

# Physicochemical properties of fortified flour based modified cassava flour blended with bacterial poly-glutamic acid

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## ABSTRACT

Cassava is one of the most important staple crops worldwide. However, cassava flour needs to meet the high-quality requirements in terms of physicochemical characteristics. The present study was designed to investigate properties of fortified cassava flour produced from co-processing of modified cassava flour with poly-glutamic acid (PGA) derived from protein of beans that had been fermented by *Bacillus natto*. Proximate analysis, cyanide content, swelling power, solubility, and viscosity of modified cassava flour (MCF) which was fortified with poly-glutamic acid (PGA) was found to indicate improvements as compared to the native flour. The modified flours were further investigated for their physicochemical properties after addition of poly-γ-glutamic acid (γ-PGA) at different levels. All flour samples showed no significant ( $p > 0.05$ ) differences in terms of lightness ( $L^*$ ), while greenness to redness ( $a^*$ ) of native flours was significantly ( $p < 0.05$ ) higher than modified flours with γ-PGA. There were significant ( $p < 0.05$ ) differences in the swelling power and solubility measured at various temperatures. From the pasting profiles, there were significant ( $p < 0.05$ ) increases in peak viscosity, final viscosity and pasting temperature of cassava flours due to addition of γ-PGA. Observation by scanning electron microscopy (SEM), pronounced cracks were observed in starch granules indicative of enzyme attack. It indicated that starch granules of modified and fortified cassava flour were depolymerized by enzymatic hydrolysis which led to cause change and degrade exterior surface of the granules within corrosion via pores of small granules.

## 1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) also known as tapioca, yucca, or manioc is considered as the third most important source of carbohydrate in tropics, after maize and rice (FAO, 2014). While cassava was once considered as the “food for the poor”, now it has become a significant world agriculture and provides a multipurpose utilization in developing countries, become a global trend economically, and a challenge towards climate change

(Howeler et al., 2013). The most common way is by processing the fresh cassava tubers into flour, so that it could be utilized in a broad range of new products to these developing countries with rapidly urbanizing societies. Due to limited functionalities, perishable, and bulky, proper strategies and technologies are required to overcome these limitations (Poonsrisawat et al., 2014).

Besides, although native flour is demanded for industrial applications, more often the industries require modified flour with improved functionality as direct application of native flour is quite challenging. In the last few decades, various methods have been developed for starch modification to achieve suitable functional properties to be utilized in various industries. Generally, there are four methods of modifications, namely physical, chemical, enzymatic, and genetic, or their combinations (Kaur et al., 2011).

Modification of starch has been extensively studied to overcome the functional limitations of native flour/starch and to increase the importance of starch for industrial applications (Kaur et al., 2012). Apart from reducing gelling tendencies, retrogradation of gel/paste texture, film formation and adhesion of native starches (Kaur et al., 2012), stabilisation of starch granule can be achieved through modification for industrial applications (Ashogbon & Akintayo, 2014). Briefly, modification often led to a change towards the starch polymer, thus increase its flexibility and change its physical and functional properties, as well as its structural properties to enhance its usefulness for food and non-food industries (Lopez et al., 2010). In an enzymatic modification, hydrolysing enzymes are mainly used for starch modification and their products are either high fructose corn syrup or glucose syrup. The enzyme used for modification is usually free from enzymatic components so that unwanted damage to the starch granule/molecule can be prevented (Kaur et al., 2012). Enzymatic modification has several advantages, such as more specific hydrolysis products, fewer by-products, higher yield, and its end products can be control for properties (Dura et al., 2014).

There are abundant of enzymes used to modify the structure of starch and achieve the desired functional properties, such as  $\alpha$ -amylase and cellulase.  $\alpha$ -Amylase is one of the

hydrolase enzymes that catalyzes the hydrolysis of  $\alpha$ -1, 4-glycosidic linkages in starch to produce products such as maltose, glucose and dextrin, while retaining the  $\alpha$ -anomeric configuration of the products (Gupta et al., 2003). Hence, the importance of  $\alpha$ -amylase is crucial due to its starch hydrolysis activity (Sundarram & Murthy, 2014).

Meanwhile, cellulases are grouped into glycoside hydrolases (GH) family and often used to catalyse the hydrolysis of glycosidic linkages depolymerising cellulose to fermentable sugars (Juturu & Wu, 2014). Moreover, cellulose can be broken down by employing cellulases to hydrolyse  $\beta$ -1,4 glycosidic bonds of the cellulose polymer (Behera et al., 2017). Bacteria have been widely explored for enzyme production. Among them, *Bacillus* sp. has become the dominant bacteria in flour modification due to its ability to produce and secrete large quantities of extracellular enzymes (Rastogi et al., 2009).

Among these methods, microbial fermentation is deemed the most cost-effective, including inexpensive raw materials, minimal environmental pollution, mild reaction conditions, and high natural product purity (Luo et al., 2016). In microbial fermentation, submerged fermentation technology remains challenging due to limited oxygen supply during fermentation process, high expenditure of raw materials, and rigorous laboratory equipment (Xu et al., 2014). On the contrary, solid-state fermentation (SSF) is more advantageous, including lower production cost, more straightforward equipment and reduced contamination risks (Pandey, 2003). Thus, SSF was chosen for  $\gamma$ -PGA production in this study (Bajaj & Singhal, 2011). In the food industry,  $\gamma$ -PGA is used as a thickener, bitterness relieving agent, cryoprotectant, encapsulation, water adsorbent, and as a nutrition supplement (Shih & Van, 2001). Therefore, it may be suggested that PGA as a food additive can be used to improve the physicochemical properties of native flour. In this research, microbial  $\gamma$ -PGA was chosen to overcome the limitation of physicochemical properties in cassava. To date, no research has been done incorporating microbial  $\gamma$ -PGA in cassava flour to improve its physicochemical properties.

The objective of this study was to fortify enzymatically modified cassava flour with

bacterial poly- $\gamma$ -glutamic acid prepared from *Bacillus natto* and to study on physicochemical properties of the fortified of the modified cassava flour. Hopefully, this research could increase the utilization of local cassava flour with value-added ingredients.

## **2. METHODOLOGY**

### **Modification Procedure**

Cassava chips (1.0 kg) were mixed with 2.0% (w/v) of enzymatic starter derived from culture of *B. natto*. The mixture was stirred and then incubated at room temperature for 24-48h, and then dried using oven at 50°C for 12-24h to reduce moisture content up to lower than 10% (Shariffa et al, 2009; Richana et al, 2010).

### **Measurement of HCN Content**

HCN content of cassava flour samples were measured by using alkali titration method (Betiku & Alade, 2011). Five % (w/v) starch slurry was prepared by soaking 10 g of cassava flour in 200 ml of distilled water for 4h in a distillation flask. The solution was then distilled into 20 ml of 0.625M NaOH using steam distillation until its volume became 150 ml. Distilled water was added and filled up to 250 ml. Eight ml of 5% KI was added into 100 ml of solution and titrated using 0.02N silver nitrate solution. The end point of titration was determined as color of the solution turned to bright yellow.

### **Proximate Analysis**

The proximate composition of the cassava flour including moisture, ash, fat, and protein contents were determined using Association of Official Analytical Chemists (AOAC, 2005) methods. Total carbohydrate content was determined by subtracting the ash, protein, and fat percentages from 100%.

## Determination of Colour Values

The colour of the cassava flour was determined by using a Chroma Meter. The Chroma meter was calibrated using its white standard calibration cover. The flour samples were placed in a plastic petri dish, lightly shaken to form a layer of 5 mm thickness, covered with the petri dish lid and the colour was read on the meter. The parameters of  $L^*$ ,  $a^*$  and  $b^*$  was considered where the  $L^*$  scale ranges from 0 black to 100 white; the  $a^*$  scale extends from a negative value to a positive value (red hue); and the  $b^*$  scale ranges from negative blue to positive yellow, were recorded and average values were computed from three randomly selected points (Rosales-Soto et al., 2016).

## Production and Extraction of $\gamma$ -PGA

Production of  $\gamma$ -PGA by solid-state fermentation of soybeans was done independently in triplicate, according to Chen et al. (2005). Firstly, raw soybeans were rinsed and soaked in clean water. Twenty grams of dehulled soybeans were weighed into 250 ml conical flasks and then autoclaved at 121°C for 20 minutes, and the cooked soybeans were allowed to cool before inoculating the substrate with 5% inoculum, mixed carefully under strictly aseptic conditions with sterile glass rods, sealed with eight layers of gauze and then incubated at 37°C for 48 hours in a static mode. Extraction of  $\gamma$ -PGA from fermented soybeans was done according to the procedure described by Chen et al. (2005). When fermentation was terminated, ten volumes of distilled water were added into the fermented soybeans. The mixture was mixed at room temperature on a rotary shaker for 1 hour at 200 rpm to dislodge the mucilage containing  $\gamma$ -PGA formed on the surfaces of the fermented substrates. The whole contents were filtered through a muslin cloth. After filtering twice using the same approach, the filtrates were pooled, and the total volume was recorded to determine the extraction yield. Approximately 10.0 ml filtrate was subjected to centrifugation at 12,000 rpm for 20 min. The resulting supernatant containing crude  $\gamma$ -PGA was poured into four volumes of cold ethanol and allowed to sit for overnight at 4 °C to precipitate the  $\gamma$ -PGA. The resultant precipitate

containing crude  $\gamma$ -PGA was collected by centrifugation at 12,000 rpm for 20 min. Then, 5 ml aliquot of distilled water was added to dissolve the precipitate and the resulting solution containing  $\gamma$ -PGA was stored prior to UV assay and fortification in modified cassava flour. To measure  $\gamma$ -PGA yield, the aqueous  $\gamma$ -PGA solution was lyophilized to obtain  $\gamma$ -PGA in powder form.

### **Water and Oil Absorption Capacity**

Water absorption of flour was measured by the centrifugation method reported by Kaur & Singh (2005). For water absorption, 3.0 g of samples were dissolved in 25ml of distilled water and placed in 50 ml pre-weighed centrifuge tubes. The mixtures were stirred at 5 minutes' intervals and held for 30 minutes, followed by centrifugation for 30 minutes at 3000 g. The supernatant was decanted, the excess moisture was removed by draining for 25 minutes at 50, and the sample was reweighed. For oil absorption, 2.5 g of flour sample was mixed with 30 ml peanut oil in pre-weighed centrifuge tubes and stirred for 1 min. After a holding period of 30 minutes, the tubes were inverted for 25 minutes to drain the oil prior to reweighing. Triplicate determinations were carried out, and the water and oil absorption capacities were expressed as grams of water or oil per gram of the sample on a dry basis.

### **Observation Using Scanning Electron Microscope**

The microstructures of the granules of native and modified flours were viewed with a field emission of the scanning electron microscope (Carl Zeiss, Germany) according to the method reported by Shariffa et al. (2017). The starch granules were stuck onto aluminium specimen stubs with double-sided adhesive tape and sputter-coated with 20-30 nm layer of gold using a sputter coater. The accelerating voltage of the SEM is 15.00 kV.

### **Statistical Analysis**

All experiments were performed at least twice with three measurements for each analysis. The data was reported in mean standard deviation. All data obtained were

subjected to analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS; version 21). Turkey's-b multiple comparisons of means were used to analyze all the cassava flour samples at the  $p \leq 0.05$  confidence level.

### 3. RESULTS AND DISCUSSION

#### Proximate Analysis

The proximate composition provides a general overview of the nutritional value of food and includes analysis of the ash, moisture, fat and protein content. Moisture content, especially in food products, is necessary to be analyzed as it can determine the shelf life of the food. High water content in food is the main factor that caused the growth of microorganisms which lead to the destruction of food (Wahyuni et al., 2017). The determination of ash content is also important as it indicated the levels of different kinds of minerals and some vitamins present in the food samples (Chinma et al., 2013). Moreover, the protein content is an essential parameter since it determined the quality of the food products. Protein may serve as an amplifier batter that affects the development of food production through the formation of gluten. Meanwhile, the content of fat in food serves as a lubricant batter in which high-fat content might cause the food products easy to be oxidized (Wahyuni et al., 2017).

Table 1 shows the proximate composition of cassava flour and  $\gamma$ -PGA blends. The moisture, ash, protein, fat, carbohydrate, and amylose contents ranged from 5.91 to 7.98%, 1.25 to 1.62%, 1.19 to 2.32%, 0.11 to 0.40%, 88.86 to 90.89% and 20.28 to 23.85% respectively. The protein and fat contents increase with increasing level of  $\gamma$ -PGA while moisture, ash and amylose contents decreased.

The moisture content estimates water content as well as the dry matter of the samples (Ojo et al., 2017). Flours with moisture content less than 14% can resist microbial growth and hence storage stability (Hayma, 2003). Therefore, the moisture content of the flour samples between 0 – 10% is within the range suitable for effective storage of flour and

further processing without the risk of contamination by microorganisms (Chinma et al., 2013). This result also indicated that the flour samples will keep well without having the caking problem if stored properly under good conditions that prevent moisture absorption (Iwe et al., 2017).

**Table 1. Proximate Composition of Native, Modified and Fortified Cassava Flour**

Flour Samples*	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
NCF	7.98±0.00 <sup>d</sup>	1.62±0.01 <sup>d</sup>	1.36±0.01 <sup>b</sup>	0.18±0.00 <sup>b</sup>	88.86±0.01 <sup>a</sup>
MCF	6.52±0.00 <sup>b</sup>	1.28±0.02 <sup>ab</sup>	1.19±0.00 <sup>a</sup>	0.11±0.00 <sup>a</sup>	90.89±0.01 <sup>d</sup>
MCF+10% PGA	5.91±0.02 <sup>a</sup>	1.30±0.03 <sup>ab</sup>	1.74±0.01 <sup>c</sup>	0.25±0.00 <sup>c</sup>	90.80±0.02 <sup>d</sup>
MCF+20% PGA	6.43±0.07 <sup>b</sup>	1.25±0.00 <sup>a</sup>	1.92±0.00 <sup>d</sup>	0.28±0.00 <sup>d</sup>	90.11±0.07 <sup>c</sup>
MCF+30% PGA	6.80±0.20 <sup>c</sup>	1.36±0.04 <sup>c</sup>	2.01±0.01 <sup>e</sup>	0.32±0.00 <sup>e</sup>	89.50±0.17 <sup>b</sup>
MCF+40% PGA	6.41±0.08 <sup>b</sup>	1.33±0.02 <sup>bc</sup>	2.28±0.00 <sup>f</sup>	0.35±0.00 <sup>f</sup>	89.64±0.07 <sup>b</sup>
MCF+50% PGA	6.51±0.09 <sup>b</sup>	1.28±0.00 <sup>ab</sup>	2.32±0.01 <sup>g</sup>	0.40±0.00 <sup>g</sup>	89.48±0.10 <sup>b</sup>

\*) NCF, Native Cassava Flour; MCF, Modified Cassava Flour, PGA,  $\gamma$ -Polyglutamic Acid.

Flours with moisture content less than 14% can resist microbial growth and hence storage stability (Ojo et al., 2017; Hayma, 2003). Therefore, the moisture content of the flour samples between 0-10% is within the range suitable for effective storage of flour and further processing without the risk of contamination by microorganisms (Chinma et al., 2013). This result also indicated that the flour samples will keep well without having the caking problem if stored properly under good conditions that prevent moisture absorption (Iwe et al., 2017).

The ash content of native cassava flour was significantly ( $p<0.05$ ) higher than the rest of the flour samples, which is 1.62%. High ash value reflects high levels of different kinds of minerals and some vitamins present in the original samples. Thus, all the other flour samples had complied with the regulation. The ash content of the flour did not show a consistent increment as the percentage of  $\gamma$ -PGA increased compared to the finding by (Iwe et al., 2017). Iwe et al. (2017) reported that the ash content of the high-quality cassava flour increased with increasing level of substitution of the flour with wheat flour. This phenomenon was probably due to the properties of  $\gamma$ -PGA which consists of mainly glutamic acid as ash content reflects the mineral content in a food sample. The observed range of ash values in modified cassava flour fortified with  $\gamma$ -PGA seems to suggest that in addition to being suitable edible biofilms, the cassava and  $\gamma$ -PGA blends may be a good source of mineral elements (Chinma et al.,



2013).

The increase in protein and fat contents can be attributed to  $\gamma$ -PGA inclusion in the cassava flour. The protein contents of modified flour fortified with 50%  $\gamma$ -PGA was significantly ( $p < 0.05$ ) higher than the other flours. However, a significant ( $p < 0.05$ ) low protein content observed from the modified cassava flour. Protein content in modified flour might be dissolved into the incubation media during the modification process (Richana et al., 2010). The low protein content may also be due to the inefficiency utilization of the carbohydrates in the flour during modification process (Rosales-Soto et al., 2016).

The increment of protein contents when the modified cassava flour was fortified with  $\gamma$ -PGA might be due to the high glutamic acid content in the  $\gamma$ -PGA. According to Tewe and Lutaladio (2004), protein content might increase upon analysis due to the presence of amino acids in the flour samples. This is because approximately 60% of total nitrogen is derived from amino acids. The presence of glutamic acid in  $\gamma$ -PGA is fairly counted in the percentage of nitrogen thus resulted in significant ( $p < 0.05$ ) increments of its contents proportional with the levels of  $\gamma$ -PGA fortification.

According to Gomez et al (2004), cassava contains just 0.1% fat. In this study, the fat contents of cassava flour and  $\gamma$ -PGA increased with an increase in the level of  $\gamma$ -PGA ranged from 0.25 to 0.40%. The carbohydrate contents of native cassava flour are significantly ( $p < 0.05$ ) lower than the other flour samples. The carbohydrate was calculated by deducting the moisture, ash, protein, and fat content of the sample with 100. The carbohydrates of flour samples ranged from 86.86 to 90.89%.

### Analysis of pH and Titratable Acidity

Table 2 showed the pH and titratable acidity (%TTA) of native, modified, and fortified cassava flours. All flour samples had a pH value between 5.53 and 6.27, with native cassava flour having a significant ( $p < 0.05$ ) higher value. The pH value of the modified cassava flour was significantly ( $p < 0.05$ ) lower than its native flour due to the incubation process which

resulted in a decrease in pH and increase of acidity. Non-significant variations of pH value were observed in modified cassava flour fortified with  $\gamma$ -PGA. These data suggest that the incorporation of  $\gamma$ -PGA into the modified cassava flour does not alter its pH value due to the neutral properties of  $\gamma$ -PGA. Although the pH value obtained from this study indicated that all the flour samples were acidic in aqueous solution, these flour samples were still of good quality since flour with a pH of 4 or less will have a sour aroma and taste (Apea-Bah et al., 2011).

**Table 2. pH and Titratable Acidity of Native, Modified and Fortified Cassava Flour**

Flour Samples	pH	% TTA
NCF	6.27±0.13 <sup>b</sup>	0.67±0.01 <sup>a</sup>
MCF	5.88±0.55 <sup>a</sup>	0.84±0.01 <sup>d</sup>
MCF+10% PGA	5.46±0.06 <sup>a</sup>	0.85±0.01 <sup>d</sup>
MCF+20% PGA	5.45±0.04 <sup>a</sup>	0.81±0.00 <sup>c</sup>
MCF+30% PGA	5.44±0.02 <sup>a</sup>	0.80±0.01 <sup>bc</sup>
MCF+40% PGA	5.44±0.02 <sup>a</sup>	0.80±0.01 <sup>bc</sup>
MCF+50% PGA	5.43±0.02 <sup>a</sup>	0.78±0.00 <sup>b</sup>

Mean values with different superscript in a column are significantly ( $p < 0.05$ ) different.

NCF, native cassava flour; MCF, modified cassava flour; PGA: poly-  $\gamma$ -glutamic acid.

Titratable acidity (TTA) results are expressed as % lactic acid.

### Determination of Color Values

Determination of color values can be an important quality parameter, which has a direct influence on the acceptability of the developed product that could be further tested with a consumer sensory study. Moreover, color may contribute to the depreciation of the commercial value of the final product (Aloys & Zhou, 2006). Results of color analyses of cassava flour samples were shown in Table 3. Cassava flour lightness ( $L^*$ ), greenness to redness ( $a^*$ ) and blueness to yellowness ( $b^*$ ) ranged from 92.64 to 93.34, -0.29 to -0.66 and 6.60 to 7.63, respectively. All flour samples showed no significant ( $p > 0.05$ ) differences in terms of  $L^*$ , while  $a^*$  of native flour was significantly ( $p < 0.05$ ) higher than modified flour.

All cassava flour samples exhibited higher values of  $L^*$  of more than 90. According to Boudries et al. (2009), flour or starch with  $L^*$  values of higher than 90 indicated a satisfactory whiteness for its purity. Regarding  $a^*$ , all flour showed negative  $a^*$  values. The  $a^*$  value was significantly ( $p < 0.05$ ) increased after the modification of native flour. Pongpaiboon et al.

(2016), the changed of color may be caused from the feature of  $\alpha$ -amylase which is dark brown liquid and the Maillard reaction between reducing sugar from the hydrolyzed flour, and the amino group in the proteins during modification. A study by Martinez et al. (2015) also reported that the color of flours after hydrolyzed with  $\alpha$ -amylase was darker than native flours. All samples had positive  $b^*$  values where native flour showed a significant ( $p < 0.05$ ) higher  $b^*$  value; this indicates that these flour samples were best described as yellow.

**Table 3. Color Determination of Native, Modified, and Fortified Cassava Flour**

Flour Samples	L*	a*	b*	WI
NCF	92.64±0.44 <sup>a</sup>	-0.66±0.01 <sup>a</sup>	7.63±0.03 <sup>c</sup>	89.42±0.28 <sup>a</sup>
MCF	92.86±0.22 <sup>a</sup>	-0.32±0.01 <sup>b</sup>	6.67±0.11 <sup>a</sup>	90.24±0.25 <sup>b</sup>
MCF+10% PGA	93.34±0.34 <sup>a</sup>	-0.35±0.03 <sup>b</sup>	6.89±0.03 <sup>b</sup>	90.43±0.24 <sup>b</sup>
MCF+20% PGA	93.29±0.26 <sup>a</sup>	-0.31±0.04 <sup>b</sup>	6.68±0.13 <sup>a</sup>	90.54±0.19 <sup>b</sup>
MCF+30% PGA	92.82±0.21 <sup>a</sup>	-0.36±0.06 <sup>b</sup>	6.74±0.08 <sup>ab</sup>	90.16±0.21 <sup>b</sup>
MCF+40% PGA	93.13±0.05 <sup>a</sup>	-0.29±0.02 <sup>b</sup>	6.60±0.04 <sup>a</sup>	90.48±0.05 <sup>b</sup>
MCF+50% PGA	92.75±0.08 <sup>a</sup>	-0.29±0.02 <sup>b</sup>	6.63±0.06 <sup>a</sup>	90.18±0.10 <sup>b</sup>

Mean values with different superscript in a column are significantly ( $p < 0.05$ ) different.

NCF: native cassava flour; MCF: modified cassava flour; PGA: poly- $\gamma$ -glutamic acid.

WI: whiteness index.

Referring to the whiteness index (WI) of cassava flour, native flour exhibited a significant ( $p < 0.05$ ) lower degree of whiteness (89.42) compared to other flour samples. During the modification of cassava flour, there was a decrease in ash and protein content (Table 3), which are among factors affecting the whiteness of flour. Through modification process, the enzymes degrade the protein content in cassava flour, convert free sugar to lactic acid thus the content of protein and free sugar in modified flour was lower than those in native cassava flour. A higher protein and free sugar in native flour may cause non-enzymatic browning during the drying process, which results in a darker color, thus reducing the whiteness of flour (Yuliana et al., 2018). Shyu & Sung (2010) reported that the addition of  $\gamma$ -PGA in sponge cake batter results in a significant increase in the WI of the resulting cake. However, the whiteness of cassava flour and  $\gamma$ -PGA blends are in the same range as modified flour, thus suggest that incorporation of  $\gamma$ -PGA in cassava flour does not affect its color parameters since the properties of  $\gamma$ -PGA being colorless.

## Water and Oil Absorption Capacity

Water absorption capacity (WAC) measures the ability of flour to absorb water and swell for application in food development. WAC of cassava flour and  $\gamma$ -PGA blends ranged from 2.18 to 2.34 g/g where native flours showed significantly ( $p < 0.05$ ) higher WAC. This may be attributed to the low protein and high carbohydrate contents as carbohydrates have been reported to greatly influence the WAC of foods (Anthony et al., 2014).

**Table 4. Water and Oil Absorption Capacity of NCF, MCF and FCF**

Flour Samples	WAC (g/ml)	OAC (g/ml)
NCF	2.34 $\pm$ 0.01 <sup>d</sup>	2.10 $\pm$ 0.00 <sup>f</sup>
MCF	2.21 $\pm$ 0.00 <sup>b</sup>	1.99 $\pm$ 0.00 <sup>b</sup>
MCF+10% PGA	2.24 $\pm$ 0.00 <sup>c</sup>	2.00 $\pm$ 0.00 <sup>c</sup>
MCF+20% PGA	2.18 $\pm$ 0.00 <sup>a</sup>	2.02 $\pm$ 0.00 <sup>e</sup>
MCF+30% PGA	2.20 $\pm$ 0.01 <sup>b</sup>	2.00 $\pm$ 0.00 <sup>bc</sup>
MCF+40% PGA	2.24 $\pm$ 0.01 <sup>c</sup>	2.02 $\pm$ 0.00 <sup>d</sup>
MCF+50% PGA	2.23 $\pm$ 0.00 <sup>c</sup>	1.98 $\pm$ 0.00 <sup>a</sup>

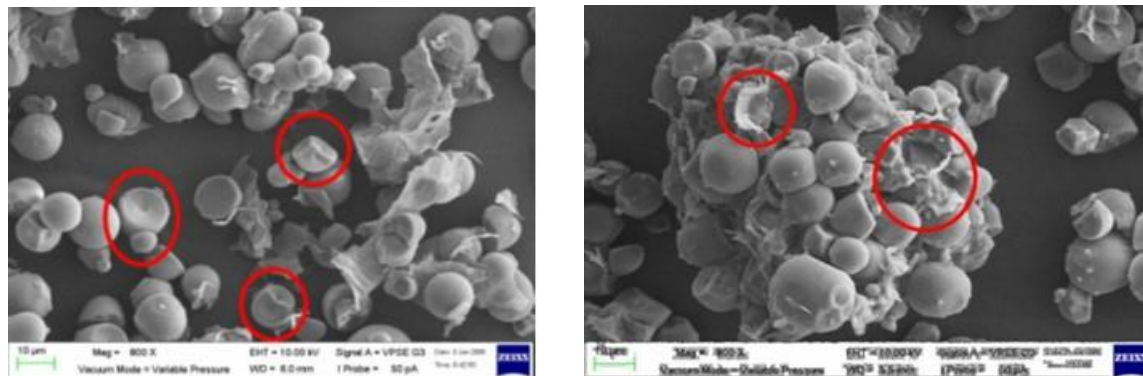
<sup>1</sup>Mean values with different superscript in a column are significantly ( $p < 0.05$ ) different.

<sup>2</sup>NCF: native cassava flour; MCF: modified cassava flour;  $\gamma$ -PGA: poly- $\gamma$ -glutamic acid.

According to Aremu et al. (2009), flour with high water absorption capacity tends to have more hydrophilic constituents in its granules, such as polysaccharides. In addition, starch that has a high proportion of amorphous material will have more sites to bind water, thus water is highly absorbed (Lawal, 2004). Therefore, the difference in WAC displayed between NCF and MCF with  $\gamma$ -PGA blends might be due to different hydrophilic carbohydrate in the component and the decrease in starch after incorporation of  $\gamma$ -PGA.

Oil absorption capacity (OAC) is a function of the lipophilic nature of the flour constituents. Based on Table 4, OAC of cassava flour and  $\gamma$ -PGA blends ranged from 1.98 to 2.10 (g/ml), showing native flour with high OAC because of hydrophobic character of protein in the flour (Ohizua et al., 2017). This is an indication that native cassava flour could be an excellent retainer of flavour and contribute significantly in terms of mouthfeel when used in food preparation (Iwe et al., 2017). Increased in OAC may also be attributed to the presence of more hydrophobic proteins which showed superior binding of lipids (Ekunseitan et al., 2016). However, the addition of  $\gamma$ -PGA in modified cassava flours shows no increment in terms of OAC. According to Lim et al. (2012), the high water-binding capacity of  $\gamma$ -PGA

resulted in more excellent moisture retention and significantly lower oil uptake. Therefore, this observation suggests that  $\gamma$ -PGA has a great potential to be used as a healthy functional oil-reducing agent in deep-oil fried products.



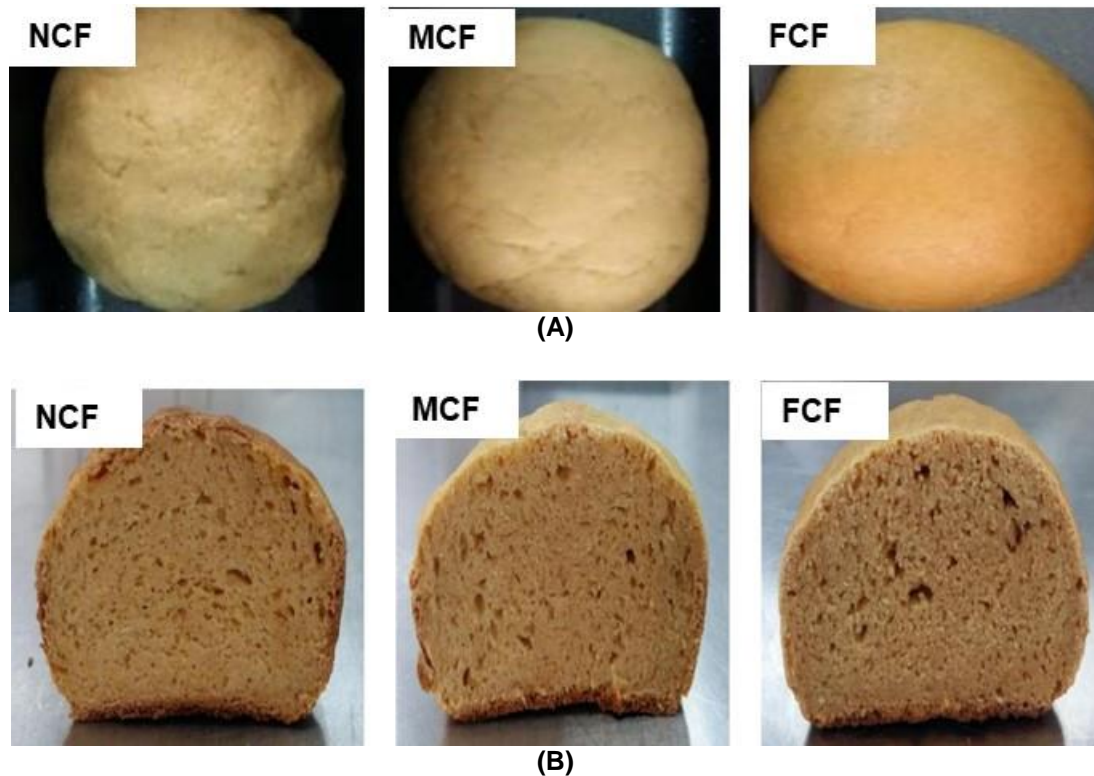
**Figure 1. SEM Observation on Starch Granules of NCF (left) Compared to FCF Prepared with PGA of *B. natto* (right)**

From the SEM micrographs, the process of corrosion and enzymatic hydrolysis on cassava starch granules occurred mainly on surface of starch granules as shown in Figure 1 (right). Qualitatively, rough surface and eroded starch granules could be observed in Figure 1 (right) of that MCF was prepared with starter culture of *B. natto* and FCF was fortified with PGA of *B. natto* compared to Figure 1 (left) of the NCF. Figure 1 (left) showed smooth surface of starch granules with irregular sections while Figure 1B and 2B as well as 1C and 2C of the FCF exhibited that some of starch granules had been broken with rough and eroded surface. Referring to Figure 1 (right), the size of some granules had become smaller, and the amount of the granules had become increasingly decreased. According to Putri, et al. (2011), the granule molecules that reside in amorphous region had been depolymerized by enzymatic hydrolysis process. According to Shariffa, et al. (2009), enzymatic hydrolysis by amylase had taken place in which the amylase was able to change and degrade exterior surface of the granules by exo and endo-corrosion occurrence.

### Analysis of the Characteristics of Produced Bread

The resulting five types of cassava flour were used in the production of bread to evaluate the functional properties of the flour in the manufacture of bakery products. The rapeseed

displacement method was used to determine the physical characteristics of the resulting bread such as expansion, total porosity, and density. The physical characteristics of the bread produced using five types of flour samples have been stated in Figure 2.



**Figure 2. The Texture of Bread Dough was Prepared from Native Cassava Flour (NCF), Modified (MCF) and Fortified Flour (FCF) before Developing (A) after Baking (B)**

Bread expansion is linked to the ability of the dough to rise because of the fermentation process that produces gases trapped in the dough network (Akanbi & Ikujeola, 2016). Table 5 showed a significant difference between the five types of cassava flour. Bread made from 70% MCF and 30% PGA showed the highest expansion value of  $1.95 \pm 0.03 \text{ cm}^3/\text{g}$  while bread made from NCF had the lowest expansion value of  $1.27 \pm 0.05 \text{ cm}^3/\text{g}$ .

**Table 5. Water and Oil Absorption Capacity of NCF, MCF and FCF**

Analysis	NCF	MCF	FCF-PGA10	FCF-PGA20	FCF-PGA30
Expansion ( $\text{cm}^3/\text{g}$ )	$1.27 \pm 0.05^a$	$1.53 \pm 0.01^b$	$1.76 \pm 0.04^c$	$1.85 \pm 0.03^d$	$1.95 \pm 0.03^e$
Total porosity (%)	$4.34 \pm 0.02^a$	$5.33 \pm 0.11^b$	$6.58 \pm 0.10^c$	$8.51 \pm 0.19^d$	$11.23 \pm 0.06^e$
Density ( $\text{g}/\text{cm}^3$ )	$0.98 \pm 0.01^e$	$0.76 \pm 0.03^d$	$0.68 \pm 0.02^c$	$0.56 \pm 0.01^b$	$0.51 \pm 0.01^a$

NCF: native cassava flour; MCF: modified cassava flour; FCF, fortified cassava flour  
FCF-PGA10, 20, 30, fortified cassava flour and 10, 20, 30%  $\gamma$ -polyglutamic acid, respectively.

Table 5 showed that fortified flour prepared from composite flour of MCF 70%, and PGA 30% had a very high amount of porosity compared to original cassava flour and modified cassava flour ( $p < 0.05$ ). A significant increasing trend of total porosity was observed in the composite flour as the degree of soybean flour substitution increased. The total porosity of the composite flour was the highest among the flour samples at  $11.23 \pm 0.06\%$  while the NCF recorded the lowest total porosity which was  $4.34 \pm 0.02\%$ .

#### 4. CONCLUSIONS

It can be concluded that the extraction of the crude enzyme starter culture is possible and has been carried out successfully. Microbial production of  $\gamma$ -PGA by the solid-state fermentation of soybean by *B. natto* is also considered fruitful as the total yield of crude  $\gamma$ -PGA from this study was comparable with previous studies. With the improvement in nutritional value and the ability of the starch to withstand heat and shear force during heating, it could be suggested that fortified cassava flour with  $\gamma$ -PGA was suitable to be use in the production of baking food product, especially bread.

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