Modification of wikau maombo flour from sweet cassava (*Manihot utillisima*) and bitter cassava (*Manihot esculenta* Crantz) with annealing method

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Abstract. Modifying wikau maombo flour (WMF) by annealing method was carried out to see its effect on the physicochemical characteristics and proximate value of WMF from bitter and sweet cassava as raw materials. The results show that the selected samples (bitter cassava: sweet cassava) of annealing-modified WMF had the swelling power, water solubility index, and viscosity of 10.30 g/g: 15.92 g/g, 10.84%: 27.11%, and 26.63 cP: 34.44 cP, respectively. Proximate analysis of water, ash, fat, protein, crude fibre, and carbohydrate contents of annealing-modified wikau maombo flour show a significant effect on heating time and heating temperature with respective values (bitter cassava : sweet cassava) of 10.37%: 11.38, 1.57%: 1.75%, 1.32%: 1.70%, 1.56%: 1.87%, 4.08%: 4.27%, and 91.42%: 92.35%. Based on these results, the selected annealing modified wikau maombo flour from bitter cassava was the sample treated at 60°C heating and 16 hours of heating time. Meanwhile, the selected sample made from sweet cassava was the one treated at 50°C heating and 16 hours of heating time.

1. Introduction

Two types of cassava plants commonly cultivated are sweet and bitter cassava because they produce more tubers with higher starch content. However, cassava has a short shelf life. Cassava is an agricultural product that is easily damaged, because it has high water content, reaching 57.71% [1]. The quality of cassava will decrease if stored at room temperature for 24 hours, especially if it is defective when harvested. In addition, bitter varieties of cassava tubers need special processing before consumption, as this type of cassava contains high levels of cyanogenic glucoside (HCN) reaching 209.3877 mg/kg [1]. This amount is toxic; thus, reducing the level of HCN is a major concern in the use of cassava [2]. Efforts to make cassava safe for consumption vary depending on the region. These methods generally aim to increase palatability, reduce toxicity, and convert fresh, perishable cassava tubers into durable products. The pre-processing of cassava before consumption aims to detoxify and...
modify the starch. Some of products like tapai, garri, lafun, and fufu are the fermented cassava products [3]. Especially in the Buton area, to minimize HCN levels, cassava is processed into wikau maombo.

Wikau maombo is food that undergoes fermentation in the processing so that enzyme activity occurs from the mold which results in a sweet and distinctive taste formed in wikau maombo products [4]. During the fermentation process, changes in chemical composition, taste, aroma, colour, and texture, which can increase levels of protein and glucose found in wikau maombo. In addition, WMF can be used as an alternative to wheat flour in various diversification of food products. The basic difference in nutritional content is that WMF does not contain gluten (a substance that only exists in wheat that determines the elasticity of food).

Flour modification can be carried out by various methods, including enzymatic, chemical, and physical modification to improve the natural properties of starch to produce better properties or several characteristics that are expected to meet certain needs. Physical modification such as annealing modification is a safe method because it does not leave chemical residues. Hydrothermal annealing modification is the physical treatment of starch granules with excessive water at a temperature under the gelatinization temperature at a predetermined time [5]. The advantage of the annealing modified flour or starch produced is that it has a higher gelatinization temperature and the produce starch is more stable to heat. Oktavianti et al. [5], reported that the best treatment of modified purple sweet potato flour was at an annealing temperature of 27 °C with a soaking time of 8 hours. Meanwhile, another research [6] reported that the dry puree of sweet potato with annealing treatment at 50 °C for 4 hours has the best characteristics with an initial gelatinization temperature of 54.3°C.

2. Materials and methods

2.1. Materials
Bitter and sweet cassava, sodium hydroxide (NaOH), n-hexane (C₆H₁₄, Merck = 99%), hydrochloric acid (HCl, Merck = 37%), sodium bicarbonate (NaHCO₃, Sigma = 99.7 %), and polypropylene plastic.

2.2. Research procedure

2.2.1. Soaking and fermentation of bitter cassava (Manihot esculenta Crantz) and sweet cassava (Manihot utilissima). The cassava was peeled and cleaned, then split into 3 parts of the axis, soaking it in seawater for 3 hours until it was soft. Then, it was rinsed with fresh water to remove the mucus formed during immersion [7]. Furthermore, the fermentation process was carried out for 3 days then the cassava was dried in an oven at 60°C.

2.2.2. Making of wikau maombo. Wikau maombo was made into flour by mashed it using a blender until it becomes flour. Then it was sieved using an 80-mesh sieve and heated in an oven at 60°C for 2 hours as explained by Hamidah [8] to stabilize the flour.

2.2.3. Optimization of annealing physical modification. Two hundred grams of wikau maombo flour which had reached a moisture content of approximately 40% was then placed in a stainless-steel basin, then incubated at 50 °C for 8 hours and 16 hours. The basin was covered with aluminum foil. Furthermore, the flour was dried using an oven at 50 °C (for ± 5 hours).

2.2.4. Physicochemical of modified wikau maombo flour. Analysis of viscosity was performed using an Oswald viscometer. This test was done by calculating the time required for the flour solution that reached the maximum point to flow from the Oswald viscometer until it reached the stopping point. Time was calculated using a stopwatch [9].

Starch suspension solution with a concentration of 1% or 0.5 g was heated in a water bath shaker at 90°C for 30 min. The suspension was centrifugated at 3000 rpm for 15 min. After that, the supernatant
was separated from the sediment and the supernatant was poured into a petri dish. The supernatant then evaporated overnight at 70°C and the precipitate was weighed. Swelling power is the ratio between the weight of the sediment left in the centrifuge tube and the dry weight of the sample. Meanwhile, the solubility is the percentage weight of starch that dissolves in water [10].

3. Results and discussion

3.1. Physical characteristic of annealing modified WMF
Several changes in starch character can occur in the annealing process, including increasing granule stability, improving crystal structure, starch chain interactions in crystalline and amorphous parts, double helices formation, increasing gelatinization temperature, narrowing the gelatinization temperature range, decreasing the ability to expand starch, and decreasing amount of dissolved amylose [11]. Annealing can induce the reorganization of the double helices structure of amylopectin and starch chains into more regular crystalline structures [12,13].

Table 1. Effect of temperature and heating time of annealing modified wikau maombo flour on the physical characteristics of solubility, swelling power, and viscosity.

| Temperature & heating duration | Bitter cassava | Sweet cassava |
|-------------------------------|---------------|--------------|
|                               | Swelling power (g/g) | Water solubility index (%) | Viscosity (cP) | Swelling power (g/g) | Water solubility index (%) | Viscosity (cP) |
| Control                       | 9.41±0.68     | 9.41±0.660  | 4.91±0.03    | 5.82±0.22     | 25.85±0.21             | 10.71±0.03 |
| 40°C 8 hours                  | 10.09±0.19    | 6.33±0.92   | 24.86±0.22  | 7.52±0.55    | 8.48±0.39              | 23.56±0.37 |
| 40°C 16 hours                 | 8.69±0.08     | 9.68±0.69   | 10.10±0.37  | 7.55±0.24    | 2.24±1.12              | 29.58±0.85 |
| 50°C 8 hours                  | 9.73±0.35     | 10.75±0.90  | 8.43±0.19   | 9.10±0.29    | 3.61±2.27              | 24.99±0.52 |
| 50°C 16 hours                 | 8.15±0.14     | 8.25±0.18   | 13.26±0.66  | 15.92±0.16   | 27.11±0.87             | 34.33±2.98 |
| 60°C 8 hours                  | 8.66±0.58     | 7.34±0.45   | 8.01±0.10   | 10.07±0.27   | 9.32±0.66              | 17.22±0.41 |
| 60°C 16 hours                 | 10.30±0.21    | 10.84±0.55  | 26.63±0.85  | 5.87±0.23    | 10.71±0.09             | 16.96±0.58 |

One of the factors observed in this study was swelling power. Amylopectin, amylose, and temperature have an influence on swelling power. The swelling power value of the annealing modified wikau maombo flour was in the range 8.15 (g/g) – 10.30 (g/g) and 5.82 (g/g) – 15.92 (g/g) for bitter and sweet cassava, respectively. This is due to the liberation of cells that occurs during fermentation [14]. The tendency of lower swelling power values at 50°C with annealing time of 16 hours for bitter cassava and at 60°C with 16 hours of annealing time for sweet cassava was thought to be caused by the stretch between the bonds of starch constituent molecules and the opening of starch constituent granules. Horndok and Noomhorm [15] explain that the rearrangement of starch molecules resulted in a decreased capacity of starch granule development. It was thought that drying after the annealing caused a retrogradation. The water that entered the granule came out caused by the influence of the drying temperature. The water that came out of the granule caused the bonds between the amylose, which were initially stretched, to tighten again and reduced the swelling power [16]. Wang et al. [17] also state that the higher the heating temperature, the more crystalline formed, which increased granule stability and reduced the granule's swelling power. Vieira and Sarmento [18] and Adebowale [19] explain that temperature influences crystalline changes and affects the swelling power of starch.

The water solubility index in the annealing modified wikau maombo flour was in the range of 6.33 (%) – 10.84 (%) and 2.24 (%) – 27.11 (%) for bitter and sweet cassava, respectively. The higher the solubility, the higher the ability of flour to dissolve in water [20]. The higher the temperature and
heating, the solubility of wikau maombo flour tends to be more unstable and the number was decreased. Kurakake (1997) in Sun et al. [21] explains that solubility decreases with increasing heating temperature as the higher the temperature used, the stronger the starch granules due to rearrangement between amylose and amylopectin. Increased interaction between amyllopectin-amyllose or amyllopectin-amylopectin results in a more stable structure that inhibits amylose from leaving the starch granule [22]. This is in line with research conducted by Klein et al. [23] where there was a decrease in solubility from 7.256% to 2.117% in rice and cassava starch as the heating temperature increased.

The viscosity value of the annealing modified maombo flour was in the range of 8.01 (cP) – 26.63 (cP) and 16.96 (cP) – 34.33 (cP) for bitter and sweet cassava, respectively. The viscosity value of modified wikau maombo flour shows that the higher the temperature and the heating time, the lower the viscosity. This was caused by the fact that in the heating process, there was a complex formation between amylose-fat which reduced the swelling power of the granules and improved the stability of the paste during heating. The higher the heating temperature, the faster the flour thickens, and the lower its resistance to flow (viscosity). The decrease in viscosity was caused by the starch granules that had undergone gelatinization and maximum swelling caused by the heating of the starch suspension from the starch granules into the hot water-soluble fraction.

### 3.2. Proximate value of modified wikau maombo flour

Wikau maombo flour in this study was modified using the annealing method. The selected annealing modified wikau maombo flour from bitter cassava was the sample treated at 60°C heating and 16 hours of heating time. Meanwhile, the selected sample made from sweet cassava was the one treated at 50°C heating and 16 hours of heating time. Proximate value analysis was carried out to determine the nutritional content of a food ingredient or food product such as moisture, ash, protein, fat, and carbohydrate contents. Information on the proximate content of a product is essential to determine the energy contained in the product.

### Table 2. The proximate values of the selected annealing modified wikau maombo flour.

| No. | Component               | Bitter cassava | Sweet cassava |
|-----|-------------------------|----------------|---------------|
|     |                         | Control        | Selected      | Control | Selected |
| 1   | Water content (%ww)     | 10.56±0.13     | 10.37±0.21    | 10.16±0.64 | 11.38±0.58 |
| 2   | Ash content (%dw)       | 1.58±0.14      | 1.57±0.26     | 1.38±0.28 | 1.75±0.22 |
| 3   | Fat content (%dw)       | 2.12±0.06      | 1.32±0.09     | 1.48±0.34 | 1.70±0.39 |
| 4   | Protein content (%dw)   | 1.57±0.07      | 1.56±0.02     | 1.62±0.09 | 1.87±0.04 |
| 5   | Crude fibre content (%dw)| 3.05±0.53     | 4.08±0.40     | 3.97±0.49 | 4.27±0.62 |
| 6   | Carbohydrate content (%dw)| 91.67±0.36       | 91.42±0.75     | 90.64±0.12 | 92.35±0.37 |

Water content determines the acceptability, freshness, and durability of food products [24]. Water content in food ingredients affects the quality and storage capacity of the products [25]. The amount of water added to food can affect the rate of damage caused by microbiological, chemical, and enzymatic processes. The low water content of a food ingredient is one of the factors that can make the food last longer. The results of proximate analysis show that the water content of the annealing-modified wikau maombo flour from bitter cassava was 10.37%, which was lower than the control’s water content of 10.56%. Meanwhile, the water content of the annealing-modified wikau maombo flour from sweet cassava was 11.38%, which was higher than the control’s water content of 10.16%. The resulting water content in annealing modified wikau maombo flour from bitter cassava decreased as the higher the temperature, the lower the water content of the modified flour produced. Englyst et al. [26] explain that the hydroxyl groups in starch granules are a major factor that influences the water retention capacity. In starchy materials, this hydroxyl group has a great ability to retain water since water is easy.
to enter the hydroxyl groups. Starch generally can absorb approximately 25% water, but if the starch has changed such as degradation or gelatinization, this percentage will be lower [24].

All starches derived from cereals and tubers contain small amounts of inorganic salts which can either be derived from the material itself or water during processing [27]. The results of the proximate analysis show that the ash contents of the annealing-modified wikau maombo flour from bitter and sweet cassava were 1.57% and 1.75%, respectively. Sudarmadji et al. [28] state that ash is one of the factors that determine the quality of a material. The determination of ash content aims to control the concentration of inorganic salts such as sodium, potassium, carbonate, and phosphate. If the ash content is high, the mineral content is also high. The decrease in ash content can be influenced by the type of material, the method of ashing, heating time, and temperature used [28].

The fat content of the annealing-modified wikau maombo flour from bitter cassava was 1.32% (dry weight), which was lower than the control fat content of 2.12% (dry weight). Meanwhile, the fat content of the annealing-modified wikau maombo flour from sweet cassava was 1.70%, which was higher than the control fat content of 1.48%. In general, this modification increased the fat content of the wikau maombo flour made from sweet cassava. This is caused by the structure of the flour and its ability to hold fat due to the dense amylopectin contained in it. Widyaningtyas and Susanto [29] explain that the increased fat content in food products is caused by trapped fat in the matrix during the roasting process. Meanwhile, Oboh et al. [30] state that the reasons for the high fat content of cassava flour cannot be explained. However, it is suspected that there is a transformation of carbohydrates into fat [31]. Glucose will be converted into Acetyl-CoA compounds first. Then, the Acetyl-CoA will be converted into malonyl-CoA through a series of processes. The Malonyl-CoA that has been formed will be converted back into free fatty acids which will later be stored in the form of triglycerides. In addition, water and fat affect the formation of crude fibre [32]. The crude fibre contents of bitter and cassava were 4.08% and 4.27%, respectively.

Protein contents in annealing-modified wikau maombo flour from bitter and sweet cassava were 1.56% (dry weight) and 1.87% (dry weight), respectively. In general, the protein content in flour products did not differ significantly. The decrease in protein levels caused by the interaction between protein and other food components during the heating process. In addition, protein components that were dissolved during the annealing process also decreased the protein levels [33].

The carbohydrate content of annealing-modified wikau maombo flour from bitter cassava was 91.42%, which was lower than the carbohydrate content of the flour made from sweet cassava (92.35%) but it was higher than the carbohydrate content of the control (87.69%) [34]. The decrease in starch carbohydrate content was due to the breakdown of the starch to simpler components [35].

4. Conclusions
The selected annealing modified wikau maombo flour from bitter cassava was the sample treated at 60°C heating and 16 hours of heating time. Meanwhile, the selected sample made from sweet cassava was the one treated at 50°C heating and 16 hours of heating time. Modification of wikau maombo flour by annealing method can increase the gelatinization temperature and produce starch that is more stable to heat. This result can be used to recommend producing wikau maombo flour with annealing modifications method on the optimum condition to get better physicochemical flour properties.

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