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Proximate Composition and Functional Properties of Modified Cassava Flour Fortified with Poly-γ-glutamic Acid

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***Abstract*: Cassava (*Manihot esculenta* Crantz) is one of the important staple crops worldwide. New products from cassava flour are still relevant to the food industry. However, the utilization of native flour is quite challenging due to its limited functional properties. Poly-γ-glutamic acid (γ-PGA) is a water-soluble, biodegradable, and non-toxic biopolymer, presents abundantly in the traditional Japanese dish called *natto*. In the present study, an enzymatic modification was carried out on cassava flour using *α*-amylase and cellulase crude enzyme. The enzyme-modified flours were further investigated for their proximate composition and functional properties after fortification of γ-PGA at different levels (10%, 20%, 30%, 40% and 50%). Native cassava flour was used as a control. The increasing level of γ-PGA resulted in a significant increase in protein (ranged from 1.19 to 2.32%) and fat (ranged from 0.11 to 0.40%) contents, while there were decreases in moisture (ranged from 5.91 to 6.80%) and ash (1.25 to 1.36%) contents. Significant differences in the swelling power and solubility were observed at various temperatures. The bulk density, water and oil absorption capacity of native cassava flour was significantly higher than the cassava flour with γ-PGA blends. All flour samples showed no significant differences in terms of lightness (L*), while greenness to redness (a*) of native cassava flours were higher than the modified cassava flour with γ-PGA.**

***Keywords*: Cassava, Chemical, Functional, Modified Flour, Pasting, Poly-γ-glutamic Acid.**

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) also known as tapioca, *yucca*, or *manioc*, is a woody shrub with tuberous root belongs to the family of Euphorbiaceae [1]. Cassava is considered as the third most important sources of carbohydrate in tropics, after maize and rice [2]. While cassava was once considered as the "food for the poor", now it has become significant world agriculture and provides a multipurpose utilization in developing countries, become a global trend economically, and a challenge towards climate change [3]. The beneficial traits of the cassava crop such as its tolerance and high resistance to soil with lack of nutrients make cassava as one of the crops that contribute to the economic importance [4].

Cassava is a traditional food security crop, often processed into a variety of traditional food products. The most common way is by processing the fresh cassava tubers into the flour-based products. However, the utilization of starchy tubers in foods depends on their physical and chemical properties. For example, the properties of starch granules influence the behavior of flour in food systems, such as viscosity and gelatinization, which affect the texture of the end product [5]. Most native starches are limited for direct application because they are unstable to changes in temperature, pH and shear forces. Besides, native starches have a strong tendency for decomposition and retrogradation [6]. Therefore, modification of starch has been extensively studied to overcome the functional limitations of native starch and to increase the

importance of starch for industrial applications [7]. Generally, there are four methods of modification, namely physical, chemical, enzymatic and genetically, or their combinations [7]. Enzymatic modifications have been studied, partly replacing the chemical and physical methods over recent decades. This is due to enzymes are safer, healthier and mild than the chemical method to the environment and food consumers [8].

Poly-γ-glutamic Acid (γ-PGA) is an unusual anionic, naturally occurring homo-polyamide that is made of D- and L-glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups [9]. γ-PGA is edible and is present abundantly in the traditional Japanese dish called *Natto*, which is made by fermenting soybean with *Bacillus* strains. To date, there are four methods to produce γ-PGA, which are chemical synthesis, peptide synthesis, biotransformation, and microbial fermentation [10]. 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 [11]. The characteristics of γ-PGA which are being edible and non-toxic to humans and environments, allow its application to several industries [12]. In the food industry, γ-PGA is used as a thickener, bitterness relieving agent, cryoprotectant, encapsulation, water adsorbent, and as a nutrition supplement [9]. At present, there are very few studies documented on the application of γ-PGA either in flour or its products. Some of the previous studies investigate the effect of γ-PGA on rheology and thermal properties of wheat dough [13], emulsion and foam activity of sponge cake paste [14] as well as oil uptake and moisture loss in doughnut products [15].

Therefore, the objective of this study was to fortify enzyme-modified cassava flour by incorporating microbial γ-PGA. Cassava flours are first hydrolyzed by enzymatic culture containing *α*-amylase and cellulase synthesised from non-pathogenic microorganisms of bacterial species, which is *Bacillus subtilis var natto*, to produce enzyme-modified cassava flour. After that, these enzyme-modified cassava flours were fortified with microbial γ-PGA. Studies on physicochemical properties of the modified flour were carried out using instrumental methods and chemical analysis.

2. MATERIALS AND METHOD

2.1 Sample Collection

Fresh cassava tubers were obtained directly from the local farm located at Kampung Tiong, Tamparuli, Sabah, Malaysia. These cassava tubers were harvested at commercial maturity of 10 to 12 months after planting and collected in the early morning hours. Fresh cassava tubers (without breaks/cuts) were sorted within the same size (20 to 30 cm long and 5 to 10 cm in diameter) by using simple random sampling and transported under ambient conditions to the postharvest laboratory at Faculty of Food Science and Nutrition, Universiti Malaysia Sabah. *Bacillus subtilis* var. natto used to produce crude enzyme and γ-PGA was procured from Veterinary Research Centre, Indonesia.

2.2 Microorganisms and Inoculum Preparation

B. subtilis natto was used to produce enzyme starter culture and solid-state fermentation of soybeans. The strain was grown and maintained on nutrient agar (0.5% peptone, 0.3% beef extract, 1.5% agar, 0.5% NaCl, (w/v), pH 7.0) slants and stored properly at 4℃. For inoculum preparation, the strain was grown on nutrient agar slants at 37℃ for 24h. A loop of fresh culture from the agar slant was transferred into a 250 ml Erlenmeyer flask containing 50 ml of sterilized nutrient broth (0.1% beef extract, 0.2% yeast extract, 0.5% peptone, 0.5% NaCl, (w/v), pH 7.0) and incubated at 37℃ at 150 rpm for 12h.

2.2 Production of Enzyme Starter Culture

The medium used to produce enzyme starter culture containing α-amylase was composed of: (%, w/v) 1.0 soluble starch, 0.4 yeast extract, 0.1 KH_2PO_4, 0.25 Na_2HPO_4, 0.1 NaCl, 0.05 $MgSO_4{\cdot}7H_2O$, 0.05 $CaCl_2{\cdot}6H_2O$, 0.001 $FeSO_4{\cdot}7H_2O$ and 0.2 $(NH_4)_2SO_4$. For the production of enzyme starter culture containing cellulase, the medium was composed of: (%, g/L) 10.0 CMC, 0.2 KH_2PO_4, 0.13 $(NH_4)_2SO_4$, 0.03 $MgSO_4{\cdot}7H_2O$, 0.03 $CaCl_2{\cdot}6H_2O$ and 1.0 peptone, and (%, mg/L) 0.5 $FeSO_4{\cdot}7H_2O$, 0.156 $MnSO_4{\cdot}H_2O$, 0.14 $ZnSO_4{\cdot}7H_2O$ and 0.14 $CoCl_2$. The above medium composition was dissolved in 1000 ml of distilled water after which 100 ml of the medium was measured into 250 ml Erlenmeyer flask and then sterilized in an autoclave at 121℃ for 20 minutes. The initial pH of the medium was adjusted to 7.0 before autoclaving. Inoculum (1% (v/v)) of *B. subtilis natto* was transferred into the production medium and then incubated in a rotary shaker at 37℃ and 150 rpm for 48 h. The fermented broth was taken after 48 hours and centrifuged at 10,000 x g for 20 minutes at 4℃. The cell-free supernatant was collected and used as an extracellular crude enzyme.

2.3 Microbial Production of Poly-γ-glutamic Acid (γ-PGA)

Production of γ-PGA by solid-state fermentation of soybeans was done independently in triplicate, according to [16]. Twenty grams (dry weight) of dehulled soybeans were weighed into 250 ml conical flasks and then autoclaved at 121℃ for 20 minutes. After autoclaving, cooked soybeans were cooled at 40-45℃ before inoculating the substrate with 5% inoculum level, mixed carefully under strictly aseptic conditions, sealed with eight layers of gauze and then incubated at 37℃ for 48 hours in a static mode. When fermentation was terminated, ten volumes of distilled water was added (w/v, based on the initial dry weight of the substrate) into the fermented soybeans. The mixture was mixed at room temperature (20 ± 2℃) on a rotary shaker for 1 hour at 200 rpm to dislodge the mucilage containing γ-PGA. The whole contents were filtered twice through a muslin cloth. A 10.0 ml filtrate was centrifuged at 12,000 rpm for 20 minutes at 4℃. The resulting supernatant containing crude γ-PGA was poured into four volumes of cold ethanol and left overnight at 4℃ to precipitate the γ-PGA. The resultant precipitate containing crude γ-PGA was collected by centrifugation at 12,000 rpm for 20 minutes at 4℃. Then, a 5 ml aliquot of distilled water was added to dissolve the precipitate and the resulting solution containing γ-PGA was stored at 4℃ for fortification in modified cassava flour.

2.4 Preparation of Cassava Flour

2.4.1 Native Cassava Flour

Raw cassava tubers were processed within 24 hours after harvesting. Tubers were peeled manually, washed with distilled water and thinly sliced (3-5 mm) using a vegetable chopper, and dried in the cabinet dryer at 45-50℃ for 24-48 hours until the moisture content reached approximately 12% or less. Dried sliced cassava tubers were dry-milled and sieved to produce cassava flour before packed in an airtight plastic bag and stored in the freezer at -20℃ until further application.

2.4.2 Enzyme-modified Cassava Flour

Modification of cassava flour was carried out according to the method reported by [17] with slight modification. Enzyme modified cassava flour was prepared by mixing 50 g of native cassava flour into 100 ml of sterilized distilled water. Then, 2% of enzymatic culture were added into the flour slurries and aseptically mixed. These pastes were covered with parafilm and then incubated at 37℃ for 24 hours in a static mode. After incubation for 24 h, the pastes were heated in a convection oven at 105℃ for 1 hour to end the enzymatic activity. Afterwards, they were dried in the cabinet dryer at 45-50℃ for 24 hours until the moisture content reached approximately 12% or less. Dried pastes were milled into flour and stored in an airtight plastic bag at 4℃ until further application.

2.4.3 Fortification of Modified Cassava Flour Fortified with γ-PGA

For the fortification of modified cassava flour with γ-PGA, γ-PGA was added after the termination of enzyme activity. After the pastes were heated in the oven at 105℃ for one hour, the pastes were rested in a desiccator at room temperature for 5 min. Addition of γ-PGA into modified cassava flour was carried out by adding 10%, 20%, 30%, 40% and 50% of γ-PGA (v/w flour) and the mixture was incubated at 4℃ for 24 hours. After 24 hours of incubation, the pastes were dried in the cabinet dryer at 45-50℃ for 24 hours, milled and stored in an airtight plastic bag at 4℃ until further analysis and application.

2.5 Proximate Analysis

2.5.1 Moisture Content

Moisture content of flour sample was determined by the oven drying method (AOAC, 2000). Aluminium dish and lid were pre-heated in an oven at 105℃. About five grams of each sample was accurately weighed into a pre-weighed aluminium dish. The samples were dried to constant weight in an oven at 105℃ overnight where the loss in weight was recorded as moisture. The percentage of moisture content was calculated using the formula below:

$$\text{Moisture (\%)} = \frac{(W2-W3)}{(W2-W1)} \text{ x } 100$$

Where, W1: weight of empty dish, W2: weight of dish and sample before drying, w3: weight of dish and dried sample

2.5.2 Ash Content

Ash content of flour samples was determined according to AOAC (2000). Porcelain crucibles were pre-heated in an oven and weighed. About 5 g of flour samples were weighed into the crucibles and heated on a Bunsen burner. After that, the crucibles were placed in the muffle furnace and maintained at 550℃ overnight. They were later cooled in desiccators and weighed. Weight of residue after incineration was recorded as the ash content. The percentage of ash was calculated using the formula below:

$$\text{Ash (\%)} = \frac{(W3-W1)}{(W2-W1)} \text{ x } 100$$

Where, W1: weight of crucible, W2: weight of crucible and sample, W3: weight of crucible and sample after ashing

2.5.3 Crude Protein Content

The protein content of the flour sample was determined by Kjeldhal method (AOAC, 2000) using Kjeltec 2300 FOSS. Two grams of flour sample was taken into a digestion tube, where after that catalysts and 15 ml of sulphuric acid (H_2SO_4) were added. The tube was then kept in the digestion rack for 2 hours until a clear solution was obtained. The cooled digestion tube was transferred into *Kjeltec*^{TM} 2300 Analyzer for automatic distillation and titration. Percentage of protein was calculated using the formula below:

$$\text{Crude protein (\%)} = \frac{\text{(Sample solution}-\text{blank solution) ml acid x N x 14.0 x 6.25}}{\text{Weight of sample (g)x 1000}} \text{ x } 100$$

Where, N = normality of acid used in titration, 14.0 = nitrogen weight, and 6.25 = conversion factor for protein

2.5.4 Crude Fat Content

The crude fat content of flour samples was determined by using Soxtec^{TM} 2050 FOSS according to AOAC (2000). About 2 g of sample was weighed into a thimble and covered with cotton wool. Then, the thimble was attached to the Soxhtec 2050. After that, 90 ml of petroleum ether was poured into the pre-weighed extraction cup and put into Soxhtec 2050. After extraction, the extraction cup was placed into an oven at 105℃ for 1 hour. Extraction cup was cooled in desiccators prior to weighing. The fat content of samples was calculated using the formula below:

$$\text{Crude fat (\%)} = \frac{(W3-W2)}{W1} \text{ x } 100$$

Where, W1: weight of sample, W2: weight of extraction cup, W3: weight of extraction cup after extraction

2.5.5 Determination of Carbohydrate

Total carbohydrate was calculated by subtracting the sum (%) of moisture, ash, crude protein, and crude fat from 100.

$$\text{\% Carbohydrate content} = 100 - (\text{\%moisture} + \text{\%ash} + \text{\%protein} + \text{\%fat})$$

2.6 Functional Properties

2.6.1 Swelling Power and Solubility

Swelling power of flours was determined by the method of [18] with modification of smaller sample and varies the temperature. A 0.1 g of sample was transferred into a pre-weighed graduated 50ml centrifuge tube. Distilled water was added into the centrifuge tube to give a total volume of 10 ml. The sample in the tube was stirred gently for 30 seconds at room temperature and then heated in a water bath at 60, 70, 80 and 90°C for 30 minutes with constant agitation. The samples were then centrifuged at 3000 rpm for 30 minutes. The supernatant was decanted in a pre-weighed evaporating dish and dried at 100°C for 20 min. Swelling power and solubility of flours were calculated as follows:

$$\text{Swelling power} = \frac{\text{Weight of residue after centrifugation (g)}}{\text{Weight of sample dry basis (g)}}$$

$$\text{Solubility} = \frac{\text{Weight of dry supernatant (g)}}{\text{Weight of sample dry basis (g)}} \text{ x } 100$$

2.6.2 Water and Oil Absorption Capacity

Water absorption of flours was measured by the centrifugation method reported by [19]. About 3.0 g of samples were dissolved in 25 ml 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.

2.6.3 Bulk Density

Bulk density was determined as described by [20]. Ten grams of flours was put into a 100 ml graduated cylinder. The cylinder was tapped gently on the laboratory bench several times until there was no further diminution of the sample level. The volume occupied by the flour sample was noted. Bulk density was calculated as follow and expressed as mass per volume.

2.6.4 Determination of Color Values

The color of the cassava flours was determined by using a Chroma Meter (Konica Minolta CR-400, Japan). 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 color 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 (green hue) 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 [21].

2.7 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). Tukey'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

3.1 Proximate Composition

The proximate composition provides a general overview of the nutritional value of food and includes analysis of the ash, moisture, fat and protein content. Table 1 shows the proximate composition of cassava flour and γ-PGA blends. The moisture, ash, protein, fat, and carbohydrate contents ranged from 5.91 to 7.98%, 1.25 to 1.62%, 1.19 to 2.32%, 0.11 to 0.40%, and 88.86 to 90.89%, respectively. The protein and fat contents increase with increasing level of γ-PGA while moisture and ash contents decreased.

Table 1: Proximate composition[1] of native, modified and fortified cassava flour

Flour Samples[2]	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
NCF	7.98 ± 0.00^{d}	1.62 ± 0.01^{d}	1.36 ± 0.01^{b}	0.18 ± 0.00^{b}	88.86 ± 0.01^{a}
MCF	6.52 ± 0.00^{b}	1.28 ± 0.02^{ab}	1.19 ± 0.00^{a}	0.11 ± 0.00^{a}	90.89 ± 0.01^{d}
MCF+10% γ-PGA	5.91 ± 0.02^{a}	1.30 ± 0.03^{ab}	1.74 ± 0.01^{c}	0.25 ± 0.00^{c}	90.80 ± 0.02^{d}
MCF+20% γ-PGA	6.43 ± 0.07^{b}	1.25 ± 0.00^{a}	1.92 ± 0.00^{d}	0.28 ± 0.00^{d}	90.11 ± 0.07^{c}
MCF+30% γ-PGA	6.80 ± 0.20^{c}	1.36 ± 0.04^{c}	2.01 ± 0.01^{e}	0.32 ± 0.00^{e}	89.50 ± 0.17^{b}
MCF+40% γ-PGA	6.41 ± 0.08^{b}	1.33 ± 0.02^{bc}	2.28 ± 0.00^{f}	0.35 ± 0.00^{f}	89.64 ± 0.07^{b}
MCF+50% γ-PGA	6.51 ± 0.09^{b}	1.28 ± 0.00^{ab}	2.32 ± 0.01^{g}	0.40 ± 0.00^{g}	89.48 ± 0.10^{b}

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

[2]NCF: native cassava flour; MCF: modified cassava flour; γ-PGA: poly- γ-glutamic acid.

The moisture content estimates water content as well as the dry matter of the samples [22]. Flours with moisture content less than 14% can resist microbial growth and hence storage stability [23]. 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 [24]. The ash content of native cassava flour was significantly ($p<0.05$) higher than the rest of the flour samples, which is 1.62%. According to [25], high ash contents results in damaged food products due to the reactions of oxidation and reduction of minerals. The ash content of the flour did not show a consistent increment as the percentage of γ-PGA increased compared to the finding by [26]. [26] reported that the ash content of the high-quality cassava flour increased with increasing level of substitution wheat flour. This phenomenon was probably due to the properties of γ-PGA which consists of mainly glutamic acid as ash content reflects the mineral content in a food sample.

The protein contents of modified flour fortified with 50% γ-PGA is significantly ($p<0.05$) higher than the other flours. The increment of protein contents when the modified cassava flour was fortified with γ-PGA might be due to the high glutamic acid content in the γ-PGA. According to [27], protein content might increase upon analysis due to the presence of amino acids in the flour samples as approximately 60% of total nitrogen is derived from amino acids. Cassava is very low in fat. According to [28], cassava contains just 0.1% fat. [29] found that flour from cassava roots contains approximately 2.5% lipids, but only half of this is extractable with conventional solvent systems, and the fatty acids in cassava are primarily saturated. In this study, the fat contents of cassava flour and γ-PGA increased with an increase in the level of γ-PGA ranged from 0.25 to 0.40%.

3.2 Functional Properties

The starch granule is an insoluble compound in water that can be hydrated at high temperatures. Hydration and swelling of starch granules lead to the thermal disordering of the crystal structures [30]. At the gelatinization temperature, the swelling of the amorphous phase or water-penetrated phase accelerates the disruption of the crystalline region in starch. This process can be described as the swelling power of the starch granules. Table 2 and 3 showed the differences in swelling power and solubility of the starches at different temperatures.

The swelling power of cassava flour and γ-PGA blends measured at a temperature from 60 – 90℃, ranged from 2.72 to 18.40 g/g, with the highest value of 2.80 to 15.70 g/g for native cassava flour at 60℃, 70℃ and 80℃, respectively. Based on Table 4.6, an increase of swelling power was observed with a 10℃ change in temperature. When the flour samples were heated at 70℃, a sudden increase in swelling power was observed. This finding is inconsistent with the previous study reported by [31], where the swelling power of cassava starch increased slowly from 60℃ to 70℃. The increments of swelling power were rapid at the temperature of 70℃. According to [32], a gradual increase of swelling with hydration and temperature indicated that a weak association forces responsible for maintaining the structure of the starch granule. Modified cassava flour with 50% of γ-PGA exhibited the greatest swelling power among all the flour samples at the temperature of 90℃ suggests that it may serve as a useful ingredient in products like sauces, baby food, baked goods and canned foods, where the processing of these foods required a high temperature [31].

Table 2: Swelling power[1] (g/g) of native, modified and fortified cassava flour

Flour Samples[2]	Temperature (℃)			
	60	70	80	90
NCF	2.80 ± 0.01^{a}	12.59 ± 0.20^{c}	15.70 ± 0.11^{b}	16.83 ± 0.74^{a}
MCF	2.76 ± 0.04^{a}	12.48 ± 0.15^{c}	12.76 ± 0.12^{a}	17.16 ± 0.05^{ab}
MCF+10% γ-PGA	2.73 ± 0.31^{a}	11.95 ± 0.07^{c}	12.88 ± 0.53^{a}	17.08 ± 0.35^{ab}
MCF+20% γ-PGA	2.72 ± 0.02^{a}	11.83 ± 0.37^{bc}	13.09 ± 0.11^{a}	17.76 ± 0.04^{ab}
MCF+30% γ-PGA	2.89 ± 0.43^{a}	11.58 ± 0.61^{abc}	13.28 ± 0.53^{a}	17.30 ± 0.02^{ab}
MCF+40% γ-PGA	2.75 ± 0.07^{a}	10.67 ± 0.14^{ab}	12.60 ± 0.09^{a}	17.73 ± 0.52^{ab}
MCF+50% γ-PGA	2.89 ± 0.18^{a}	10.55 ± 0.46^{a}	12.61 ± 0.01^{a}	18.40 ± 0.05^{b}

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

[2]NCF: native cassava flour; MCF: modified cassava flour; γ-PGA: poly- γ-glutamic acid.

Table 3: Solubility[1] (%) of native, modified and fortified cassava flour

Flour Samples[2]	Temperature (℃)			
	60	70	80	90
NCF	6.40±0.28^{b}	13.41±0.32^{b}	15.33±0.85^{b}	9.61±1.23^{abc}
MCF	3.79±0.20^{a}	8.20±0.10^{a}	10.17±0.45^{a}	7.37±1.02^{ab}
MCF+10% γ-PGA	4.37±0.03^{a}	8.68±1.13^{a}	11.28±0.73^{a}	8.33±0.36^{ab}
MCF+20% γ-PGA	3.69±0.36^{a}	8.80±1.23^{a}	11.13±0.19^{a}	6.92±0.06^{a}
MCF+30% γ-PGA	4.52±0.13^{a}	8.63±0.42^{a}	9.78±0.33^{a}	9.26±0.45^{abc}
MCF+40% γ-PGA	4.50±0.15^{a}	8.83±0.41^{a}	10.45±0.27^{a}	9.91±1.10^{bc}
MCF+50% γ-PGA	3.95±0.19^{a}	8.50±0.17^{a}	10.68±0.97^{a}	11.48±0.22^{bc}

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

[2]NCF: native cassava flour; MCF: modified cassava flour; γ-PGA: poly- γ-glutamic acid.

The solubility of starch samples highly depended on the source, amylose and amylopectin content, extraction procedure, and thermal stability of the samples. Besides, solubility is linked to wet and heat treatment of starch samples [33]. The solubility of all the flour samples increased significantly with the increase in temperature, ranged between 3.69 to 15.33 %. Based on Table 3, the solubility (%) of native flour was significantly ($p<0.05$) higher than the other flour samples when heated at the temperature of 60 - 80℃. However, when the flour samples were subjected to heating at temperature of 90℃, inconsistent variations of the percentage of solubility displayed by all the flour samples.

Water absorption capacity (WAC) measures the ability of flour to absorb water and swell. Based on Table 4, WAC of cassava flour and γ-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 [34]. According to [35], flours with high water absorption capacity tends to have more hydrophilic constituents in its granules, such as polysaccharides. In addition, a starch that has a high proportion of amorphous material will have more sites to bind water, thus water is highly absorbed [36]. Therefore, the difference in WAC displayed between native cassava flour and modified cassava flour with γ-PGA blends might be due to different hydrophilic carbohydrate in the component and the decrease in starch after incorporation of γ-PGA.

Oil absorption capacity (OAC) is a function of the lipophilic nature of the flour constituents. Table 4 showed that OAC of cassava flour and γ-PGA blends ranged from 1.98 to 2.10 (g/ml), showing native flour with high OAC as a result of hydrophobic character of protein in the flour [37]. This indicated that native cassava flour could be an excellent retainer of flavor and contribute significantly in terms of mouthfeel when used in food preparation [26]. However, no increment of OAC when γ-PGA was added in modified cassava flours. According to [15], the high water-binding capacity of γ-PGA resulted in more excellent moisture retention and significantly lower oil uptake. Therefore, this observation suggests that γ-PGA has a great potential to be used as a healthy functional oil-reducing agent in deep-oil fried products.

Table 4: Functional properties of native, modified and fortified cassava flour

Flour Samples[2]	WAC (g/ml)	OAC (g/ml)	Bulk density (g/cm^3)	L*	a*	b*
NCF	2.34±0.01^{d}	2.10±0.00^{f}	0.52±0.02^{a}	92.64±0.44^{a}	-0.66±0.01^{a}	7.63±0.03^{c}
MCF	2.21±0.00^{b}	1.99±0.00^{b}	0.60±0.03^{bc}	92.86±0.22^{a}	-0.32±0.01^{b}	6.67±0.11^{a}
MCF+10% γ-PGA	2.24±0.00^{c}	2.00±0.00^{c}	0.57±0.02^{ab}	93.34±0.34^{a}	-0.35±0.03^{b}	6.89±0.03^{b}
MCF+20% γ-PGA	2.18±0.00^{a}	2.02±0.00^{e}	0.63±0.01^{bc}	93.29±0.26^{a}	-0.31±0.04^{b}	6.68±0.13^{a}
MCF+30% γ-PGA	2.20±0.01^{b}	2.00±0.00^{bc}	0.64±0.03^{bc}	92.82±0.21^{a}	-0.36±0.06^{b}	6.74±0.08^{ab}
MCF+40% γ-PGA	2.24±0.01^{c}	2.02±0.00^{d}	0.65±0.03^{bc}	93.13±0.05^{a}	-0.29±0.02^{b}	6.60±0.04^{a}
MCF+50% γ-PGA	2.23±0.00^{c}	1.98±0.00^{a}	0.69±0.03^{c}	92.75±0.08^{a}	-0.29±0.02^{b}	6.63±0.06^{a}

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

[2]NCF: native cassava flour; MCF: modified cassava flour; γ-PGA: poly- γ-glutamic acid.

The bulk density (g/cm^3) of flour (Table 4) is the density measured without application of any compression [38]. It is usually used to determine flour expansion and the porosity of products [39]. Moreover, it is a crucial factor considered during raw material handling, packaging requirements and application in the food industry, especially in wet processing [40]. The values of the bulk density, which varied from 0.52 to 0.69 g/ml increased as the incorporation level of γ-PGA increased. Modified flour with 50% γ-PGA shows a significant ($p<0.05$) higher bulk density while native flour had the lowest bulk density. The high bulk densities observed for the modified flour incorporated with γ-PGA indicated these flour samples required less space to occupy and require fewer packaging materials per unit weight resulting in the reduction of packaging cost.

Results of color analyses of cassava flour samples were shown in Table 4. 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 with γ-PGA. All cassava flour samples exhibited higher values of L* of more than 90. According to [41], 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 (green on hue axis). The a* value was significantly ($p<0.05$) increased after the modification of native flour. According to [42], the changed of color may be caused from the feature of α-amylase which is dark brown liquid and the Maillard reaction between reducing sugar from the hydrolysed flour, and the amino group in the proteins during modification. [17] also reported that the color of flours after hydrolyzed with α-amylase was darker and more reddish 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.

4. CONCLUSION

The present studies demonstrated that incorporation of microbial γ-PGA influenced the proximate composition of modified cassava flour. Protein and fat contents increased as levels of γ-PGA increased. Cassava flour with 50% of γ-PGA exhibited a significantly higher amount of protein and fat compared to other flour samples. However, cassava flours with γ-PGA blends displayed a significantly lower amount of moisture and ash contents, compared to their native counterparts. In terms of the functional properties, modified cassava flour and γ-PGA blends differ significantly from the properties exhibited by the native flour. Modification with enzyme and addition of γ-PGA caused a reduction in swelling power and solubility of the starch granules. The decreased in swelling power is favored as low swelling power suggest that starch granules have a strong binding force. Moreover, low swelling power indicated that the starch granules are highly resistant to breaking during cooking and gel formed. Incorporation of γ-PGA in modified cassava flour did not affect the lightness of the modified cassava flour. Expansion of the study is suggested to understand better the application of modified cassava flours incorporated with γ-PGA in food product development. Therefore, it would be interesting that future studies could focus more on using these flour blends in food production, such as baked products and weaning food.

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