Fortification of modified Cassava Starch with Bacterial Polyglutamic Acid derived from *Bacillus natto*

Hevenney Vianie Heroney [1] Joanna Ho Swee Ling [2], and Joko Sulistyo [3*]

[1,2] *Universiti Malaysia Sabah, Kota Kinabalu, Malaysia*
[3] *Universitas Ciputra Surabaya, Indonesia*

**Corresponding author: joko.sulistyo@ciputra.ac.id*

Journal of Tourism, Culinary, and Entrepreneurship

e-ISSN: 2776-0928

Publisher:
School of Tourism,
Universitas Ciputra Surabaya,
Indonesia

Keywords:
Bacillus natto
PGA
Fortified Cassava Starch
Physicochemical
Functional Properties

Received: *January 29, 2025*
Revised: *March 17, 2025*
Accepted: *March 31, 2025*

ABSTRACT

Fortification of cassava starch with bacterial polyglutamic acid (PGA) offers the potential to enhance its nutritional value. The fortified starch was determined for its physicochemical and functional properties after addition of varying levels PGA. The findings showed that with increasing PGA levels, there was a significant ($p < 0.05$) decrease in bulk density, swelling power, and viscosity (peak, trough, breakdown, setback, and final), while water and oil absorption capacities and solubility index increased. In addition, increasing PGA content significantly ($p < 0.05$) increased protein content approximately 48.71% and fat content 72.5%, while moisture and ash content decreased 13.10% and 8.1%, respectively. Scanning electron microscopy (SEM) showed obvious cracks in starch granules and increased binding of PGA protein molecules to the surface of starch granules, resulting in the formation of larger agglomerates. This study focused on the assessment of the effects of fortification of cassava starch with PGA bacteria on its functional and sensory properties.

1. INTRODUCTION

Cassava (Manihot esculenta Crantz) (Alves, 2002) has emerged as an important agricultural resource with diverse applications in developing countries. Its global economic relevance continues to grow, despite challenges posed by climate change (Howeler et al., 2013). Important attributes of this crop, including its resistance to nutrient-poor soils and environmental stresses, significantly enhance its economic value (Poonsrisawat et al., 2014). However, its limited functionality, perishability, and bulkiness necessitate the development of effective strategies and technologies to overcome these limitations. Various methods have been developed

for the modification of starch to achieve desired functional properties for industrial use (Kaur et al., 2012), thereby increasing its flexibility and altering its physical, functional, and structural properties. This enhances its utility in the food industries (Lopez et al., 2010).

In enzymatic modification, hydrolyzing enzymes are primarily used to modify starch. The enzymes employed are typically free from additional enzymatic components to prevent unwanted damage to the starch granules or molecules (Poonsrisawat et al., 2014). Enzymatic modification offers several advantages, including more specific hydrolysis products, fewer by-products, and higher yields (Dura et al., 2014).

Various enzymatic modification methods are used to alter starch structure and achieve specific functional properties. One such enzyme is α-amylase, a hydrolase that catalyzes the hydrolysis of α-1,4-glycosidic bonds in starch, resulting in products such as maltose, glucose, and dextrin, while preserving the α-anomeric configuration of the product [Gupta et al., 2003; Juturu and Wu, 2014; Behera et al., 2017). Bacteria have been extensively studied as sources of enzymes for producing α-amylase and cellulase. Among these, *Bacillus natto* is notable for its predominant use in cassava starch modification due to its ability to produce substantial amounts of extracellular

Bacillus natto is a major producer of polyglutamic acid (PGA), which can be isolated from bacterial culture media filtrates. The PGA is a biodegradable and water-soluble biopolymer consisting of D- and L-glutamic acid units linked by amide bonds between the α-amino and γ-carboxyl groups. This versatile biopolymer, synthesized by bacteria, has a wide range of applications. It is also edible and is present in significant amounts in natto, a traditional Japanese dish made by fermenting soybeans with Bacillus species. Currently, four methods are available for the production of PGA: chemical synthesis, peptide synthesis, biotransformation, and microbial fermentation (Rastogi et al., 2009).

While bacterial fermentation of PGA is well-documented for its benefits in the food industry, including thickening, reducing bitterness, and cryoprotection (Sanda et al., 2001; Luo et al., 2016), research on its incorporation into native cassava starch remains limited. Most studies have concentrated on PGA's applications in broader food processing contexts or its ability to enhance other types of starch. However, little attention has been given to exploring how PGA could improve the physicochemical properties of native cassava starch, a widely used ingredient with often less-than-ideal functional characteristics.

Native cassava starch, despite its widespread use, often has limitations in its physicochemical properties, affecting its functionality in various food products. These limitations

include issues with texture, stability, and nutrient content. Incorporating PGA could potentially enhance the starch's quality and performance in food applications. However, this specific application of PGA has not been thoroughly explored in current research.

The novelty of this research lies in the innovative integration of microbial PGA into native cassava starch. While previous studies have explored the general benefits and applications of PGA in the food industry, this research uniquely focuses on modifying cassava starch through enzymatic treatments combined with bacterial PGA. By using α-amylase and cellulase derived from *B. natto* to alter cassava starch granules and subsequently fortifying the starch with bacterial PGA, this study addresses a previously unexplored area. This approach not only aims to enhance the physicochemical properties of cassava starch but also provides a new perspective on utilizing PGA to improve specific food ingredients.

2. MATERIALS AND METHODS

Bacillus natto Starter Culture

B. natto was used to prepare starter cultures and for soybean fermentation. The strain was cultured on nutrient agar slants containing 0.5% peptone, 0.3% beef extract, 1.5% agar, and 0.5% NaCl (w/v) at pH 7.0, and stored at 4°C. For inoculum preparation, the strain was grown on nutrient agar slants at 37°C for 24 h. All fresh cultures were then transferred into 250 mL Erlenmeyer flasks containing 50 mL of sterilized nutrient broth. Incubated at 37°C on a rotary shaker at 150 rpm for 12 h (Shih and Van, 2001). The medium for α-amylase production consists 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 cellulase production, the media contained (% g/L): 10.0 CMC, 0.2 KH_2PO_4, 0.13 $(NH_4)_2SO_4$, 0.03 $MgSO_4$ $7H_2O$, 0.03 $CaCl_2$ $6H_2O$, and 1.0 peptone, along with (% mg/L): 0.5 $FeSO_4$ $7H_2O$, 0.156 $MnSO_4$ $7H_2O$, 0.14 $ZnSO_4$ $7H_2O$, and 0.14 $CoCl_2$. Both media were dissolved in 1,000 mL of distilled water, with 100 mL aliquots poured into 250 mL Erlenmeyer flasks. The flasks were autoclaved at 121°C for 15 min, and the initial pH was adjusted to 7.0. Approximately one loop *B. natto* was added to the production medium and incubated at 37°C on a rotary shaker at 150 rpm for 48 h. After fermentation, the broth was centrifuged at 10,000 rpm for 15 min at 4°C, and the cell-free supernatant was collected as crude extracellular enzymes (Shih and Van, 2001).

Microbial PGA Preparation

The PGA production by solid-state soybean fermentation was carried out following the

method described by Lim et al. (2012). Twenty grams of peeled soybeans were placed in a 250 mL conical flask and sterilized by autoclaving at 121°C for 15 min. After sterilization, the cooked soybeans were cooled to 30–40°C before being inoculated with 3-5% inoculum. The substrates were mixed carefully under aseptic conditions, covered with eight layers of gauze, and incubated at 37°C for 48 h.

After fermentation, distilled water was added to the fermented soybeans at a ratio of 10:1 (v/w). The mixture was homogenized using a rotary shaker for 1 h at 200 rpm to remove the mucilage containing PGA. The suspension was filtered through cheesecloth, and 10 mL of the filtrate was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant containing PGA was combined with cold ethanol and allowed to settle overnight at 4°C. The precipitated containing PGA was collected by centrifugation at 12,000 rpm for 20 min at 4°C. The pellet was then dissolved in 5 mL of distilled water.

Cassava Starch Modification

Modification of cassava starch was carried out following the procedure described by (Martinez et al., 2015). To prepare the enzyme-modified cassava starch, 50 g of native cassava starch was mixed with 100 mL of sterilized distilled water. Next, 2% enzymatic starter culture was added to the slurry and mixed under aseptic conditions. The mixture was then incubated at 37°C for 24 h. After incubation, the paste was heated in a convection oven at 105°C for 1 h to stop the enzymatic activity. The processed paste was then dried in a cabinet dryer at 45–50°C for 24 h until the moisture content was reduced to about 12% or less. The dried paste was ground into a fine starch, which was stored in airtight plastic bags at 4°C for further use.

Fortification of Cassava Starch

Fortification of MCS with PGA was carried out after heat treatment. After the paste was heated in an oven at 105°C for 1 hour, it was cooled in a desiccator at room temperature for 5 minutes. Fortification was carried out by adding PGA at concentrations of 10% to 50% (v/w). The mixture was then incubated at 4°C for 24 h and the paste was dried at 45–50°C for 24 h, and ground into starch, and stored in a plastic bag at 4°C for further use (Chen et al., 2005).

Swelling Power and Solubility Determination

Swelling power of starch was measured using a modified method based on Martinez et al. (2015) and Kaur et al. (2011). Approximately 0.1 g of starch sample was placed into a 50 mL

centrifuge tube, and distilled water was added to make a total volume of 10 mL. The mixture was gently stirred for 30 s at room temperature and then heated in a water bath at 60°C to 90°C for 30 min with constant stirring and then centrifuged at 3,000 rpm for 30 min. The supernatant was carefully poured into a pre-weighed evaporation dish and dried at 100°C for 20 min.

Water and Oil Absorption Capacity

Water absorption was determined following the method described by Brites et al. (2008). A 3.0 g sample was dissolved in 25 mL of distilled water and transferred into a 50 mL centrifuge tube. The mixture was stirred for 5 min, allowed to stand for 30 min, and then centrifuged at 3,000 rpm for 30 min. The supernatant was decanted, and excess water was removed by drying the sample for 25 min at 50°C before weighing. Approximately, 2.5 g of starch sample was mixed with 30 mL of cooking oil in a centrifuge tube and stirred for 1 min. The mixture was allowed to stand for 30 min, after which the tube was inverted for 25 min to dry off excess oil before weighing. Water and oil absorption capacities were expressed as grams of water or oil absorbed per gram of dry sample.

Analysis of Proximate

The moisture, ash, protein, fat, and carbohydrate contents of the starch samples were analyzed using standard methods. The moisture content was determined using the oven drying method (Reddy and Bhotmange, 2013). A preheated aluminum crucible with its lid was placed in an oven at 105°C. Five grams of sample was accurately weighed into the crucible, dried at 105°C overnight to constant weight, and the weight loss was recorded as the moisture content.

The ash content was measured by preheating a porcelain crucible in an oven, weighing, and then adding 5 g of sample. The sample was burned on a burner and then placed in a muffle furnace at 550°C overnight. The weight of the residue was recorded as the ash content.

The protein content was determined using the Kjeldahl method as described by Kaur and Singh (2005) with a KjeltecTM 2300 Analyzer. Two grams of starch sample was placed in a digestion tube along with catalyst and 15 mL of sulfuric acid (H2SO3). The mixture was digested for 2 hours to obtain a clear solution, then cooled and transferred to the analyzer for distillation and automatic titration.

The fat content was analyzed using the SoxtecTM 2050 Analyzer following the AOAC method (AOAC, 2000). Two grams of sample were weighed into a thimble and covered with cotton. Ninety milliliters of petroleum ether were added to the extraction dish, which was then

inserted into the Soxtec system. The extraction dish was dried in an oven at 105°C for 1 h, cooled in a desiccator, and weighed.

Carbohydrate content was calculated by subtracting the sum of the percentages of moisture, ash, protein, and fat from 100, using the formula: Carbohydrate (%) = 100 – (% moisture + % ash + % protein + % fat).

Bulk density was assessed using the method described by Ashogbon and Akintayo (2012). Ten grams of starch was added to a 100 mL graduated cylinder. The cylinder was tapped gently on the laboratory bench until the sample level was stable. The final volume of starch was recorded as the bulk density.

Scanning Electron Microscopy (SEM)

The microstructures of the granules of native and modified starchs were viewed with a field emission of the scanning electron microscope according to the method reported by Reddy and Bhotmange (2013). 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.

3. RESULTS AND DISCUSSION

Proximate analysis provides an overview of the nutritional content of food, assessing components such as ash, moisture, fat, and protein. Table 1 presents the proximate composition of cassava starch combined with PGA. The ranges of moisture, ash, protein, fat, and carbohydrate contents were approximately 6.95%, 1.44%, 2.12%, 0.23%, and 89.88%, respectively. Higher PGA levels were associated with increased protein and fat content, while moisture and ash contents decreased.

Moisture content indicates the amount of water and dry matter in the sample (Ojo et al., 2017). Starch with moisture levels below 14% is less prone to microbial growth, which enhances its shelf life. Thus, the starch sample is within the ideal range for safe storage and further processing. This helps minimize the risk of microbial contamination (Chinma et al., 2013). Additionally, this suggests that the starch samples will remain free from caking if stored properly under conditions that prevent moisture uptake (Iwe et al., 2017).

Native cassava starch exhibited a significantly higher ash content ($p<0.05$) at 1.62% compared to other starch samples, reflecting a greater concentration of minerals. However, this value exceeded the regulatory limit, whereas the other samples remained within acceptable

standards. Unlike the findings of Iwe et al. (2017), the ash content did not consistently increase with higher levels of PGA. Iwe et al. (2017) observed that substituting cassava starch with wheat starch led to a rise in ash content in high-quality cassava starch. The differences may be due to the composition of PGA, especially glutamic acid. Ash content, which is often associated with mineral content in foods, suggests that variations in PGA composition may affect the ash content of starch. The ash value of MCSenriched with PGA suggests its potential as a source of minerals and material for edible biofilms (Chinma et al., 2013).

The increase in protein and fat content in the blends can be attributed to the addition of PGA to the cassava starch. The protein content of wheat starch modified with PGA fortification was significantly higher ($p<0.05$) compared to other starch samples. However, the protein content in MCS remained much lower ($p<0.05$). It is possible that some of the protein in the MCSdissolved into the incubation media during the modification process. Additionally, the low protein content may be due to the inefficient utilization of carbohydrates in the cassava starch during modification (Rosales-Soto et al., 2016). The observed increase in protein content when PGA was added to the MCS could be due to the high amino acid content in PGA.

Tewe & Lutaladio (2004) indicates that the amino acid content may appear higher after analysis due to the presence of amino acids in the starch sample, since about 60% of the total nitrogen in starch comes from amino acids. The presence of glutamic acid in PGA contributes to the nitrogen percentage, leading to a significant increase ($p<0.05$) in protein content corresponding to the levels of PGA fortification.

Cassava is naturally very low in fat. As noted by Gomes et al. (2005), cassava contains only 0.1% fat. Morgan and Choct (2016). It has been found that starch made from cassava tubers contains about 2.5% lipids, although only about half of this can be extracted using conventional solvent systems. Fatty acids in cassava are mostly saturated. In this study, the lipid content of cassava starch and PGA increased with higher PGA levels, ranging from 0.25% to 0.40%. The carbohydrate content of native cassava starch was significantly lower ($p<0.05$) compared to other starch samples. Carbohydrate content was calculated by subtracting the moisture, ash, protein, and fat content from 100, with the starch samples showing carbohydrate contents ranging from 86.86% to 90.89%.

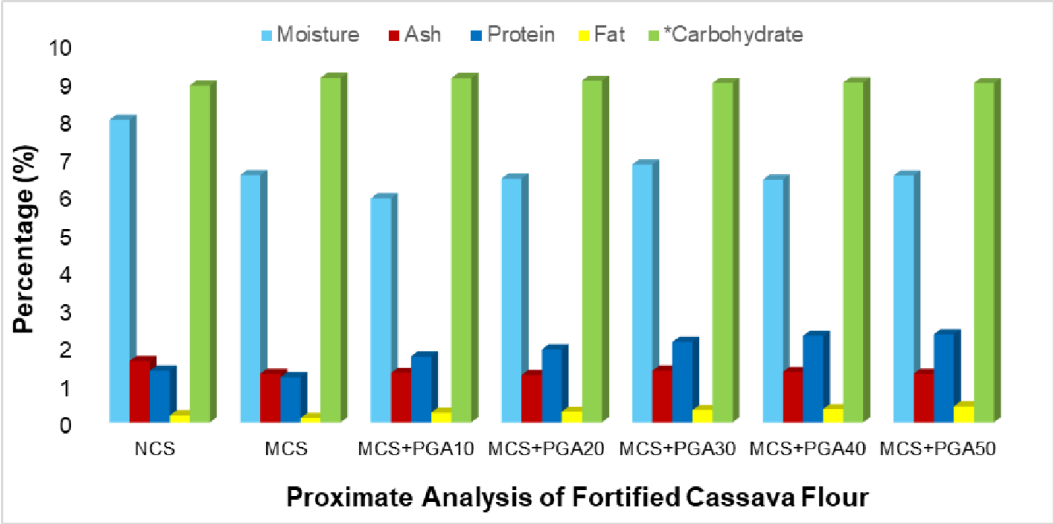

Figure 1. Proximate Analysis of Native, Modified and Fortified Starch

Starch granules are insoluble in water but can undergo hydration when exposed to high temperatures, hydration and swelling of starch granules cause thermal disruption of its crystal structure (Brites et al., 2008). When the gelatinization temperature is reached, the swelling of the amorphous or water-penetrated regions accelerates the destruction of the crystalline regions in starch. This phenomenon is referred to as the swelling power of starch granules. Fig. 1 and 2 illustrate the variation of swelling power and solubility of starch at different temperatures.

Physicochemical Properties of Fortified Cassava Starch

Starch granules are water-insoluble compounds that can become hydrated when exposed to high temperatures. As these granules hydrate and swell, their crystalline structures undergo thermal disruption (Brites et al., 2008). Upon heating, water infiltrates the more accessible amorphous regions within the starch granules, leading to their hydration and limited swelling. When the gelatinization temperature is reached, swelling intensifies in these water-penetrated or amorphous regions, causing further disruption of the starch crystal area, known as the swelling power of starch granules.

The swelling power of cassava starch and PGA mixture, measured at temperatures from 60 to 90°C, varied from 2.72 to 18.40 g/g. The highest values, ranging from 2.80 to 15.70 g/g, were observed for native cassava starch at temperatures from 60, 70, and 80°C. As shown in Figures 1 and 2, a 10°C increase in temperature led to an increase in swelling power. In particular, a sudden increase in swelling power was observed when the starch samples were heated to 70°C. This finding is in contrast to a previous study by Gbadamosi & Oladeji (2013), who reported

a gradual increase in swelling power of cassava starch from 60 to 70°C.

The increase in swelling power was very rapid at 70°C. Peroni et al. (2006) stated that the gradual increase in swelling power with hydration and temperature indicated weak associative forces responsible for maintaining the structure of starch granules. MCS containing 50% PGA showed the highest swelling power among all starch samples at 90°C, indicating its potential usefulness as an ingredient in food products that require high-temperature processing (Gbadamosi & Oladeji, 2013).

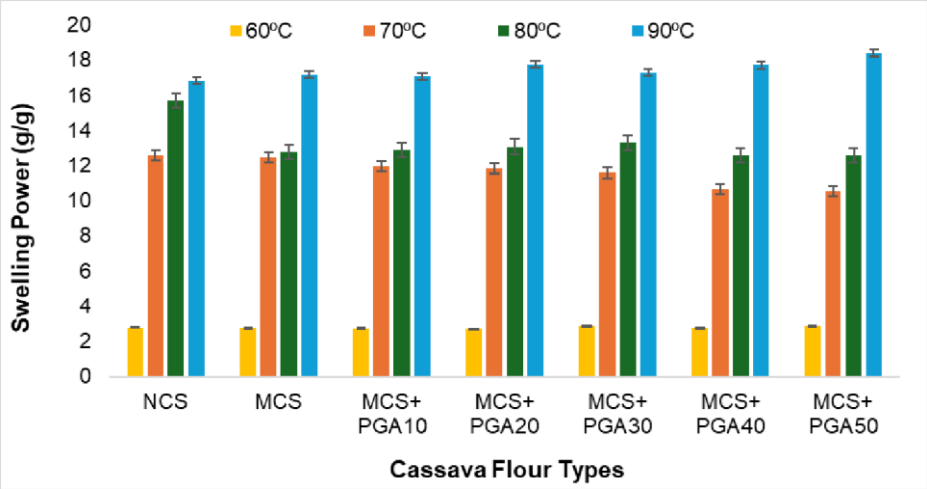

Figure 2. Swelling Power of Native, Modified and Fortified Cassava Starch

Starch solubility is influenced by several factors such as starch source, amylose and amylopectin content, extraction method, and thermal stability, as well as wet and heat treatments (Gu et al., 2016). Solubility increased significantly with temperature, ranging from 3.69% to 15.33%. Figure 2 showed that native starch has a significantly ($p<0.05$) higher solubility than other samples at 60–80°C, but the solubility varies inconsistently at 90°C.

A similar pattern was observed in a study by Shariffa et al. (2010), where the solubility of native tapioca starch was 6.56% after 24 hours of hydrolysis. The decrease in solubility suggests that the interactions between amylose and/or amylopectin and amylopectin helices had strengthened, leading to a more stable granule structure that inhibits amylose from leaching out (Martinez et al., 2015).

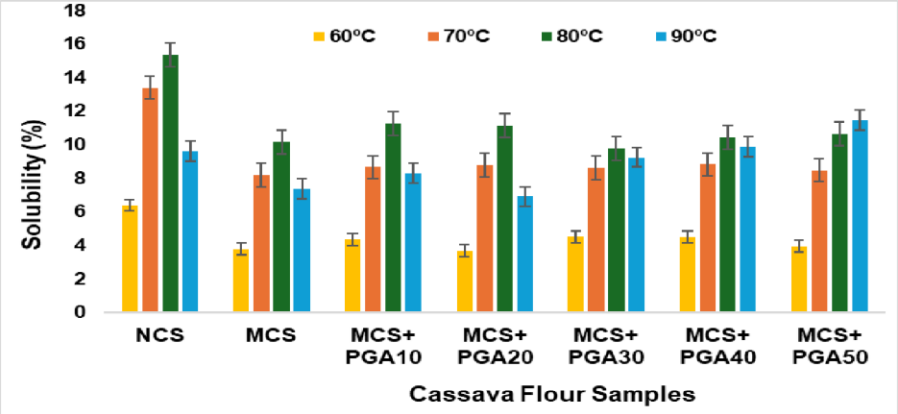

Figure 3. Solubility of Native, Modified and Fortified Cassava Starch

Morphological Observation by SEM

In this study, native cassava starchs were hydrolyzed using a mixture of extracellular α-amylase and cellulase from Bacillus natto. The mode of attack and structural changes in the hydrolyzed cassava starch were examined using scanning electron microscopy (SEM). Fig. 3 and 4 present SEM images of native, modified, and fortified cassava starch granules.

Native starch granules are typically round and smooth, with some showing irregular, truncated shapes, indicating structural weaknesses that make them prone to enzymatic degradation (Li et al., 2017; Shariffa et al., 2009). Hydrolyzed starch granules, as shown in Figure 3(B), exhibit extensively eroded surfaces, with enzymatic activity primarily affecting the granule exteriors. This action is uneven, selectively degrading some granules while leaving others mostly intact, resulting in roughened surfaces (Shariffa et al., 2017). These findings are consistent with prior studies by Sharifa et al. (2009), Rocha et al. (2010), Chen et al. (2011), and Li et al. (2017). O'Brien and Wang (2008) observed that enzyme-induced erosion can create a more extensive and deeper porous structure within starch granules. This extensive hydrolysis results in the formation of pores and pinholes on the granule surfaces.

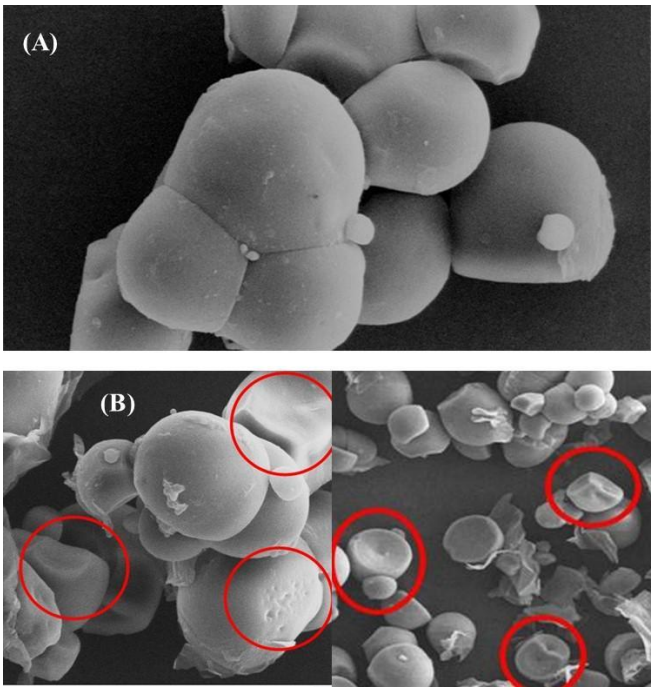

Figure 4. SEM Image of Native (A) and Modified Cassava Starch Granules (B)

The enzymatic erosion observed on the surface of starch granules indicates that hydrolysis occurs primarily through exocorrosion, meaning that the granules are degraded from the outside. In contrast, endocorrosion affects the interior of the granules, causing the formation of small holes that allow enzymes to penetrate deeper into the granules (Rocha et al., 2010). In addition, the hole-shaped granules may also be the result of granule swelling followed by collapse, which occurs when starch granules swell in concentrated enzyme solutions (Chen et al., 2011).

Fig. 4 shows that the starch granules of MCS with PGA have been partially or almost completely covered with a slime layer derived from PGA. Starch granules are surrounded on their surface by protein molecules, where enzymes form cross-links between proteins and starch molecules (Mohamad Ramlan et al., 2004). Therefore, as the ratio of PGA to starch increases, more layers of protein molecules are added to the surface of the starch granules to form larger globules. Based on Fig. 4, PGA-enriched starch showed large agglomeration where MCS granules appeared integrated in a compact structure surrounded by PGA protein molecules. This agglomeration may be due to the involvement of transglutaminase enzyme leading to the interaction between PGA and MCS with the formation of new intramolecular covalent agglomeration. This is because transglutaminase is a type of enzyme used for protein cross-linking purposes including in fortified starch (Marco et al., 2007).

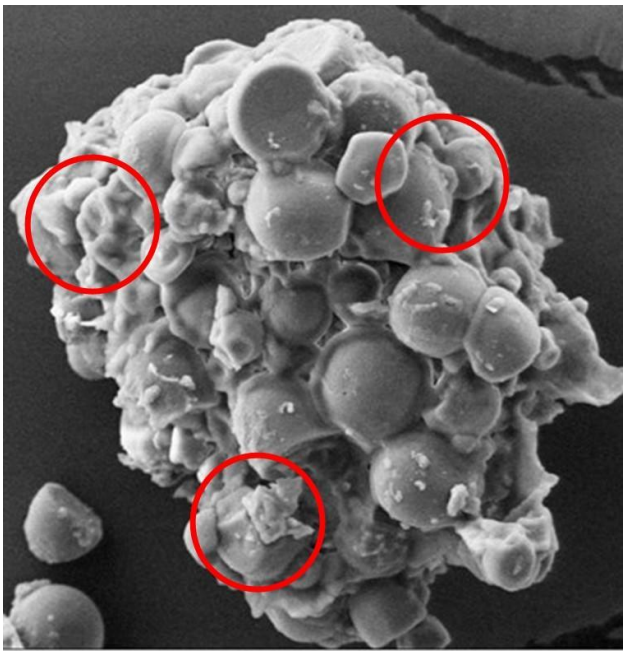

Figure 5. SEM Image of Starch Granules of Fortified Cassava Starch

Functional Properties of Fortified Cassava starch

Water absorption capacity (WAC) indicates the ability of starch to absorb water and expand. As shown in Table 3, the WAC of cassava starch and PGA blend ranged approximately 2.26 g/g, with native starch showing significantly higher WAC ($p<0.05$). This higher WAC is likely due to the lower protein content and higher carbohydrate levels in the native starchs, as carbohydrates are known to significantly affect WAC (Anthony et al., 2014). Aremu et al. (2009) noted that starchs with high water absorption capacities generally contain more hydrophilic constituents, such as polysaccharides, within their granules. Additionally, starch with a higher proportion of amorphous material has more sites available for water binding, resulting in greater water absorption (Lawal, 2004).

The difference in water absorption capacity (WAC) between native cassava starch and cassava starch modified with PGA mixture was due to the variation of hydrophilic carbohydrates and the reduction of starch content after the addition of PGA. The oil absorption capacity (OAC), which is influenced by the lipophilic nature of the starch was approximately 2.04 g/ml. The high OAC in native cassava starch, which is associated with its hydrophobic protein content, improves the retention of flavor and texture of food (Ohizua et al., 2017; Iwe et al., 2017). However, the addition of PGA did not increase the OAC in the modified starch. In contrast, the high WAC in PGA increased moisture retention and reduced oil absorption (Lim et al., 2012).

Bulk density (g/cm³) refers to the density of starch measured without any compression applied (Chandra et al., 2015). Bulk density is commonly used to assess starch expansion and product porosity (Shafi et al., 2016). In addition, bulk density is a key factor in raw material handling, packaging requirements, and food industry applications, especially in wet processing (Ajanaku et al., 2012). In this study, the bulk density values ranged from approximately 0.61 g/ml, increasing with higher levels of PGA addition. The high bulk density observed in modified starch containing PGA indicates that these samples occupy less space and require less packaging material per unit weight, leading to reduced packaging costs.

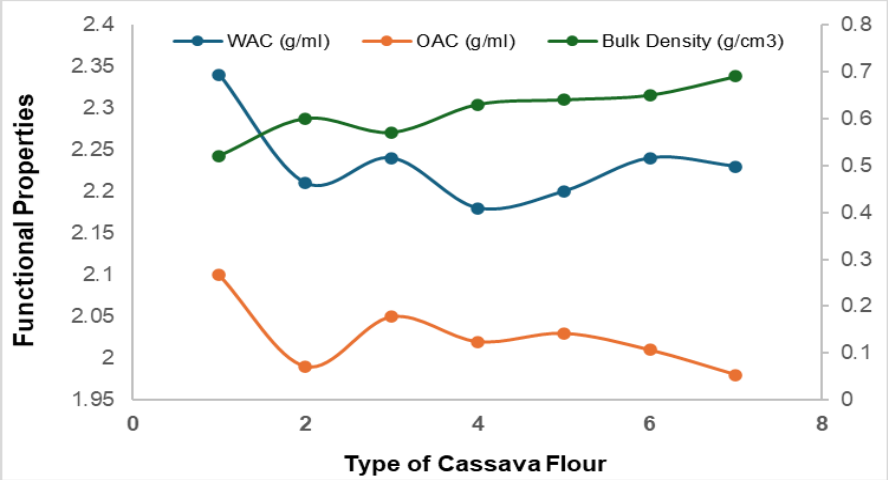

Figure 6. Functional Properties of Native, Modified, and Fortified Cassava Starch

NCS showed higher emulsion capacity compared to other starch samples, consistent with the findings of Iwe et al. (2017), who reported an emulsion capacity of 41.52% for cassava. However, modification of native starch significantly reduced its emulsion capacity ($p<0.05$) by 30%. The emulsion capacity of modified starch enriched with 50% PGA was even slightly lower, at 27.50%. Shyu & Sung (2010) reported that incorporating PGA into food systems can effectively slow down the separation of oil and water in emulsions.

Adegunwa et al. (2017) suggested that differences in the emulsion capacity (EC) of proteins might be due to their solubility behavior, as emulsifying properties are mainly related to protein hydrophobicity (Kaushal et al., 2012). Other factors that can influence the EC value of a food sample include pH and concentration. The ability of proteins to enhance emulsion formation and stabilization is particularly important in developing food products such as cakes, and frozen desserts (Adegunwa et al., 2017). Based on these findings, MCS fortified with PGA may not be

ideal for products that require strong emulsifying and stabilizing properties.

The pasting profile of starch is a critical parameter that significantly impacts the quality and sensory attributes of food. It plays a key role in determining the texture, digestibility, and overall functionality of starch-based food products (Ajanaku et al., 2012). Table 1 highlights the pasting properties of cassava starch blended with PGA, examining parameters such as peak viscosity, trough, breakdown, final viscosity, setback, peak time, and pasting temperature.

Table 1. Pasting Properties of Native, Modified and Fortified Cassava starch

Starch Types[2]	Peak Viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Set Back (RVU)	Pasting Temp. (°C)
NCS	317.00±3.29^{d}	141.05±1.94^{ab}	175.96±1.36^{d}	197.63±2.54^{b}	56.59±0.59^{b}	75.15±0.00^{a}
MCS	236.92±0.23^{c}	147.38±0.90^{bc}	88.04±1.00^{c}	194.46±0.30^{b}	47.09±1.18^{a}	78.35±0.00^{b}
MCS+PGA10	228.46±1.36^{c}	153.88±5.13^{c}	82.10±0.23^{bc}	202.17±2.47^{b}	48.30±2.65^{a}	78.33±0.04^{b}
MCS+PGA20	232.71±1.24^{bc}	146.54±1.82^{bc}	86.17±3.06^{c}	197.42±2.35^{b}	52.38±2.06^{ab}	77.95±0.49^{b}
MCS+PGA30	232.21±1.82^{bc}	155.88±1.83^{c}	74.33±2.83^{a}	203.13±1.59^{b}	57.25±0.24^{b}	78.03±0.46^{b}
MCS+PGA40	227.46±1.82^{ab}	155.29±2.53^{c}	77.17±2.71^{ab}	201.34±0.83^{b}	55.04±1.71^{b}	78.33±0.04^{b}
MCS+PGA50	222.38±1.12^{a}	135.54±0.76^{a}	76.83±0.35^{ab}	186.65±4.45^{a}	56.05±1.94^{b}	77.93±0.60^{b}

The peak viscosity values observed ranged from 222.38 to 317.00 RVU, with native cassava starch demonstrating a significantly higher peak viscosity ($p<0.05$) compared to the other samples. This observation aligns with findings by Ojo et al. (2017), indicated that the starch is well-suited for applications requiring strong gel strength and elasticity. Interestingly, the cassava starch blended with 50% PGA recorded the lowest peak viscosity, suggesting it may be the most manageable option for cooking purposes (Adebowale et al., 2005).

Final viscosity measures the stability of cooked starch at 50°C, with values in this study ranging from 186.65 to 203.13 RVU. Modified starch with 50% PGA had significantly lower final viscosity. Setback, the viscosity after cooling to 50°C, reflects the retrogradation or reordering of starch molecules and affects product texture. High setback values are associated with syneresis during freeze/thaw cycles. In this study, setback values ranged from 47.09 to 57.25 RVU.

The findings of this research have the potential to influence both the food industry and public health. By offering a method to enhance the nutritional and functional properties of a widely consumed staple, an addresses key challenges in nutrition, food processing, and sustainability. The broader adoption of PGA-fortified cassava starch could lead to healthier diets, improved food

products, and more resilient food systems, making a meaningful impact on both local and global scales.

4. CONCLUSION

This study demonstrated that fortifying cassava starch with microbial PGA significantly influenced its proximate composition, leading to increased protein and fat content proportional to the PGA concentration. Among the samples, cassava starch fortified with 50% PGA showed the highest levels of protein and fat. The functional properties of the modified starch also differed notably from those of native cassava starch. Specifically, the enzymatic modification and addition of PGA decreased the swelling power of starch granules while increasing their solubility. This reduction in swelling power suggests enhanced binding strength and greater resistance to damage during cooking and gel formation. The cassava starch fortified with 50% PGA showed the lowest peak viscosity and reduced final viscosity, making it easier to handle during cooking processes. Further research is recommended to explore the potential of this fortified cassava starch in products such as baked goods and complementary foods.

5. ACKNOWLEDGMENTS

This research work was financially supported by the Internal Project Fund of the Department of Research and Community Development, Universitas Ciputra Surabaya, Indonesia. We also thank the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia for providing research facilities for analysis.

6. REFERENCES

Alves, A.A.C. (2002). Cassava botany and physiology, Hillocks, R.J., Thresh, J.M., Bellotti A. Ed., *Cassava: Biology, Production and Utilization*, Wallingford, UK: CABI, pp. 67-89.

Adebowale, Y.A., Adeyemi, I.A., Oshodi, A.A. (2005). Functional and physicochemical properties of starchs of Mucuna species. *African Journal of Biotechnology*. *4*, 1461-1468.

Adegunwa, M.O., Adelekan, E.O., Adebowale, A.R., Alamu, E.O. (2017). Evaluation of nutritional and functional properties of plantain (*Musa paradisiaca*) and tigernut (*Cyperus esculentus*) starch blends for food formulations. *Cogent Chemistry*. *3*, 1383707.

AOAC, *Official methods of analysis, 18th edition*. (2000). Association of Official Analytical Chemists. Washington, D.C.

Anthony, N.M., Sawi, M.K., Aiyelaagbe, O.O., Taiwo, A., Winnebah, T., Fomba, S.N. (2014). Proximate characteristics and complementary assessment of five organic sweet potatoes cultivars and cowpea varieties. *International Journal of Engineering Science*. *3*(9), 38-42,

Aremu, M.O., Basu, S.K., Gyar, S.D., Goyal, A., Bhowmik, P.K., Datta, B.S. (2009). Proximate composition and functional properties of mushroom starchs form *Ganoderma* spp.,

Omphalotus olearius and *Hebeloma mesophaeum* used in Nasarawa Stata, Nigeria. *Malaysian Journal of Nutrition*. *15*(2), 233-241.

Ashogbon, A.O., Akintayo, E.T. (2012). Morphological, functional and pasting properties of starches separated from rice cultivars grown in Nigeria. *International Food Research Journal*. *19*(2), 665-671.

Ajanaku, K.O., Ajanaku, C.O., Edobor-Osoh, A., Nwinyi, O.C. (2012). Nutritive value of sorghum ogi fortified with groundnut seed (*Arachis hypogaea* L.). *American Journal of Food Technology*. *7*, 82-88.

Boudries, N., Belhaneche, N., Nadjemi, B., Deroanne, C., Mathlouthi, M., Roger, B., Sindic, M. (2009). Physicochemical and functional properties of starches from sorghum cultivated in Sahara of Algeria. *Carbohydrate Polymers*. *78*(3), 475-480.

Behera, B.C., Sethi, B.K., Mishra, R.R., Dutta, S.K., Thatoi, H.N. (2017). Microbial cellulases diversity and biotechnology with reference to mangrove environment. *Journal of Genetic Engineering and Biotechnology*. 15, 197-210.

Brites, C.M., Lourenço, D.S.C.A., Bagulho, A.S., Beirão-da-Costa, M.L. (2008). Effect of wheat puroindoline allels on functional properties of starch. *European Food Research Technology*. *226*, 1205-1212.

Chandra, S., Singh, S., Kumari, D. (2015). Evaluation of functional properties of composite starchs and sensorial attributes of composite starch biscuits. *Journal of Food Science and Technology*. *52*(6), 3681-3688.

Chen, X., Chen, S., Sun, M., Yu, Z. (2005). High yield of polyglutamic acid from *Bacillus subtilis* by solid-state fermentation using swine manure as the basis of a solid substrate. *Bioresource Technology*. *96*, 1872-1879.

Chen, Y., Huang, S., Tang, Z., Chen, X., Zhang, Z. (2011). Structural changes of cassava starch granules hydrolysed by a mixture of α-amylase and glucoamylase. *Carbohydrate Polymers*. 85, 272-275.

Chinma, C.E., Ariahu, C.C., Abu, J.O. (2013). Chemical composition, functional and pasting properties of cassava starch and soy protein concentrate blends. *Journal of Food Science and Technology*. *50*(6), 1179-1185.

Dura, A., Blaszczak, W., Rosell, C.M. (2014). Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymers*. 101, 837-845.

Gbadamosi, S.O., Oladeji, B.S. (2013). Comparative studies of the functional and flavour-chemical properties of isolated cassava, cocoyam and breadfruit starches. *International Food Research Journal*. *20*, 2273-2277.

Gomes, E., Souza, S.R., Grandi, R.P., Silva, R.D. (2005). Production of thermostable glucoamylase by newly isolated *Aspergillus flavus* and *Thermomyces lanuginosus. Brazilian Journal of Microbiology*. 36, 75- 82.

Gu, H., Yao, H., Wang, F. (2016). Structural and physico-chemical properties of resistant starch from Chinese chestnut (*Castanea mollissima*) prepared by autoclaving treatment and pullulanase hydrolysis. *Journal of Food Processing and Preservation*. *42*(1), pp. e13364.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K., Chauhan, B. (2003). Microbial α-amylase: a biotechnological perspective, *Process Biochemistry*. *38*, 1599-1616.

Howeler, R.L., Thomas, N., Holst Sanjuan, K., Sanjuan, K.H., Quiros, H., Isebrands, J., Martinez Oliva, E., Lagos Molina, S.M., et al. (2013). *Save and grow cassava: A guide to sustainable production intensification*. FAO, Roma, Italia.

Iwe, M.O., Michael, N., Madu, N.E., Obasi, N.E., Onwuka, G.I., Nwabueze, T.U., Onuh, J.O. (2017). Physicochemical and pasting properties high quality cassava starch and wheat starch blends, *Agrotechnology*. *6*(3), 1-8.

Juturu V., Wu, J.C. (2014). Microbial cellulases: Engineering, production and applications. *Renewable and Sustainable Energy Reviews*, *33*, 188-203.

Kaushal, P., Kumar, V., Sharma, H.K. (2012). Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (*Colocasia esculenta*), rice (*Oryza sativa*) starch, pigeonpea (*Cajanus cajan*) starch and their blends. *LWT - Food Science and Technology*. *48*(1), 59-68.

Kaur, M., Singh, N. (2005). Studies on functional thermal and pasting properties of starchs from different chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry*. *91*, 403-411.

Kaur, M., Oberoi, D.P.S., Sogi, D.S., Gill, B.S. (2011). Physicochemical, Morphological and Pasting Properties of Acid Treated Starches from Different Botanical Sources. *Journal of Food Science and Technology*. *48*(4), 460-465.

Kaur, B., Ariffin, F., Bhat, R., Karim, A.A. (2012). Progress in starch modification in the last decade, *Food Hydrocolloids. 2*, 398-404.

Lawal, O.S. (2004). Composition, physicochemical properties and retrogradation characteristics of native, oxidized, acetylated and acid-thinned new cocoyam (*Xanthosoma sagittifolium*) starch, *Food Chemistry*. *87*, 205-218.

Lim, S.M., Kim, J.J., Shim, J.Y., Imm, B.Y., Sung, M.H. (2012). Effect of poly-γ-glutamic acids (PGA) on oil uptake and sensory quality in doughnuts. *Food Science and Biotechnology*. *21*(1), 247-252.

Li, Y., Li, C., Gu, Z., Hong, Y., Cheng, L., Li, Z. (2017). Effect of modification with 1,4-α-glucan branching enzyme on the rheological properties of cassava starch. *International Journal of Biological Macromolecules*. *10*3, 630-639.

Lopez, O.V., Zaritzky, N.E., Garcia, M.A. (2010). Physicochemical characterization of chemically modified corn starches related to rheological behavior, retrogradation and film forming capacity. *Journal of Food Engineering. 100*(1), 160-168.

Luo, Z., Guo, Y., Liu, J., Qiu, H., Zhao, M., Zou, W., Li, S. (2016). Microbial synthesis of poly-γ-glutamic acid: current progress, challenges, and future perspectives. *Biotechnology for Biofuels.* 9, 134-145.

Marco, C., Pérez, G., Ribotta, P., Rosell, C.M. 2007. Effect of microbial transglutaminase on the protein fractions of Rice, Pea and Their Blends. *Journal of the Science* of *Food* and *Agriculture*. *87*(14), 2576-2782.

Martinez, M.M., Pico, J., Gomez, M. (2015). Physicochemical modification of native and extruded wheat starchs by enzymatic amylolysis. *Food Chemistry*. *167*, 447-453.

Mohamad Ramlan, B.M.S., Nobuyuki, M., Koji, T., Kazuhiro, Y., Higasa, T., Matsumura, Y., Shigeru, M. (2004). Gelling properties of soybean β-conglycinin having different subunit compositions. *Bioscience, Biotechnology* and *Biochemistry. 68*(5), 1091–1096.

Morgan, N.K., Choct, M. (2016). Cassava: Nutrient composition and nutritive value in poultry diets. *Animal Nutrition*. *2*(4), 253–261.

Ohizua, E.R., Adeola, A.A., Idowu, M., Sobukola, O.P., Afolabi, T.A., Ishola, R.O., Ayansina, S.O., Oyekale, T.O., Falomo, A. (2017). Nutrient composition, functional, and pasting properties of unripe cooking banana, pigeon pea, and sweet potato starch blends", *Food Science and Nutrition*. *8*, 750-762.

O'brien, S., Wang, Y.J. (2008). Susceptibility of annealed starches to hydrolysis by α-amylase and glucoamylase. *Carbohydrate Polymers*. *72*(4), 597-607.

Ojo, M.O., Ariahu, C.C., Chinma, E.C. (2017). Proximate, functional and pasting properties of cassava starch and mushroom (*Pleurotus pulmonarius*) starch blends. *American Journal of Food Science and Technology. 5*(1), 11-18.

Peroni, F.H.G., Rocha, T.S., Franco, C.M.L. (2006). Some structural and physicochemical characteristics of tuber and root starches. *Food Science and Technology International*. *12*(6), 505-513.

Poonsrisawat, A., Wanlapatit, S., Paemanee, A., Eurwilaichitr, L., Piyachomkwan, K., Champreda, V. (2014). Viscosity reduction of cassava for very high gravity ethanol

fermentation using cell wall degrading enzymes from *A. aculeatus*, *Process Biochemistry. 49*, 1950-1957.

Pongpaiboon, W., Srichamnong, W., Chaiyakul, S. (2016). Physical properties and resistant starch content of rice starch residues hydrolysed by α-amylase. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*. *10*(10), 722-727.

Rastogi, G., Muppidi, G.L., Gurram, R.N., Adhikari, A., Bischoff, K.M., Hughes, S.R., Apel, W.A., Bang, S.S., Dixon, D.J., Sani, R.K. (2009). Isolation and characterization of cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine. *Journal of Industrial Microbiology and Biotechnology*. *36*(4), 585-598.

Reddy, K., Bhotmange, M. (2013). Isolation of starch from rice and its morphological study using scanning electron microscopy. *International Journal of Agriculture and Food Science Technology*. *4*(9), 859-866.

Rosales-Soto, M.U., Gray, P.M., Fellman, J.K., Mattinson, D.S., Unlu, G., Huber, K., Powers, J.R. 2016. Microbiological and physico-chemical analysis of fermented protein-fortified cassava (*Manihot esculenta*) starch. *Food Science and Technology*. *66*, 355-360.

Rocha, T.S., Carneiro, A.P.A., Franco, C.M.L. (2010). Effect of Enzymatic Hydrolysis on Some Physicochemical Properties of Root and Tuber Granular Starches. *Ciência e Tecnologia de Alimentos*. *30*(2), 544-551.

Sanda, F., Fujiyama, T., Endo, T. (2001). Chemical synthesis of poly-gamma-glutamic acid by polycondensation of gamma-glutamic acid dimer: synthesis and reaction of poly-gamma-glutamic acid methyl ester. *Journal of Polymer Science: Polymer Chemistry*. *39*, 732-741.

Shafi, S., Wani, I.A., Gani, A., Sharma, P., Wani, H.M., Masoodi, F.A., Khan, A.A., Hamdani, A.M. (2016). Effect of water and ether extraction on functional and antioxidant properties of Indian horse chestnut starch. *Food Measurement*. *10*, 387-395.

Shariffa, Y.N., Karim, A.A., Fazilah, A., Zaidul, I.S.M. (2009). Enzymatic hydrolysis of granular native and mildly heat-treated tapioca and sweet potato starches at sub-gelatinization temperature. *Food Hydrocolloids*. *23*, 434-440.

Shariffa, Y.N., Uthumporn, U., Karim, A.A., Zaibunnisa, A.H. (2017). Hydrolysis of native and annealed tapioca and sweet potato starches at sub-gelatinization temperature using a mixture of amylolytic enzymes. *International Food Research Journal*. *24*(5), 1925-1933.

Shih, I.L., Van, Y.T. (2001). The production of poly-γ-glutamic acid from microorganisms and its various applications. *Bioresource Technology*. *79*(3), 207-225.

Tewe, O.O., Lutaladio, N. (2004). *Cassava for livestock feed in sub-Sarahan Africa*. Rome, Italy, FAO.

Shyu Y.S., Sung, W.C. (2010). Improving the emulsion stability of sponge cake by the addition of γ-polyglutamic acid. *Journal of Marine Science and Technology*. *18*(6), 895-900.