Effect of processing methods on α-amylase and pasting properties of cassava flour

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Abstract

Cassava flour is usually produced at household level by various methods and used for direct consumptions in many countries. In this study, cassava flour was prepared by different methods (peeled vs unpeeled, grated vs sliced, pressed vs unpressed and reconstitution of starch and fibrous residues) and their paste viscosity properties were investigated by a Rapid Visco Analyzer (RVA). The results suggest inconsistent paste properties, in particular peak (PV1) and final viscosity (FV1), as influenced by the method of preparation. Paste viscosity properties of these flour samples were further determined in the presence of silver nitrate ($AgNO_3$), i.e. an enzyme inhibitor and less variation in paste properties was observed. The degree of change in peak and final viscosity of flour (ΔP and ΔF), which implied the amount of enzyme activity was the lowest in the reconstituted flour, followed by grated-peeled-pressed sample. When considering the paste viscosity of flour without the effect of enzyme, the effect of processing methods on paste viscosity of cassava flour was still observed, presumably caused by the different chemical compositions. Reconstituted flour, with the highest starch content (94.5% dwb) had the highest peak viscosity (356 RVU) while sliced flour samples with the lowest starch contents (81.9-83.5% dwb) had the lowest peak viscosity (257-266 RVU).

Keywords : Cassava flour, Alpha-Amylase, Paste Viscosity, Starch content

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Introduction

Cassava (*Manihot esculenta* Crantz) is a root crop planted and consumed commonly as a major food source in several regions, especially in developing countries. In Africa, the production of cassava reached 101.6 million tons in 2003 made cassava become the most important root crops in the world (FAO, 2013) and the main food source that provides calories. The roots of cassava are very rich in starch, which makes them an important source of dietary energy. They can be used diversely in a wide range, i.e. from the household level as an important staple food upto an industrialized level for a production of food, paper, textile, plywood, pharmaceuticals, cosmetics, bio-ethanol and biodegradable plastics. Cassava flour is another product made from fresh cassava roots, being white to off-white in color, odourless, tasteless, and containing no foreign matter and fungus with the moisture of 10-12% (Oti, 2010). With those characteristics and good viscosity property, cassava flour is used popularly in many diets as well as to substitute other commercial flour especially wheat flour in bakery, noodle and breakfast cereal products (Loreto, 1992; Akingbala *et al.*, 2011). According to the work conducted by Annor-Frempong *et al*. (1996), cassava flour was convinced that had possibility to be used as an additive in crushed meat products.

The flour can be produced from fresh roots of sweet (i.e. low cyanide) or bitter (i.e. high cyanide) varieties (Chotineeranat *et al*., 2006; Sriroth *et al*., 2009), through a process consisted of different steps including 1) peeling, 2) chipping, slicing or grating followed by dewatering, 3) drying and then 4) milling. The actual processing practice, however, varies depending on geographical original, the flour quality and the end applications. The roots can be processed in various practices such as peeled or partially peeled; fresh or boiled; soaked or unsoaked in water; with or without fermentation. Interestingly, reconstitution of extracted starch and fibrous residues was introduced in order to produce flour with low and safe cyanide contents (Ooye *et al*.,2014).

The influence of processing on cassava flour quality has been extensively investigated. For instance, the effect of chipping method (size and equipment), drying method (sun drying vs. oven drying, drying temperature), fermentation process (natural or culture addition) and milling methods (hammer mill, pin mill, roller mill) on flour quality including chemical composition, cyanide content, microbiological quality, paste viscosity profile and cooking quality have been elucidated (Padmaja, 1995; Fernadez *et al*., 1996; Jones *et al*., 1996). Usually, flours contain almost the same components as are present in the raw materials, except the moisture and fiber. The components that are often found in flours include starch, non-starch polysaccharide, sugar, protein, lipid, and inorganic materials. Moreover, some starch degrading enzymes present in fresh cassava roots have been reported (Tangphatsornruang *et al*., 2005). Comparisons in properties of cassava flour and its corresponding starch have been reported by Moorthy *et al*. (1996), Niba *et al*. (2002) and Padonou *et al*. (2005). Similarly, Charoenkul *et al*. (2011) reported that the paste viscosity and setback of cassava flour form different varieties, being classified by mealy, firm and mealy & firm according to the texture of cooked roots, were positively correlated to starch content and negatively correlated to α-amylase activity, while protein, lipid and fiber did not show correlation with pasting parameters. Nevertheless, these flour samples were obtained from low-cyanide cassava varieties i.e. sweet varieties and processed by milling of peeled, dried chips.

The α-amylase can hydrolyze starches especially when starch is gelatinized during cooking process. The α-Amylase is an endo-acting enzyme which is capable to hydrolyze starch molecules randomly inside the glucan chains, leading dramatically decrease in the molecular weight as well as paste viscosity. During cassava flour production, this enzyme found in fresh roots may not be completely removed or inactivated. Consequently, the enzyme residues may be left over in finished products and introduce inconsistent quality of flour products. Our previous work has demonstrated the α-amylase activity in cassava flour produced by using a household process, as determined by a falling number, a method usually used to determine the amylase activity in wheat flour and by a colorimetric method, using an α-amylase specific substrate, namely non-reducing-end blocked *p*-nitrophenyl maltoheptaoside (Yonkoksung *et al*., 2013). Furthermore, a simple method by determining the change in paste viscosity (i.e. ΔP, peak viscosity and ΔF, final viscosity by a Rapid Visco Analzyer, RVA), of flour in the absence and presence of α-amylase inhibitor (i.e. silver nitrate, $AgNO_3$) was applied to measure the enzyme activity and the results indicated a good correlation between changes in viscosity and colorimetric method (Yonkoksung *et al*., 2013).

Objective

Our previous work has demonstrated the effect of α-amylase on the quality of cassava flour from different high-cyanide cassava varieties, prepared by grinding of peeled roots. In this work, the effect of different processing methods of preparing cassava flour from bitter cassava varieties on paste properties and α-amylase activity was investigated. Furthermore, the chemical compositions of flour prepared from different methods were analyzed to elucidate their effects on paste properties, when considered without the effect of enzyme.

Materials and Methods

Materials

Fresh cassava roots of the variety Huay Bong 60 (HB60) were obtained from the Thai Tapioca Development Institute (TTDI) experimental station, located Huay Bong district, Nakhonratchasima province, Thailand under the support of Faculty of Agriculture, Kasetsart University, Thailand.

Cassava flour preparations

Different processing methods which are employed at the household production were applied to prepare cassava flour.

Grating method

Fresh roots, peeled or unpeeled were washed thoroughly with tap water and cut into small pieces. The samples were then grated and incubated for 2 h. This was further used to prepare pressed and unpressed samples. For grated-unpressed samples, the mash was dried immediately in a hot air oven at 50°C for 24 h. The dried samples were hammer-milled and passed through a 100-mesh sieve to obtain fine powder. These flour samples are referred to as the Grated-Peeled-Unpressed-Cassava Flour and Grated-Unpeeled-Unpressed-Cassava Flour. For grated-pressed samples, the grated mash after incubation was pressed with a hydraulic press prior to drying and milling in a same manner as unpressed samples. These flours were referred to as the Grated-Peeled-Pressed-Cassava Flour and Grated-Unpeeled-Pressed-Cassava Flour (Chotineeranat, *et al*., 2006).

Slicing method

Fresh roots, peeled or unpeeled, were washed and sliced to 2-3 cm thicknesses. Sliced cassava was spread thinly on the tray and then dried in an oven at a temperature of 50°C for 24 h. The dried samples were hammer-milled to fine powder and passed through a 100-mesh sieve. These flours were referred to as the Sliced-Peeled-Cassava Flour and Sliced-Unpeeled-Cassava Flour (Lamptey *et al*., 2008).

Reconstitution method

Fresh roots were peeled, washed, cut and grated with water and the ground root slurry was then sieved to separate the starch milk from the fibrous pulp. Starch milk was settled for 6 h before the supernatant was decanted. Sedimented starch was collected, washed thoroughly with water and dried at 50°C for 24 h. The fibrous residues were also dried immediately at a temperature of 50°C for 24 h. The dried starch and fibrous residues were then milled, mixed and sieved through a 100-mesh sieve to obtain the flour. This flour was referred to as the Reconstituted Cassava Flour (Padmaja and Moorthy, 1999).

Analytical methods

Chemical composition of cassava flours

Proximate analysis of compounds, i.e. the moisture, ash and crude fiber contents, in the cassava flours were performed by the standard AOAC methods (1990). Starch contents were determined by the enzyme method using α-amylase/amyloglucosidase method (Megazyme kit, McCleary *et al*., 1997).

Color determination

Colorimetric measurements of flour samples were determined in triplicate using a spectrophotometer (CM 3500 d, Minolta Camera Co., Ltd, Tokyo, Japan). The CIE color values were recorded as *L** = lightness (0 = black, 100 = white), *a** (-*a** = greenness, +*a** = redness) and *b** (-*b** = blueness, +*b** = yellowness).

Paste viscosity determination by a Rapid Visco Analyzer (RVA)

i) In the absence of an enzyme inhibitor, $AgNO_3$

The paste viscosity properties of the flour suspension in distilled water (9.2% dwb, 2.5 g flour in 25 g distilled water) were evaluated using a Rapid Visco Analyzer (RVA4, Newport Scientific, Australia). The paddle speed was set at 960 rpm for the first 10s, then at 160 rpm for the rest of the analysis. The suspension was heated from 50 to 95°C at a rate of 12°C/min and held at 95°C for 2.5 min. The paste was then cooled down to 50 °C at 12°C/min, and held at this temperature for 2 min until the end of the experiment. The viscosity at different stages of the RVA profile were recorded, including peak viscosity (PV1), trough viscosity, i.e. a minimum viscosity at 95°C (H1), final viscosity at 50°C (FV1). The breakdown (BDV = PV-H) and setback from trough (FV-H) were also reported.

ii) In the presence of an enzyme inhibitor, $AgNO_3$

To determine the α-amylase activity in flour samples, a Rapid Visco Analyzer was used to evaluate the paste viscosity profile in the presence of silver nitrate ($AgNO_3$), which acts as a potent enzyme inhibitor. In this experiment, flour samples were suspended in 15 mM $AgNO_3$ instead of distilled water and the test was performed under the same condition as previously described in (Dixon and Webb, 1964; Collado and Corke, 1999)

Statistical analysis

All the determinations were done in duplicate and the data were statistically analyzed by one way ANOVA and Duncan's multiple range test. Only significant terms ($p<0.05$) were retained in the models.

Results and Discussion

Chemical compositions

The chemical compositions of all flour samples were summarized as shown in Table 1. In which, in overall chemical composition were significant difference ($p < 0.05$). The starch contents were in the range of 82-89% (dwb) except the reconstituted flour (95% dwb), which were lower than commercial cassava starch (> 99% dwb). Undoubtedly, the flour samples contained higher fiber (0.33-1.65% dwb) and ash (0.37-1.83% dwb) contents than commercial starches (ash content < 0.2% dwb). When considering the effect of processing methods on chemical compositions of produced flour, it was obvious that the reconstituted flour had the highest starch content and the lowest ash content. During the preparation of starch for flour reconstitution, starch was washed with water, therefore some of soluble inorganic salts and minerals were removed. The ash content of flour obtained by slicing (1.62-1.83%) was higher than those obtained by grating (1.07-1.24%).

Table 1 Chemical composition of cassava flours from different processing methods*

Processing method	Content (%, dry basis)		
	Starch	Crude fiber	Ash
Grating method:			
Grating (peeled-pressed)	88.8±2.0 [ab]	0.59±0.2 [c]	1.17±0.0 [c]
Grating (peeled-unpressed)	87.7±4.3 [bc]	0.59±0.1 [c]	1.07±0.0 [d]
Grating (unpeeled-pressed)	85.9±0.5 [bc]	1.65±0.0 [a]	1.21±0.0 [c]
Grating (unpeeled-unpressed)	86.9±2.9 [bc]	0.96±0.1 [b]	1.24±0.0 [c]
Slicing method:			
Slicing (peeled)	81.9±2.1 [c]	0.33±0.1 [d]	1.83±0.0 [a]
Slicing (unpeeled)	83.5±3.8 [bc]	0.58±0.0 [c]	1.62±0.0 [b]
Reconstitution method	94.5±0.1 [a]	0.68±0.0 [c]	0.37±0.0 [e]

*Mean values in the same column with different letters are significantly different ($p < 0.05$).

During grating, the plant tissue was broken so that soluble minerals might be lost with some released water. In contrast, the fiber contents of cassava flours prepared by grating method were higher than slicing method (0.6-1.6% and 0.3-0.6%, respectively). The dried, sliced samples, after milled, might contain large pieces of fibrous pulp that were separated out by sieving. In contrast, the grated flour samples were subjected to more mechanical forces during grating and hammer-mill and might contain more small pieces of fibrous residues. The fiber contents of peeled flour samples were certainly less than unpeeled ones for both grated and sliced samples.

Pasting properties

The paste viscosity profiles of cassava flour, as determined by an RVA in the absence of $AgNO_3$, were recorded as shown in Figure 1a. It is obvious that cassava flour prepared by different methods had significantly different paste viscosity profiles. Flour prepared by slicing method provided the paste with the lowest peak, trough and final viscosity (166-184, 18-32 and 27-45 RVU, respectively), compared to those prepared by grating method (237-240, 44-77 and 64-110 RVU, respectively) (Table 2). The paste viscosity of reconstituted flour was the highest; the peak, trough and final viscosity were 329, 78 and 117 RVU, respectively. The variation in peak, trough and final viscosity, indicated as the difference between the maximum and minimum values, of all flours were 163, 60 and 90 RVU, respectively. All flour had similar pasting temperatures.

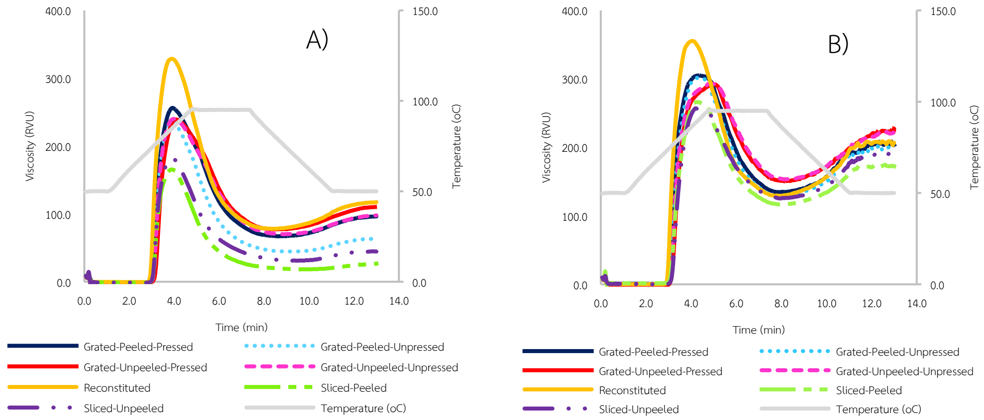

Figure 1 Paste viscosity profile of cassava flour prepared from different processing methods, as determined by a Rapid Visco Analyzer in the absence (A) and presence (B) of an enzyme inhibitor, i.e. silver nitrate ($AgNO_3$).

When the paste viscosity of cassava flour was determined in the presence of $AgNO_3$ (Figure 1b), the paste viscosity including peak, trough and final viscosity of all flour samples were increased significantly which were 257-266, 119-126, 173-190 RVU, respectively for sliced samples; 293-305, 128-153, 199-226 RVU, respectively for grated samples and 356, 126 and 207 RVU, respectively for reconstituted flour. During heating, starch becomes gelatinized and makes it more prone to enzyme attack, leading to lower paste viscosity. In contrast, in a presence of $AgNO_3$, upon gelatinization, starch was not hydrolyzed due to an inhibitory effect caused by $AgNO_3$, resulting in higher viscosity of cooked paste.

Table 2 Paste viscosity profile of cassava flour prepared from different processing methods, as determined by a Rapid Visco Analyzer in the absence and presence of an enzyme inhibitor, i.e. silver nitrate ($AgNO_3$)*

Processing method	Viscosity (RVU)			
	Peak vis. (PV1)	Final vis. (FV1)	Peak vis. (PV2)	Final vis. (FV2)
Grating method:				
Grating (peeled-pressed)	257±0.1 ^{b}	96±1.2 ^{c}	305±1.0 ^{b}	204±4.8 ^{bc}
Grating (peeled-unpressed)	237±0.2 ^{c}	64±3.6 ^{d}	301±0.5 ^{bc}	199±0.1 ^{c}
Grating (unpeeled-pressed)	239±0.8 ^{c}	110±0.6 ^{b}	293±0.6 ^{d}	226±3.1 ^{a}
Grating (unpeeled-unpressed)	240±1.0 ^{c}	98±1.8 ^{c}	297±1.4 ^{cd}	225±2.9 ^{a}
Slicing method:				
Slicing (peeled)	166±3.2 ^{e}	27±1.0 ^{f}	266±0.9 ^{e}	173±0.6 ^{e}
Slicing (unpeeled)	184±0.5 ^{d}	45±1.4 ^{e}	257±4.3 ^{f}	190±4.0 ^{d}
Reconstitution method	329±0.2 ^{a}	117±0.1^{a}	356±0.6 ^{a}	207±0.8 ^{b}

*Mean values in the same column with different letters are significantly different ($p < 0.05$). PV1 is Peak viscosity in absence of enzyme inhibitor, PV2 is Peak viscosity in presence of enzyme inhibitor, FV1 is Final viscosity in absence of enzyme inhibitor, FV2 is Final viscosity in presence of enzyme inhibitor.

Furthermore, it is interesting to note that less variation in paste viscosity of cassava flour was observed with no action of α-amylase enzyme, when flour was cooked. The variation range in peak, trough and final viscosity, reported as the difference between the maximum and minimum values, of all flour when evaluated in the presence of $AgNO_3$ reduced significantly (99, 34 and 53 RVU for peak, trough and final viscosity, respectively). This implied the substantial influence of α-amylase on the paste properties of cassava flour and different processing methods might yield the different amount of enzyme residues in produced flour.

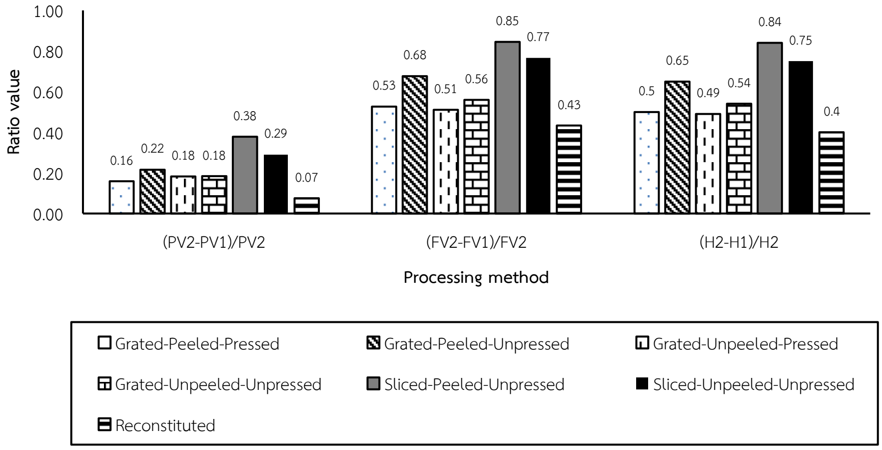

Figure 2 Changes in peak viscosity (ΔP), final viscosity (ΔF) and trough viscosity (ΔH) of cassava flour prepared from different processing methods, as determined by a Rapid Visco Analyzer

Note: Changes in paste viscosity are the ratios of the difference between the defined paste property of flour in the presence (e.g. Peak viscosity, PV2) and absence (e.g. Peak viscosity, PV1) of $AgNO_3$ to peak viscosity of flour in the presence of $AgNO_3$; [e.g. ΔP = (PV2-PV1)/PV2)]

As earlier demonstrated in the previous work (Yonkoksung *et al*., 2013), the changes in paste viscosity, reported as the ratio of the difference between the paste property in the presence and absence of $AgNO_3$ to the value obtained in the presence of $AgNO_3$, of cassava flour by a Rapid Visco Analzyer were well correlated with the amount of α-amylase activity, when determined calorimetrically using a specific substrate, Figure 2 represents changes in peak, trough and final viscosity of cassava flour prepared by different methods. Changes in peak, trough and final viscosity of cassava flour prepared by slicing method (0.29-0.38, 0.75-0.84 and 0.77-0.85, respectively) were higher than those prepared by grating (0.16-0.22, 0.49-0.65 and 0.51-0.68, respectively) and reconstitution (0.07, 0.40 and 0.43, respectively) methods. This implied that the reconstitution method yielded the product with the least enzyme activity. The slicing method gave the flour with the highest enzyme residues, as compared to those obtained by grating method. During grating, the plant cells were broken and those soluble enzymes might be lost with some released water. Drying of sliced or grated cassava at a low temperature could not effectively inactivate the enzyme.

When considering the paste viscosity of flour without the effect of enzyme, the effect of processing methods on paste viscosity of cassava flour was still observed, presumably caused by the different chemical compositions. Reconstituted flour, with the highest starch content

(94.5% dwb) had the highest peak viscosity (356 RVU) while sliced flour samples with the lowest starch contents (81.9-83.5% dwb) had the lowest peak viscosity (257-266 RVU).

Color of cassava flour

Color is one of several important qualities of flour for food applications. The color of flour depends on the origin of raw materials. Corn flour is yellow while sweet potato flour of some varieties is orange in color. The color of cassava flour is usually off-white to yellowish. The color of cassava flour depends on the composition of roots as well as the process of making flour. Table 3 summarizes the color of cassava flour prepared by different methods. The brightness (*L**) of cassava flour ranged from 90.8-97.1, of which the reconstituted flour had the highest value. The brightness of unpeeled samples was lower than the peeled one. The yellowness of flour products was indicated by the positive values of *b**, which was the lowest in reconstituted flour due to washing. Cassava flour prepared by slicing method had lower whiteness index than those obtained by grating and reconstitute methods.

Table 3 Color determination of cassava flours from different processing methods*

Processing method	*L**	*a**	*b**	Whiteness index
Grating method:				
Grating (peeled-pressed)	94.89±0.08^{c}	0.15±0.01^{e}	9.16±0.08^{e}	89.51±0.04^{c}
Grating (peeled-unpressed)	95.54±0.02^{b}	0.06±0.01^{f}	8.84±0.01^{f}	90.10±0.00^{b}
Grating (unpeeled-pressed)	91.19±0.04^{f}	1.45±0.04^{a}	11.18±0.06^{c}	85.70±0.03^{e}
Grating (unpeeled-unpressed)	91.49±0.01^{e}	1.37±0.01^{b}	10.22±0.06^{d}	86.63±0.04^{d}
Slicing method:				
Slicing (peeled)	93.24±0.01^{d}	0.69±0.01^{d}	13.19±0.01^{b}	85.16±0.01^{f}
Slicing (unpeeled)	90.88±0.03^{g}	1.01±0.02^{c}	14.20±0.08^{a}	83.09±0.09^{g}
Reconstitution method	97.14±0.01^{a}	-0.22±0.02^{g}	4.46±0.00^{g}	94.70±0.01^{a}

*Mean values in the same column with different letters are significantly different ($p < 0.05$).

This could be explained by two possible reasons. First, sliced samples contained more soluble components, e.g. sugar, amino acid which might undergo non-enzymatic browning. Secondly, the enzymatic browning might be taken place at a greater extent due to more enzymes, i.e. polyphenol oxidase and peroxidase available in the cells of plant tissue (Kamuf *et al*., 2003; Richard-Forget and Gauillard, 1997). Flour that was prepared from unpeeled roots had the lower whiteness index than flour prepared from peeled roots as the peel contained a lot of phenolic compounds, a substrate for enzymatic browning.

Conclusion

Cassava flour with consistent quality is an important factor for successfully developing its applications at the commercial scale. There are many factors that can affect cassava flour quality. This work demonstrates the effect of processing, typically applied at the household production, on the paste viscosity of cooked flour. Further investigation suggests the variation in paste property of flour is greatly caused by the residues of some starch-degrading enzymes left over in the products. In addition, chemical compositions of produced flour can partly affect the viscosity of cooked paste. The reconstitution method provides flour with the highest paste viscosity, the lowest enzyme residues and the highest whiteness. When compared between grating and slicing methods, flour obtained by grating methods has better quality in term of paste viscosity, enzyme residues and whiteness.

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