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Dynamic Rheological, Thermal, and Structural Properties of Starch from Modified Cassava Flour (MOCAF) with Two Cultivars of Cassava

Sifat Rheologi, Termal, dan Struktur Pati MOCAF dari Dua Jenis Singkong

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Abstract

The objectives of this study were to investigate alteration in the starch properties of modified cassava flour from two different local cassava cultivars from Indonesia at different fermentation times, provide structural information for starch molecules, and characterize the dynamic rheological and thermal properties. Resistant starch, non-resistant starch, and total starch levels in the cassava cultivars were also evaluated. Changes in the starch properties of *Cimanggu* and *Kaspro* (local cultivars of cassavas from Indonesia) were compared following different fermentation times (0, 12, and 24 hours). The properties of starch from modified cassava flour were influenced by the fermentation time, although both cassava varieties showed the same general characteristics. Their levels of resistant starch, non-resistant starch, and total starch were 33.28% to 51.74%, 20.51% to 23.72%, and 53.81% to 72.25% (g/100 g dry sample), respectively. The starch granules for the non-fermented samples were oval, polygonal, and round shapes; however, during fermentation, the starch granules became more porous and developed cracks of various sizes. The starches of both varieties showed an onset temperature between 67.85 and 70.17 °C, a peak temperature of 69.33 °C to 71.62 °C, and an end set temperature of 68.62 °C to 70.95 °C, with enthalpy values ranging from 1.25 mJ/mg to 1.74 mJ/mg.

Keywords: cassava, cultivar, dynamic rheological, fermentation, modified cassava starch

Abstrak

Tujuan dari penelitian ini adalah untuk mengetahui perubahan sifat pati tepung singkong termodifikasi dari dua varietas singkong lokal Indonesia dengan lama fermentasi yang berbeda, memberikan informasi struktur molekul pati, dan melakukan karakterisasi sifat rheologi dan termal dinamis dari pati. pati resisten, pati non-resisten, dan kadar pati total dalam varietas singkong. Perubahan sifat pati Cimanggu dan Kaspro (varietas singkong lokal Indonesia) dibandingkan setelah dilakukan fermentasi pada waktu yang berbeda (0, 12, dan 24 jam). Sifat pati dari tepung singkong termodifikasi dipengaruhi oleh lama fermentasi, meskipun kedua varietas singkong menunjukkan karakteristik umum yang sama. Kadar pati resisten, pati non-resisten, dan total pati masing-masing 33,28% – 51,74%, 20,51% – 23,72%, dan 53,81% – 72,25% (g/100 g sampel kering). Granula pati dari sampel non-fermentasi berbentuk oval, poligonal, dan bulat. Namun, selama fermentasi, granula pati menjadi lebih berpori dan mengembang dengan terdapat retakan pada berbagai ukuran. Pati dari dua varietas menunjukkan onset temperature *(suhu awal) antara 67,85 °C dan 70,17 °C, suhu puncak 69,33 °C – 71,62 °C, dan suhu akhir 68,62 °C – 70,95 °C, dengan nilai entalpi berkisar dari 1,25 mJ/mg - 1.74 mJ/mg.*

Kata Kunci: *dinamik rheologi, fermentasi, pati singkong termodifikasi, singkong, varietas*

INTRODUCTION

According to the annual production area, cassava (*Manihot esculenta* Crantz) is the sixth most necessary necessary food crop (Food and Agriculture Organization of the United Nations Statistics Database, 2013). In 2018, cassava production in Indonesia achieved 19,341,233 tons with a total harvest area of 792,952 ha (Jonathan et al., 2019). Cassavas consist of two cultivars based on the cyanide acid (HCN) content, referred to as sweet and bitter cultivars. Sweet cassavas (such as the *Mentega*, *Ketan*, and *Cimanggu*, Indonesian local cassava cultivars) have HCN contents less than 50 µg/g HCN on a fresh weight basis. In contrast, bitter cassavas (such as the *Malang 6* and *Kaspro*, also local cultivars from Indonesia) have HCN contents up to 400 µg/g of fresh weight basis (Peprah et al., 2020). According to Nugraheni et al. (2015), higher HCN contents in cassavas correlate with a more bitter taste and higher starch content (and vice versa). Therefore, the tapioca industry generally uses high-HCN cultivars (bitter cultivars).

Modified cassava flour (MOCAF) is a potential product derived from modifying cassava flour. MOCAF is processed from modified cassava cells by microbial fermentation using lactic acid bacteria (LAB), which predominate during cassava flour fermentation. The growing microbes produce pectinolytic and cellulolytic enzymes that can damage cassava cell walls so that they release starch granules. The substrate has an essential role in fermentation or enzyme bioprocesses. MOCAF production is high enough for broad utilization as a food ingredient or raw material for various types of food. Thus, MOCAF has a broad spectrum of applications, and the scope is almost the same as wheat flour, corn flour, and rice flour (Subagio et al., 2022).

MOCAF can be made from both sweet and bitter cassava cultivars. MOCAF from sweet cassava is used to make cakes and biscuits because of its mild taste and aroma. MOCAF from bitter cassava cultivars can also be used, although the taste preference was lower than the sweet one. Each cassava variety had unique starch characteristics in physicochemical and morphological properties, so MOCAF produced from a different cassava cultivar allegedly has different characteristics too. This research was conducted with *Cimanggu* and *Kaspro*, local cassava cultivars from Indonesia.

The starch modification process during MOCAF production makes MOCAF characteristics different from tapioca, which causes MOCAF functional changes by increased viscosity, gelation ability, hydration ability, water-holding capacity, and solubility. Chemically, MOCAF has a higher starch content (85–87%) than tapioca (82–85%). The viscosity value (mPa.s) of MOCAF is also higher than tapioca. The MOCAF viscosity is stable at various pH and temperature levels compared to tapioca (Putri et al., 2022).

The resistant starch (RS) properties can affect MOCAF quality. RS can enhance the MOCAF nutritional profile by adding dietary fiber, thus making it healthier (Dewi et al., 2020). RS also can absorb water and help prolong the shelf life of MOCAF by preventing it from drying quickly (Firdaus et al., 2017). It also can affect the textural properties of the foods made with MOCAF, such as leading to a change in stickiness, firmness, and flexibility (Mwizerwa et al., 2017). The food may also benefit gut health because RS acts as a prebiotic, providing nutrition for beneficial gut bacteria (Fuentes-Zaragoza et al., 2011). RS is a natural component present in many food resources. Sterilizing, drying in ovens, or drying at high temperatures, increased the RS levels (Pereira et al., 2016). The fermentation of natural RS reduced the intestinal pH, and the fermentation time influenced the tubers' RS content, where a longer processing time reduced the RS contents in many food samples and a shorter processing time helped retain more RS (Bavaneethan et al., 2015). Studies related to RS and starch granules' rheological, thermal, and morphological properties have been performed by Vamadevan & Bertoft (2015).

Therefore, this study aimed to verify the changes in the properties of MOCAF starches using two different cassava cultivars, provide structural information on the starch molecules and examine the starches' dynamic rheological and thermal properties. Such information may be helpful when considering starch application in the food industry and determining its RS contents. Thus, the effects of varying the cassava type and fermentation time during MOCAF starch production are investigated.

METHODS

MOCAF Preparation

The raw materials used in this research were two varieties of fresh local cassava (8.5–9 months old; *Cimanggu* and *Kaspro*) from Gumuk Mas Village (Jember District, East Java, Indonesia). Cassava flour is made by manually peeling using a special cassava peeler, washing using clean water, and chopping into slices with a 1-1.5 mm thickness. Next is soaking the slices with clean water in a basin with active compounds A (0.05% citric acid) and B (made by soaking 1-ounce fresh cassava slices in water mixed with enzymes and microbial culture for 24 hours). The fermentation time were 0, 12, and 24 hours. This soaking time affects the resulting MOCAF quality. Then the slices are soaked in a solution of active compound C (0,2% salt solution) for 10 minutes to wash the scum from the cassava, which can cause brown color during drying. The slices were sun-dried for 5 hours daily for 3–4 days. The dried cassava slices were ground, milled, and sieved through a 100-mesh sieve (Diniyah et al., 2018).

MOCAF Starch Sample Preparation

Starch was extracted from MOCAF by the precipitation and centrifugation method by Schmiele et al. (2015) with modifications. Briefly, the MOCAF was weighed, distilled water was added at a ratio 1: 10, then blended and filtered with a filter cloth, then passed through a 200-mesh sieve. The filtrate was centrifuged for 5 minutes in a centrifuge (ThermoFisher Scientific, Germany), and the resulting precipitate was dried at 40–50 °C for 24 hours in an oven (Memmert, Germany).

Analysis

Determination of RS

The RS content in MOCAF starch was determined using the Megazyme Resistant Starch Assay Kit (Liu et al., 2022; Zhang et al., 2012). Each sample was mixed with pancreatic α-amylase and amyloglucosidase solution, and the resulting solution was incubated in a shaking water bath (37 °C, 16 hours). During this incubation, the non-resistant starch (NRS) is solubilized and hydrolyzed to glucose by the two enzymes. The reaction was stopped by adding an equal volume of aqueous ethanol and centrifuging to separate the digested starch (supernatant) from the nondigested starch (residue). The supernatant was diluted with sodium acetate buffer, incubated, dissolved in KOH in an ice bath, mixed with sodium acetate buffer, and hydrolyzed to glucose with amyloglucosidase. The glucose oxidase/peroxidase reagent was added to a sample aliquot, and the solution was incubated (50 °C for 20 minutes). The absorbance was then measured at 510 nm using a spectrophotometer. The quantities of RS and NRS were calculated based on a calibration curve derived using standard glucose solutions.

Determination of Starch Granule

The granule morphology of MOCAF starch from *Cimanggu* and *Kaspro* cassava var. was studied using scanning electron microscopy (SEM). MOCAF starch was sprinkled onto double-sided cellophane tape attached to circular aluminum stubs and then coated with a thin layer of gold (Hummer Sputter Coater, Techincs EMS, Inc., VA). The sample was examined and photographed (Miniscope TM 3000 Hitachi, Japan). Twelve granules were randomly selected, and their sizes were measured, as described previously by Ali et al. (2016) and Agnes et al. (2017), with modifications.

Determination of the Dynamic Rheological Behavior

The viscoelastic behavior of the MOCAF starch gel was determined in duplicate. The samples were placed into a Magnetostrictive Rheometer System (Mx-2000; MG-Rheo, Japan). The experiment was performed at a constant deformation of 0.5% strain. The mechanical spectra were obtained by recording the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta = G''/G'$) as a function of the frequency (ϖ), as described by Tang & Liu (2017), with modifications.

Determination of Thermal Starch Properties

Differential scanning calorimetry (DSC) was used to evaluate the thermal properties of MOCAF starches. The thermal characteristics of the starches were studied using a 6100 SII EXSTAR 6000 DSC instrument (Seiko, Japan). MOCAF starch samples were mixed with distilled water (10% by volume) in a vial to yield a paste and to create a condition with excess water. The pan was hermetically sealed, and an empty pan was used as the reference. The analysis was performed over a 30–100 °C temperature range and a measurement speed of 2 °C/minutes. The transitions were characterized in terms of the onset temperature (To), peak temperature (Tp), and the end set temperature (Te), and the change in enthalpy (ΔH) associated with gelatinization, as described by Schmiele et al. (2015), with modifications. Each sample was analyzed in duplicate.

Statistical Analysis

The analysis was conducted on six treatments with third replication. The data were processed using Analysis of Variance. In cases where significant differences were observed, the data were further analyzed using the least significant difference test at 95% ($p = 0.05$). Data analysis was performed using SPSS 15.0 software.

RESULTS DAN DISCUSSION

Table 1 shows the RS, NRS, and total starch contents of MOCAF starch from the *Cimanggu* and *Kaspro* varieties based on the fermentation time. The average RS content of MOCAF starch from the *Cimanggu* and *Kaspro* varieties differed depending on the fermentation time. It ranged from 33.28 to 51.74% (dry sample), whereas the NRS content ranged from 20.51 to 23.72% (dry sample). In addition, the MOCAF total starch content from the *Cimanggu* and *Kaspro* varieties differed with the fermentation time and ranged from 53.81 to 72.25% (dry sample).

Table 1: RS, NRS, and total starch from MOCAF starch produced with different fermentation times

Sample	RS (%)	NRS (%)	Total Starch (%)
SM *Cimanggu* var. (fermentation 0 hours)	36.77 ± 0.006^c	$23.73^a \pm 0.042$	60.50 ± 0.004^b
SM *Cimanggu* var. (fermentation 12 hours)	40.48 ± 0.004^d	$23.25^a \pm 0.088$	63.73 ± 1.846^a
SM *Cimanggu* var. (fermentation 24 hours)	33.28 ± 0.001^a	$20.53^a \pm 0.048$	53.81 ± 0.146^d
SM *Kaspro* var. (fermentation 0 hours)	48.36 ± 0.002^e	$23.64^a \pm 0.033$	72.00 ± 1.204^a
SM *Kaspro* var. (fermentation 12 hours)	51.74 ± 0.002^f	$20.51^a \pm 0.067$	72.25 ± 1.637^a
SM *Kaspro* var. (fermentation 24 hours)	33.92 ± 0.002^b	$22.43^a \pm 0.110$	56.35 ± 3.519^c

[a] Mean of values ± standard deviation, SM = starch MOCAF

[b] Different superscripted letters in the same column indicate cases where the values are significantly different ($p \geq 0.05$)

The total starch content in MOCAF starch increased from 0 to 12 hours of fermentation with both the *Cimanggu* and *Kaspro* varieties but decreased after 24 hours of fermentation. The statistical analysis showed no significant difference between total starch unfermented with 12 hours of fermentation (*Kaspro*). During fermentation, the total starch significantly decreased from 12 to 24 hours. It may be due to changes in the starch's chemical nature. After 24 hours, the total starch was eliminated, following the enzyme action decrease. The highest total starch level of 72.25 ± 1.64% (dry sample) was observed in *Kaspro* variety MOCAF starch after 12 hours of fermentation. The varieties between *Cimanggu* and *Kaspro* significantly differed with non-fermentation (0 hours). The lowest total starch level of 53.81 ± 0.15% (dry sample) was observed in *Cimanggu* variety MOCAF starch after 24 hours of fermentation. In the same case, during the fermentation of sorghum, starch tends to decrease after 24 hours and significantly decreases throughout fermentation because degraded become amylose, amylopectin, and other small molecules (maltodextrin, for example.) (Adiandri & Hidayah, 2019; Nasrin & Anal, 2014). The decreasing total starch content in fermented MOCAF was indicated by the MOCAF enzyme activity, hydrolyzing the starch component during the fermentation. The enzyme randomly hydrolyzes the linear bond of α-1,4 glycosidic in amylose (Setiarto et al., 2018).

The highest RS content was observed in MOCAF starch from the *Kaspro* variety with 12 hours of fermentation (51.74 ± 0.002% dry sample), followed by MOCAF starch with 0 hours of fermentation (48.36 ± 0.002% dry sample) and 24 hours of fermentation (33.92 ± 0.002% dry sample). The RS contents of MOCAF starch in different varieties and fermentation time showed significant differences ranging from 33.28 ± 0.001 to 51.74 ± 0.002%. The RS content increased from 0 to 12 hours of fermentation time. Through the 12 hours of MOCAF fermentation, the organic acids, enzymes, and other substances produced in the fermentation process can degrade the protein and fat-wrap the surface of MOCAF starch granules to reduce the steric hindrance among the starch granules and fully expose them. The accumulation of organic acids in the fermented substrate forms empty holes on the surface of starch granules, thereby dissolving the amylose wrapped in the starch granules. Amylose and other substances produced by the fermentation degrade the starch molecules to a different degree, thereby weakening or damaging the amorphous region, that is, the non-crystalline region of starch granules, and entirely dissolving out the amylose also increasing the retrogradation value. Therefore, MOCAF amylose and the MOCAF RS content can significantly increase fermentation (12 hours), making it easier to produce resistant starch (Ge et al., 2020). The RS contents decreased with an increasing period of fermentation (12 to 24 hours), during which time amylase was leached from the granules, which increased the starch solubility and thereby increased its susceptibility to fermentation (Bavaneethan et al., 2015). Differences in the RS contents from different varieties were due not only to the chemical/compositional parameters (e.g., amylose and PO4 contents) but also to the physical/structural characteristics of the starch (i.e., crystallinity pattern, size, and granule shape, molecular interactions, and arrangements) (Bavaneethan et al., 2015; Kittipongpatana & Kittipongpatana, 2015). Part of this reduction can be attributed to the breakdown of starch into simpler sugars by enzymes of the fermenting microflora. The RS content is reduced in fermentation, also due to a loss in the structural integrity of starch granules and degradation of the amorphous regions (Nasrin & Anal, 2014).

The microstructures of MOCAF starch granules from the *Cimanggu* and *Kaspro* varieties were examined as a function of the fermentation time (Figure 1). The micrographs showed granules with different shapes from MOCAF starch of the *Cimanggu* and *Kaspro* varieties following different fermentation times. The MOCAF starch granules for all samples were clearly visualized; they had oval, polygonal, and round shapes. The surface morphological differences were observed between the granules, and some granules' surfaces appeared to have an oval shape on one end and a polygonal shape on the other (non-fermented granules). Some granules were found to have cracks and pores as the fermentation time increased.

SEM observations showed that the starch granules had different morphological appearances before (0 hours) and after (12 and 24 hours) fermentation, as shown in Figure 1. MOCAF starch granules with a diameter ranging from 30 to 50 µm had small to large, rounded, irregular shapes, which appeared as oval and truncated ellipsoidal shapes. Partial hydrolysis resulted in the breakage of the starch surface pores (Li et al., 2017). Non-fermented MOCAF starch granules had smooth surfaces with irregular shapes in some portions. This result also aligns with the research by Agnes et al. (2017) that most all wall cassava starches are round or oval shapes with a flat surface on one side containing a conical pit. Mtunguja et al. (2016) reported that the volume percent distribution of the smallest starch granule size ranges from 25-48 µm and the largest in the 12-25 µm range. It means that MOCAF starch granules can be classified into small size distribution. The implication of starch granule size and distribution correlates with the structure of MOCAF's amylose and amylopectin fractions. Tang et al. (2001) stated that the number-average degree of amylopectin polymerization for barley decreased with the granule size decrease, and amylose polymerization was the same for small and large granules. Another experiment concluded that in barley, large granules contained smaller, less branched amylose polymers (Takeda et al., 1999).

Table 2 showed that the gelatinization enthalpy of MOCAF starch without fermentation (0 hours fermentation) was higher than the *Cimanggu* variety (1.67 mJ/mg) and the *Kaspro* variety (1.74 mJ/mg) after fermentation (1.33; 1.64 mJ/mg for the *Cimanggu* variety and 1.25; 1.46 mJ/mg for the *Kaspro* variety at 12 and 24 hours fermentation time, respectively).

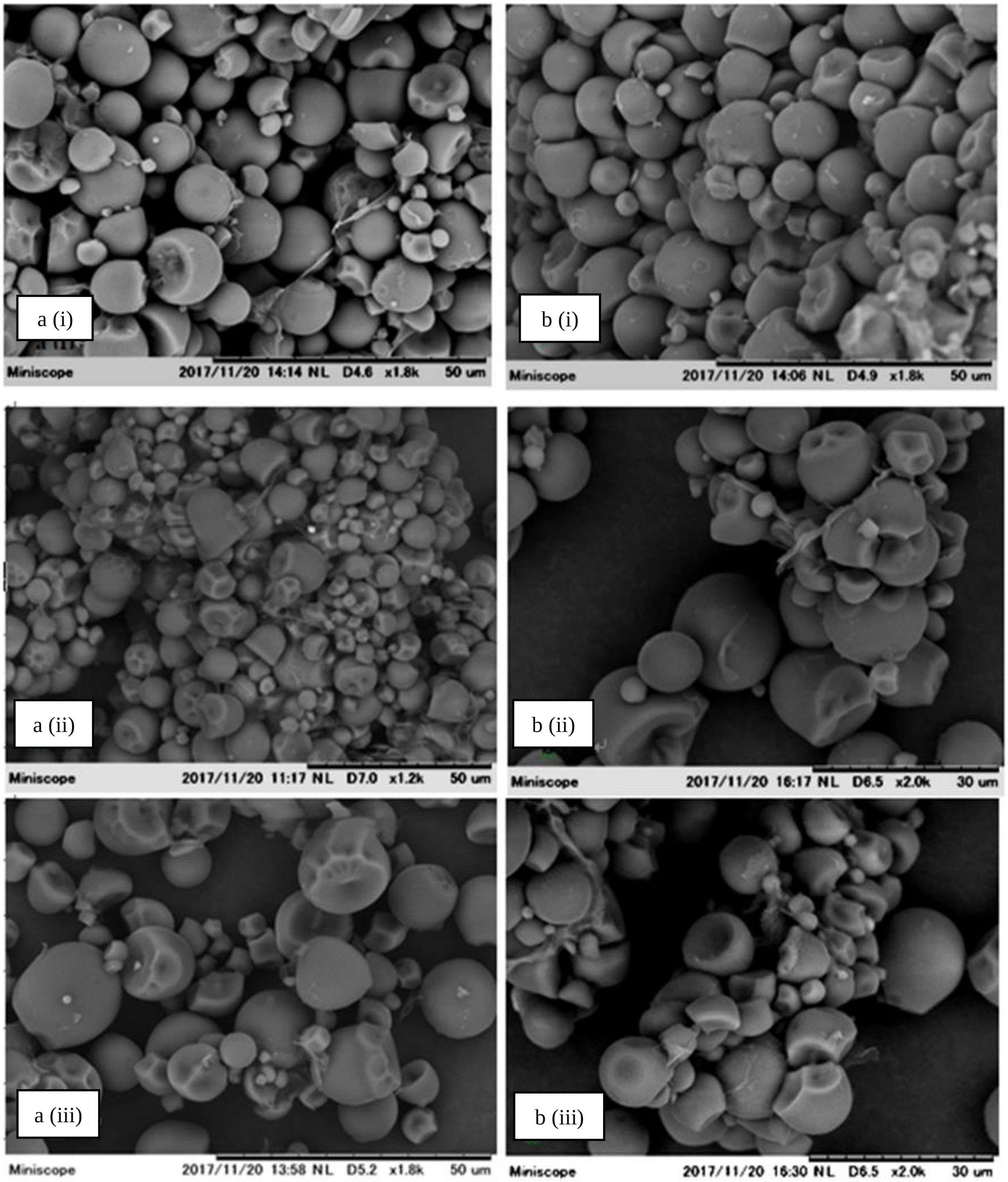

Figure 1. The Structure of MOCAF Starch Granules from the *Cimanggu* Variety (a) and the *Kaspro* Variety (b) Revealed Differences in Their Shapes and Morphologies at 0 hours (i), 12 hours (ii), or 24 hours (iii) Fermentation Time. A Magnification of 1800× was Used for SEM.

Table 2: Gelatinization properties of native (non-fermented) and fermented MOCAF starches produced with different fermentation times

Cassava Variety	Fermentation Time (hours)	Transition Temperature (°C)			Gelatinization Enthalpy (mJ/mg)
		To	Tp	Tc	
Cimanggu	0	69.79±0.02^{a}	70.16±0.00^{cd}	70.54±0.03^{a}	1.67±0.13^{a}
	12	70.17±0.04^{a}	70.95±0.21^{d}	71.62±0.21^{a}	1.33±0.24^{a}
	24	69.50±0.07^{a}	70.13±0.42^{c}	70.77±0.32^{a}	1.64±0.34^{a}
Kaspro	0	68.18±0.08^{a}	69.61±0.07^{b}	71.47±0.25^{a}	1.74±0.05^{a}
	12	67.85±0.16^{a}	68.62±0.14^{a}	69.33±0.37^{a}	1.25±0.17^{a}
	24	68.54±0.19^{a}	68.95±0.36^{a}	69.45±0.40^{a}	1.46±0.28^{a}

Results are means ± standard deviation. Data in the same column with different letters are significantly different ($p < 0.05$)

The gelatinization enthalpy (ΔH) decreased with increasing fermentation times in both cassava varieties at 12 hours, which was attributed to the preferential hydrolysis of the amorphous regions and the improvement of the double helix order (Hong et al., 2022). Similarly, de de Oliveira et al. (2014) showed that the gelatinization enthalpy of cassava starch was modified when the amount of HCl used during modification was decreased. The ΔH of MOCAF starches decreased due to disrupting hydrogen bonds among double helices in the crystalline and non-crystalline regions of the starch granules due to fermentation (Kittipongpatana & Kittipongpatana, 2015). Then, it was increased at 24 hours of fermentation, and the gelatinization enthalpy (ΔH) increased caused by the more and more stable crystalline region in starch granules during fermentation (Zong et al., 2022). The gelatinization temperatures of starch samples can vary due to modified methods and different genetic origins (Bet et al., 2017). Chisenga et al. (2019) stated that the gelatinization enthalpy could be different due to variations in amylose contents and the level of starch granule crystalline. Varieties of cassava (*Cimanggu* and *Kaspro*) are different botanical sources, so they have different properties, including amylose content and starch granule crystalline. Table 2 shows that the Tp values between *Cimanggu* and *Kaspro* varieties were significantly different. The *Cimanggu* variety had the highest gelatinization temperatures (To, Tp, and Tc). This result indicated higher stability of *Cimanggu* starch crystallites upon heating (Aprianita et al., 2009). The gelatinization temperatures in different botanical flour and starch sources also showed significant differences. The research by Aprianita et al. (2009) showed that gelatinization temperatures of yam flour and starch ranged from 74.21 to 84.67 °C and 69.18 to 81.5 °C, respectively. Compared to taro and sweet potato, yam flour and starch had a narrower gelatinization temperature range. The structure of amylopectin also could be due to the gelatinization enthalpy influence since the core of starch granule of crystallinity is related to gelatinization enthalpy.

Figure 2 shows changes in the storage modulus (G′) and loss modulus (G′′) for MOCAF starch of the *Cimanggu* and *Kaspro* varieties accordingly to the fermentation time. Thermal properties were analyzed using Differential Scanning Calorimetry (DSC). The DSC for the starches is shown in Table 2. The DSC results showed that MOCAF starch had a To of 67.85–70.17 °C, a Tp 68.62–70.95 °C, and a Tc of 69.33–71.62 °C, with an enthalpy between 1.25–1.74 mJ/mg.

Generally, the magnitudes of G′ reported were greater than those of G′′. The MOCAF starches from both cultivars exhibited rheological behaviors like a strong gel network. This tendency agrees with a report on potato starch after cross-linking (Heo et al., 2017). The dynamic modulus values (G′ and G′′) of MOCAF starch granules with increasing fermentation times compared to native starch granules (i.e., non-fermented granules, 0 hours of fermentation). The variation recorded in the storage modulus of MOCAF starch from the sweet cultivar may be caused by an intrinsic factor. The loss factor (tan δ) was determined directly as the G′′/G′ ratio. The tan δ values of the starches were between 0.01 and 0.20. Tan δ values < 1 indicated predominantly elastic behavior, while those >1 indicated predominantly viscous behavior. The tan δ values for the MOCAF starches samples were in the range of 0.01–0.20 (tan < 1), indicating that more of the samples studied were elastic than viscous. Li et al. (2017) study showed that modification improved rheological properties but less sensitivity to processing conditions (such as a low pH, a high temperature, or shearing) than native starch granules.

The results of DSC showed different values following starch modification, indicating that the thermal characteristics obtained differed from those of native starch. Similarly, in another study of modified cassava starch, after 1 hour of treatment, the To and gelatinization enthalpy values decreased (de Oliveira et al., 2014).

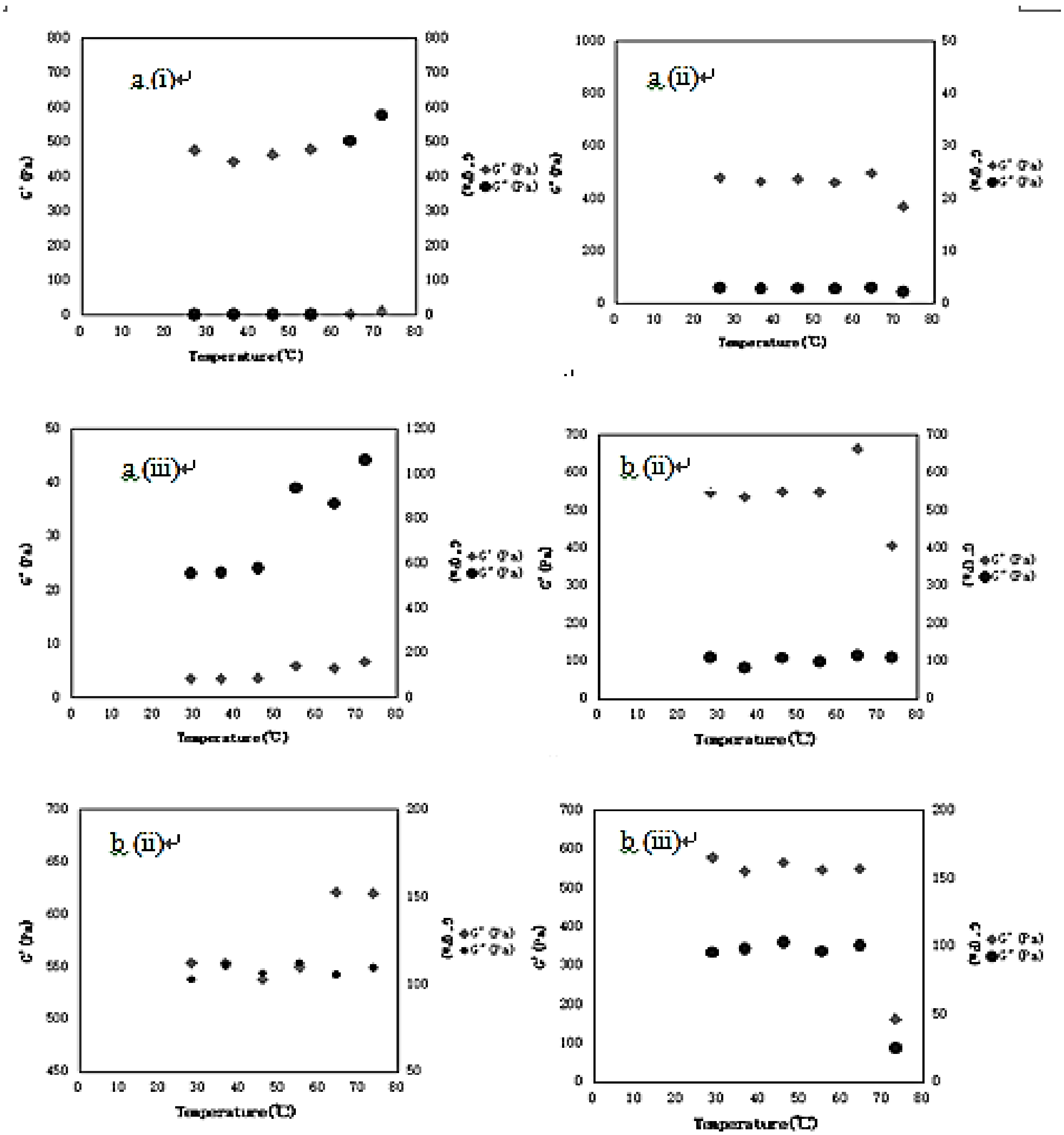

Figure 2. Graphs Showing the Storage Modulus (G′) and Loss Modulus (G″) Values of MOCAF Starch from the *Cimanggu* (a) and *Kaspro* (b) Varieties at Various Fermentation Times of 0 hours (i), 12 hours (ii), or 24 hours (iii).

Application in The Food Industry

The development of processing MOCAF methods improved with fermentation time, and cassava varieties (Cimanggu and Kaspro) treatment could greatly facilitate the development of new MOCAF applications in the food industry. Understanding the dynamic rheological and thermal properties of the MOCAF starches can provide valuable insights into their stability and processibility.

The rheological properties of MOCAF starch greatly influence the texture, appearance, and processability of many food products (Novelina et al., 2023). For example, the rheological properties of starch affect the texture and cooking qualities of gluten-free pasta and noodles to be firm yet tender, don't stick together, and hold up well to cooking (Padalino et al., 2016). In dessert products, the rheological properties of starch can impact the creaminess, mouthfeel, and product stability (Tarrega & Costell, 2006). MOCAF's favorable rheological properties make it suitable for vegetarian and vegan meat alternatives, where it can contribute to a meat-like texture and help bind the ingredients together (Paredes et al., 2022).

MOCAF starches with high thermal stability might be more suitable for food products that require high-temperature cooking. For example, breads and bakery products typically require high-temperature cooking. High thermal stability in the starch ensures that the product maintains its structure and texture during baking (Patel et al., 2005). Starches with high thermal stability can help deep-fried foods (such as donuts) achieve a crispy exterior while maintaining a soft interior (Zhang et al., 2014). This type of starch is also needed to withstand extrusion cooking which involves high temperatures and pressure in producing extruded snacks (such as cereal puffs). The starches also contribute to the unique textures of extruded snacks (Moscicki et al., 2013). Canned food which contains starch as a thickener needs starch with high thermal stability to maintain the product's texture and consistency during the sterilization process involving high temperature to ensure a long shelf-life.

The starch molecules' structural information could help predict and control the digestibility, gelatinization, retrogradation, and other functional properties (Cornejo-Ramírez et al., 2018). Some food products could benefit from high RS MOCAF starch. For example, using MOCAF with high RS content can make bakery products more nutritious with a lower glycemic impact (Di Rosa et al., 2023). Foods that support gut health (such as functional yogurts) might benefit from using MOCAF with high RS content because RS can act as a prebiotic (Xu et al., 2021). Meanwhile, MOCAF with lower RS content might be more suitable for baby food industries because the products require easily digestible ingredients (Susilowati et al., 2021). Instant foods industries like instant noodles might prefer using MOCAF with lower RS content because it can hydrate more quickly and easily (Afifah & Ratnawati, 2017).

CONCLUSIONS

The analyses carried out in this study indicated that the treatment of cassava varieties and the fermentation time influenced the characteristics of the resulting MOCAF starches, although the same general characteristics were observed, independently of the cassava variety. The RS, non-RS, and TS starch abundances ranged from 33.28% to 51.74%, 20.51% to 23.72%, and 53.81% to 72.25% (dry sample), respectively. The MOCAF starch granules were clearly visualized for all samples, and the non-fermented granules had oval, polygonal, or round shapes, although the starch granules developed increasing numbers of pores with cracks as the fermentation time increased. MOCAF starches from two varieties showed a To of 67.85–70.17 °C, a Tp of 68.62–71.62 °C, and a Tc of 69.33–71.62 °C, with an enthalpy of 1.25–1.74 mJ/mg. °

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