour retained bread volume and sensory characteristic, as well as decreased glycemic index of bread by about 20 points. This work aimed to strengthen purple yam flour textural characteristics by improving gelatinisation profile using annealing at various times (1, 6, 12, 18, 24 hours), and temperatures (40, 45, 50, 55, 60°C). Annealed purple yam flour was then incorporated into bread making, to substitute wheat flour by 50%. In vivo study on blood glucose level was carried out using a commercial kit, in which result was used to calculate glycemic index. Result showed that annealing decreased peak viscosity (191 cP) and final viscosity (355 cP), as well as improved peak time (13 min). Temperature and time during annealing significantly (P<0.05) affected viscosity. Bread with 50% subtitution of annealed yam flour showed higher hardness (22 N), and lower bread volume (532 cm^3). The use of arabic gum reduced hardness of substituted bread. Blood glucose level profile of volunteers after consumption of substituted bread did not substantially differ from that of normal bread, and this led to glycemic index of 93.19. Annealing did not seem to be a sufficient method to improve hypoglycemic effect of purple yam flour, which may correlate to its starch nature.

Introduction

Some yam species (*Dioscorea* spp.), such as *D. opposita* (Hsu *et al*., 2007) and *D. japonica* are important elements in traditional Chinese medicine to strengthen gastrointestinal tract, cure diabetic, and improve general health. Some more species were also studied for their health promoting effect, including *Dioscorea opposita* (Gao *et al*., 2007; Hsu *et al*., 2007; Zhang *et al*., 2011), *D. alate* (Lin *et al*., 2006), and *D. polygonoides* (McAnuff *et al*., 2005). *Steroidal sapogenin* (McAnuff-Harding *et al*., 2006) has been attributed as substance responsibles for the effect, which was also found in some species such as *D. japonica* (Yang and Lin, 2008), *D. villosa* (Sautour *et al*., 2006), *D. zingiberensis* (Peng *et al*., 2011), *D. alata* (Chen *et al*., 2003, Lin *et al*., 2006), and *D. dumentorum* (Undie and Akubue, 1986). Sapogenin reduced blood glucose levels in diabetic rats (McAnuff *et al*., 2005; Sautour *et al*., 2006; Yang and Lin, 2008). The effect in reducing blood glucose level was also due to the presence of α-glycosidase inhibitor (Zhang *et al*., 2011), and some polysaccharides in *D. alata* capable of lowering sucrase activity to inhibit glucose absorption (Chen *et al*., 2003).

D. alata var. *purpurea* had been incorporated into noodle making to give hypolipidemic and antioxidative effects, while maintaining sensory characteristics (Lin *et al*., 2006). Our previous work showed that 20% substitution of purple yam flour into wheat bread mixture resulted in similar volume and sensory evaluation to that of wheat bread, and was better volume than those of bread substituted with 20% of sorghum, pumpkin, and taro flour (Purwandari *et al*., 2011a). Whilst, glycemic index of bread substituted with 20% purple yam flour was 80.78, suggesting there was hypoglycemic effect of purple yam flour substitution. Similarly, substitution of 20% *D. esculenta* flour into bread also did not alter its sensory evaluation (Suprianto, 2009). Substitution was improved to reach 30% by incorporation of arabic gum (Milanita, 2010), without adversely affecting sensory assessment. However, highest substitution so far was reported by Nindjin *et al*. (2011), where 70%

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yam starch added into bread mixture resulted in same volume as normal bread (Nindjin *et al*., 2011).

As bread does not contain sugar, it is suitable for diabetic diet. One of the most essentials characteristics of bread is its texture, including porosity, hardness and volume. Bread structure was closely correlated to gelatinization profile of starch (Miyazaki *et al*., 2006). Here, elastic, springy, shear resistant, gas retention ability of gluten network is important to determine bread volume (Mondal and Datta, 2008; Sikora *et al*., 2010). The use of non-wheat flour diluted gluten and thus weaken the network (Nindji *et al*., 2011), and reduce bread volume. As non-wheat flour or starch may have high water binding nature, it may cause competition for water in the sistem, to retard formation of sufficient gluten network (Nindjin *et al*., 2011).

Both flour and starch of yam (*Dioscorea* sp.) showed higher shear and temperature resistance, compared to those of taro and sweet potato (Aprianita *et al*., 2009). Among three species examined (white *D. alata*, *D. esculenta*, and *D. alata* var. *purpurea*), the latter was more resistant to shear and heat (Purwandari *et al*., 2010). However, wheat dough stability was reduced by addition of yam flour (Nindji *et al*., 2011; Purwandari *et al*., 2011). Therefore, appropriate method to improve yam starch textural properties is needed. One of possible methods in strengthen textural characteristics of starch is annealing. Annealing is a hydrothermal method commonly used for the purpose, it includes heating flour/starch under gelatinisation temperature in high moisture content around 60% (Jayakody and Hoover, 2008). The process increased crystallinity, strengthened amylose-amylose and amylopectin-amylopectin bonds, to give more stable nature of the flour/starch. Increase in heat and shear resistance and crystalinity may be followed by increase in enzyme resistance (Chung *et al*., 2009), although some works showed the opposite (O'Brien and Wang, 2008). Annealing changed proportion of resistant starch (RS), slowly digestible starch (SDS), or rapidly digestible starch (RDS), but may increase or decrease expected glycemic index (Chung *et al*., 2009).

This work studied gelatinisation profile of purple yam flour as affected by annealing, as well as determined its substitution effect on bread texture and glycemic index.

Materials and Methods

Experimental design

Two factors used in the experiment were temperature (40, 45, 50, 55, 60°C) and time (1, 6, 12, 18, and 24 hours) during annealing (Chung *et al*., 2000). Experimental design was carried out following Respons Surface Methodology (RSM) for two factors and five levels such as shown in Table 1 (Myers and Montgomery, 2002), and data were analysed using Minitab™ statistical package. Response Surface Methodology (RSM) is a useful method for modelling and process optimization. In this work, RSM was employed to determine optimum conditions to give highest textural characteristics of flour. Parameters measured were water absorption index (WAI), water solubility index (WSI), and gelatinization profile (peak viscosity, final viscosity, and peak time) of annealed flour; hardness and porosity of bread substituted with 50% annealed yam flour; and glycemic index of substituted bread.

Table 1. Experimental design according to response surface methodology

Run	X_1	X_2	Temperature (°C)	Time (hours)
1	-1	-1	45	6
2	-1	1	45	18
3	1	-1	55	6
4	1	1	55	18
5	0	0	50	12
6	0	0	50	12
7	0	0	50	12
8	0	0	50	12
9	0	0	50	12
10	1.414	0	60	12
11	-1.414	0	40	12
12	0	1.414	50	24
13	0	-1.414	50	1

Preparation of flour

Purple yam flour was prepared using method described previously (Purwandari *et al*., 2010). Yam tuber bought from local market was peeled, sliced 1-2 mm thick, then quickly soaked in 1% sodium metabisulphite solution for 5 minutes. Slices were then drained, followed by sun-drying until fully dry as indicated by cracking sound when chips were broken. Drying was usually completed in a day (8-9 hours) during hot sunny day. Whenever drying could not be finished in the same day, chips were kept in a refrigerator to avoid any possible fermentation. The chips were then ground using electric grinder, and sieved in 80 mesh. Flour was then kept in an airtight container.

Annealing

Annealing was done following a method used by Chung *et al*. (2000). Purple yam flour was added by 60% distilled water, then kept in a water bath at temperature and time according to experimental design. Upon completion of annealing, the flour was oven-dried at 40-50°C in 5 hours, ground, sieved with 80 mesh sieve, and stored in an airtight container.

Bread making

Flour used in bread making was the most temperature and shear resistant flour as indicated by textural parameters, i.e. flour treated with highest temperature and longest time of annealing. Bread mixture consisted of 400 g flour containing 50% w/w annealed purple yam flour, 1 g salt, 8 g skim milk, 16 g margarine, 1.2 g baker yeast, and arabic gum at different levels (1, 2, 3%). Fermentation of the dough took 15 min in a fermentor, and it was then kneaded twice, where each kneading was done until dough did not stick on side of container. Dough was then put in the oven at 170-180°C for 45 min. Higher yam flour content tends to demand more moisture. Therefore, bread mixture containing 50% yam flour was added about 100 mL more water than the water quantity added to control bread.

Physical properties and gelatinisation profile of flour

Determination of water absorption and water solubility index of flour was carried out according to previous method (Hsu *et al*., 2003). Gelatinisation profile was determined using Rapid Visco Amylography (RVA) technique in a Brabender® Viscoamylograph.

Hardness and volume of bread

Hardness of bread was analysed using Tensile Strength measurement method on a Texture Analyser (Imada, Japan). Bread volume was measured by first weighing the whole loaf of bread (W_1). Then samples from different parts of bread (center, upper, bottom, and sides of bread, with two samples from every part) were taken by cutting bread into several 2 × 2 × 2 cm cubes, then every cube was weighed (W_2). Bread volume was calculated using following equation:

Bread volume = $(W_1/W_2) \times 8$ cm^3

Bread volume from samples was averaged.

Measurement of glycemic index

Glycemic index of bread was analysed by first measuring blood glucose level of volunteers, using method described by Marques *et al*. (2007), which was according to an AOAC method. As many as 10 healthy volunteers with no diabetic family background, non-smoking, aged 18-23 years old, and with body mass index of 17-25, were asked to involve in the experiment by first filling informed consent. None of the volunteer was alcohol drinking, under medication nor consuming supplement. They were fasted 12 hours before consuming 95 g bread that equals to 50 g carbohydrate. Blood glucose level of each volunteer was measured every 15 minutes after consumption of bread until 2 hours, using a commercial kit consists of Gluco DR™/AGM-2100 Biosensor, and GlucoDr™ AGS Biosensor Strip. Normal white bread was used as control. Experiment was repeated three times, with at least three days interval for each experiment. Data were then plotted in a time against blood glucose level graph with time as X-axis. Area under curve was determined as increment area for every blood glucose level of each bread. Then, glycemic index was calculated as the proportion of area under curve of sample bread over that of control bread, times 100.

Results and Discussion

Physical properties and gelatinisation profile of flour

WAI and WSI values were expected to be lower in annealed flour than in native flour. This also what was shown in our results, where WAI and WSI were smaller at high temperature and long time annealing, although the values were peaking at medium level of annealing temperature and time (Figure 1a, b). Therefore, regression coefficients for WSI were negative for temperature and positive for time, although statistically there was no significant ($P>0.05$) effect of either factor studied (Table 2). Similarly, regression coefficients of both factors were negative for WAI (Table 2), indicated negative correlation between the two factors and WSI or WAI. R^2 values were rather low for both parameters, with 71.1% for WAI and 64% for WSI (Table 2). The increase in WAI was possibly due to some structural changes causing hydrophilic end to be exposed thus allowing starch/flour to absorb more water (Altan *et al*., 2009). Whilst, reduce WAI at higher temperature maybe due to decomposition, degradation, or dextrinization of starch (Altan *et al*., 2009), causing some reduce in water absorption capability. The increase in WSI may be caused by gelatinization to facilitate solubilisation of starch (Altan *et al*., 2009). In contrast, reduce in WSI was probably due complexation of structure at high temperature (Altan *et al*., 2009).

Annealing lowered gelatinisation profile parameters, as also shown by previous works (Chung *et al*., 2000; Jayakody and Hoover, 2008; O'Brien and Wang, 2008; Chung *et al*., 2009). Peak viscosity decreased by increasing annealing temperature and time (Figure 1). Therefore, high temperature and long time annealing gave lowest value of peak viscosity

Table 2. Regression coefficients of WAI, WSI, peak viscosity, final viscosity, and peak time as analysed using response surface methodology

Parameter	Factor[a)]	Regression Coefficient	P [b)]	R^2
WSI	Constant	63.84	0.00**	55.00%
	Temperature	-0.85	0.37	
	Time	0.54	0.56	
	Temperature*Temperature	-1.62	0.13	
	Time*time	-1.97	0.08	
	Temperature*Time	-1.15	0.39	
WAI	Constant	9.53	0.00**	71.10%
	Temperature	-0.01	0.85	
	Time	-0.04	0.54	
	Temperature*Temperature	-0.16	0.05	
	Time*time	-0.15	0.06	
	Temperature*Time	0.24	0.03*	
Peak Viscosity	Constant	683.60	0.001**	82.00%
	Temperature	-278.00	0.031*	
	Time	-441.10	0.004**	
	Temperature*Temperature	-130.10	0.280	
	Time*time	206.90	0.105	
	Temperature*Time	151.70	0.334	
Final Viscosity	Constant	1178.80	0.000***	78.7%
	Temperature	-363.26	0.026*	
	Time	-481.79	0.007***	
	Temperature*Temperature	-213.09	0.166	
	Time*time	127.66	0.385	
	Temperature*Time	72.00	0.704	
Peak Time	Constant	13.00	0.000***	40.9%
	Temperature	-0.00	1.000	
	Time	0.02	0.104	
	Temperature*Temperature	-0.01	0.412	
	Time*time	-0.01	0.412	
	Temperature*Time	-0.00	1.000	

[a)]* indicated interaction between two different factors or quadratic function between same factors
[b)]* indicated significant difference at P<0.05, ** indicated significant difference at P<0.01, *** indicated significant difference at P<0.001.

Table 3. Volume and hardness of bread substituted by 50% annealed purple yam flour

Bread type	Volume (cm^3)*	Hardness (N)*
Substituted bread, 1 % arabic gum	413.27±21.6474[a]	23.2[b]
Substituted bread, 2 % arabic gum	430.95±21.6474[a]	23.0[b]
Substituted bread, 3 % arabic gum	512.20±21.6474[b]	22.0[b]
Control bread	783.43±21.6474[c]	9.9[a]

*Numbers with same superscripts in one coloumn indicated no significant difference (P>0.05)

(191 cP), while low temperature (50ºC) and short time (1 hour) annealing resulted in highest peak viscosity (2126 cP). Similarly, low peak viscosity was resulted from annealing high temperature and long time. As a consequence, negative regression coefficients were shown by either annealing temperature (-363.3) or time (-481.8). Annealing time gave greater effect than temperature to final viscosity, significantly (P<0.05). Peak time was reduced by annealing, to reach 13 min (Table 2). Annealing in wheat flour or wheat starch prolonged peak time (Yun *et al.*, 2010). Native purple yam flour showed peak time of 10.7 min, and reduced to 8.7 min in composite wheat flour containing 20% of yam flour (Purwandari *et al.*, 2011b). In its native form, purple yam flour had longer peak time as compared to other local flours, such as taro (8.53 min), and sweet potato (5.21 min) (Aprianita *et al.*, 2009). The increase in peak time indicated more stable starch in the flour. Annealed purple yam flour had better heat and shear stability than native yam flour (Zaidul *et al.*, 2007), due to more organised structure of amylopectin and amylose led to higher crystallinity (Jayakody and Hoover, 2008). As highest

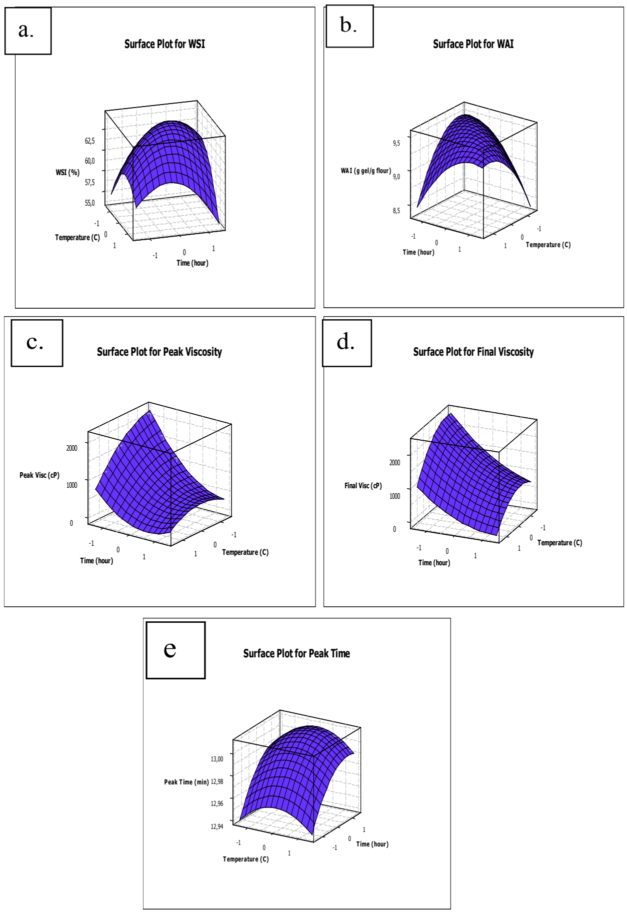

Figure 1. Surface plot for (a) WAI, (b) WSI, (c) peak viscosity, (d) final viscosity, and (e) peak time of annealed purple yam flour as affected by annealing temperature and time

temperature resistance was shown by flours annealed at high temperature and long time, flour annealed at highest temperature and longest time was then used in bread making, to substitute 50% wheat flour.

Volume and hardness of substituted bread

Bread substituted with 50% annealed purple yam flour was analysed for its volume and hardness, by incorporating arabic gum at 1, 2, or 3% level. Bread volume containing 0, 1, 2, or 3% arabic gum were 783.43±21.6474, 413.27±21.6474, 430.95±21.6474, 512.20±21.6474 cm^3, respectively, to indicate significant (P<0.05) role of gum arabic on bread volume. However, volume of substituted bread was still only 65% volume of wheat flour bread (Table 3). Arabic gum added into bread mixture at 3% level resulted in significantly (P<0.05) larger bread volume than that of lower level of arabic gum addition. Arabic gum influenced viscoelastic properties of dough, by reducing peak viscosity, peak time, and final viscosity (Bhattacharya *et al.*, 2006; Alam *et al.*, 2009; Barcenas *et al.*, 2009), while also reduced the tendency of retrogradation as shown by lower values of setback and breakdown viscosity (Barcenas *et al.*, 2009). Dough stability during cooking was

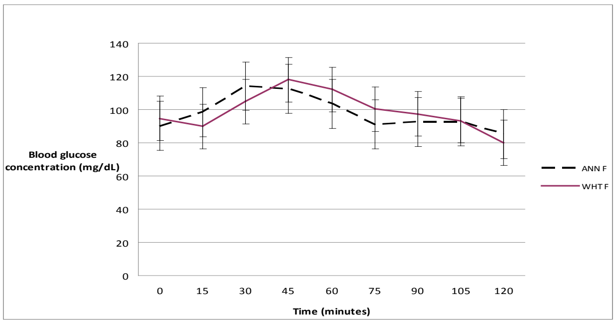

Figure 2. Blood glucose level of volunteers as affected by bread consumption. WHT F is wheat flour bread, and ANN F is bread with 50% substituton of annealed purple yam flour. Bars indicated standard error of every measurement

improved by addition of arabic gum (Alam *et al*., 2009). The effect of arabic gum was better than other hydrocolloids in improving viscoelasticity of bread dough (Barcenas *et al*., 2009). In this case, HPMC (hydroxypropylmethylcellulose) or CMC (carboxymethylcellulose) weakened gluten network of dough, and reduced viscoelasticity (Alam *et al*., 2009; Barcenas *et al*., 2009).

Apart from arabic gum, there was a probable influence of yam mucilage on gelatinisation profile of purple yam flour, as shown by lower elastic moduli (G'), and higher temperature to rise the moduli (Yeh *et al*., 2009). This characteristic of mucilage was considered for giving capability of yam flour to replace 5% fat in sausage (Tan *et al*., 2007), as well as to be used in noodle making (Lin *et al*., 2006), without adversely affecting texture and sensory properties of the products (Lin *et al*., 2006; Tan *et al*., 2007).

Nevertheless, the presence of arabic gum and mucilage did not make susbtituted bread volume became comparable to that of normal bread. This may indicate that 50% substitution level of yam flour in bread is too high, which consequently caused too much dilution of gluten in bread mixture that cannot be compensated by arabic gum and mucilage in yam. Nevertheless, yam (*D. alata*) starch could substitute wheat flour at 60% without affecting bread volume (Nindjin *et al*., 2011). Starch had higher viscoelasticity than its corresponding flour (Aprianita *et al*., 2009), which may due to the presence of several non-starch components in flour. Purple yam flour contained 31% amylose which was considered as high (Dias *et al*., 2010), that may weaken viscoelastic characteristics (Hung *et al*., 2005).

Higher hardness of substituted bread is a consequence of the reduce in bread volume. Hardness of substituted bread containing 1-3% arabic gum ranged from 22-33 N (Table 2), which was around twice harder than that of normal bread (9,9 N). There are several possible explanations for the reduce in bread volume apart from dilution of gluten in bread mixture. First, as annealing may increase resistant starch content in flour (Chung *et al*., 2009), bread volume may be reduced (Korus *et al*., 2009). Secondly, the presence of ungelatinised resistant starch caused discrete gluten network around starch granule, while normal bread showed continue and unbroken gluten network surrounding starch granule in bread dough (Korus *et al*., 2009). Although we did not measure resistant starch, slowly digestible starch, nor rapidly digestible starch in annealed purple yam flour, there was a possibility of increasing resistant starch in yam flour as previously reported (Chung *et al*., 2009), as indicated by more resistant nature of annealed yam flour. However, it was not clear whether the increase in hardness of yam substituted bread was due to the increase in resistant starch or the presence of ungelatinised yam starch as a result of annealing.

Glycemic index of substituted bread

Blood glucose level of volunteers upon consumption of bread substituted with 50% annealed purple yam flour did not differ substantially from those consuming normal white bread (control bread) (Figure 2). Consumption of either bread led to similar peak of blood glucose level at 45 min after consumption. Similarly, at the end of second hours, blood glucose level as caused by consumption of two bread types was also the same. As a result, glycemic index of substituted flour was as high as 93.19. This result may suggest that there were some other factors influence glycemic index. Although annealing increased crystallinity of starch, it was not always accompanied by the increase in resistant starch (Chung *et al*., 2009). This may explain why, despite the increase in temperature and shear resistance of annealed yam flour, the glycemic index was practically unchanged, which may indicated there was no substantial amount of resistant starch formed during the process. Annealing may even elevate susceptibility to enzyme digestion in gastrointestinal tract, due to formation of pores on surface of granule, to facilitate enzyme entering the granule and digest it, without altering granule shape (O'Brien and Wang, 2008). We did not notice any microscopic change of annealed starch granule, a phenomenon which may similar to what was reported by O'Brien and Wang (2008). Another factor on digestibility is amylose content. Amylose content affected enzyme susceptibility in annealed starch (Dias *et al*., 2010), where high amylose (32%) starch had higher digestion rate than low amylose content (Dias *et al*., 2010). Amylose content in purple yam flour was high (31%), which may in turn facilitate enzymic digestion in the gut.

As result showed, apparently annealing was not suitable method to reduce glycemic index of purple yam flour, which was likely due to its high amylose content and possibly its susceptibility to form pores on granule surface during annealing. Therefore, it is important to find other effective ways to improve glycemic index such as cross-linking using enzyme. It was also important to explore other functional benefits of purple yam flour, such as antioxidant, hypolipidemic effect, and immune modulation.

Conclusion

Annealing of purple yam flour improved its gelatinisation profile. Substituted bread containing 50% annealed purple yam flour was twice harder than normal bread, with relatively unaltered glycemic index compared to control bread.

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