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CHEMICAL PROPERTIES, PARTICLE SHAPE, AND SIZE OF FERMENTED LOCAL WHITE CORN FLOUR OF ANOMAN FS VARIETY

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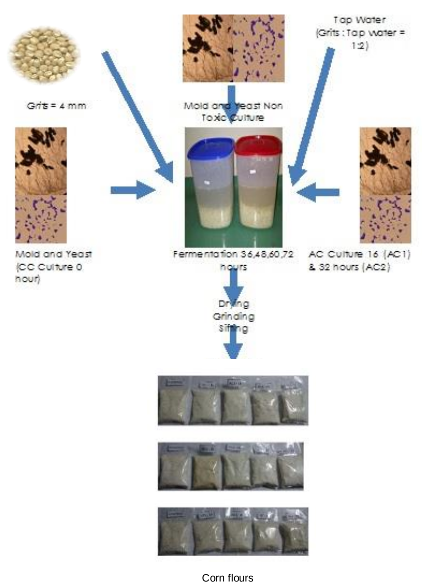
Graphical abstract



Abstract

This research aims to study the chemical characteristics, the shape and size of the particles from local white corn flour fermented with indigenous yeast and mold. The fermentation processes studied are 1) fermentation with the addition of complete yeast and mold at 0 hour of fermentation and addition of amylolitic cultures at 16 hours of fermentation (AC1); 2) fermentation with the addition of complete yeast and mold at 0 hour, amylolitic cultures at 16 hours and 32 hours (AC2); and 3) fermentation with the addition of non-toxic culture at 0 hour, amylolitic cultures at 16 hours and 32 hours (NTS). Observations are conducted at 36, 48, 60, and 72 hours of fermentation. The results show that the addition of AC1, AC2, and NTS starters during fermentation generally lowers the moisture, ash, protein, fat, and amylose and pH value of the corn flour. Lowered chemical contents of local white corn flour due to the fermentation process is beneficial, both in terms of nutritional contents and functional properties. Another advantage is that the flour does not smell sour. All of the flours have the same granule shape as the control has, but addition of the starter AC1, AC2, and NTS makes smaller corn flours than the control.

Keywords: Fermentation, indigenous yeast mold, local white corn, chemical properties, particle shape & size

1.0 INTRODUCTION

Corn is the second important source of carbohydrates after rice in Indonesia. The national maize production during the last three years increased from 18,506,287 tons (2013), to 19,008,426 tons (2014), and 19,612,435 tons (2015) [1]. Various types of local corn have grown in Indonesia and the local white corn varieties are developed as the superior varieties by the Indonesian Ministry of Agriculture. The advantages of the white corn are: containing higher starch than that of the yellow one, having attractive white color, and higher productivity than the yellow corn and more resistant to drought [2].

On the other hand, white corn has a weakness, which is having hard grains. The common way to soften the grains of corn is soaking it before process it further. Soaking process changes the native properties of flour produced [3] because there is a fermentation process during soaking. Various microorganisms are alive and active, and metabolizing the food composition. Ref [4] found 15 isolates were alive when soaking the white corn grits of Anoman 1 and Pulut Harapan varieties. The isolates included lactic acid bacteria, molds, and yeasts. Overall, of the 15 isolates, one yeast and four molds were found amylolytic.

The utilization of 15 isolates for fermentation until 72 hours has caused the chemical composition of flour changed. In general, they have increased the water contain, but have decreased ash, protein, fat, crude fiber, pH value, and amylose contains [5]. In addition, the presence of protein and fat has caused the amylose to form bonds with proteins and fats into amylose-protein and amylose-fat bonding which has increased the gel hardness [6]. The decreased protein, fat and crude fiber contains in corn flour after fermentation process has caused the gel of starch become softer and easier to apply to another processed product. The addition of lactic acid bacteria has caused the pH of flour decrease significantly. It also has made the flour smell sour and less desirable [5]. Besides that, the fermentation process has also changed the physical and functional properties of flour produced [7].

Based on the above information, it is necessary to do further research using a different formulation of the starters to produce white corn flour that is more acceptable. To increase the activity of the amylolytic microorganisms, they are added after 32 hours of fermentation, as well as after 16 hours. In addition, to produce the flour that does not contain potential toxic microbes, one of the formulations does not use the microbes.

2.0 METHODOLOGY

2.1 Corn

Corn type used in this research was local white corn Anoman FS variety that was obtained from the Cereal Crops Research Institute, Maros, Sulawesi, Indonesia. The corn was made into grits for a standardized fermentation process. Kernels of corn were washed with drinking water (corn: water = 1:4 w/v) and drained on a sieve. After that, the kernels were grinded using pin disc mill and sieved to produce grits with a diameter of ≥4 mm. The grits were then washed with drinking water (grits: water = 1:4 w/v) for 30 minutes and then drained and ready for fermentation.

2.2 Microorganisms

Microorganisms used as a starter culture prepared were Penicillium chrysogenum, Penicillium citrinum, Aspergillus niger, Rhizopus stolonifer, Rhizopus oryzae, Fusarium oxysporum, Acremonium strictum, Candida famata, Kodamaea ohmeri, Candida krusei/ incospicua. The microorganisms used were previously isolated and identified from a spontaneous fermentation of corn grits [4].

2.3 Culture Preparation and Enumeration

One loop of each mold was streaked onto fresh Potato Dextrose Agar (PDA) slant and then incubated at 30 °C for five days. After five days, molds were harvested by scrapping, suspended in 10 mL sterile water and appropriately diluted for enumeration using hemacytometer. Yeast culture was prepared as above, but incubation was carried out at 30 °C for two days. Yeast enumeration was also carried out using hemacytometer [7].

2.4 Fermentation with Added Starter Culture

Five-day-old molds and two-day-old yeast in sterile water made up the complete starter culture. For amylolytic starter culture, Penicillium citrinum, Aspergillus niger, Acremonium strictum, and Candida famata were used. Each microorganism was inoculated aseptically into a container (15 L) containing maize grits and drinking water (1:2 w/v), in which the initial number of each microorganism was 10^6 CFU/mL.

The fermentation studied included three treatments of fermentation with added starter, which are: (1) AC1, a complete starter culture containing 10 microbes (mold and yeast) previously isolated from the spontaneous fermentation added at the beginning of fermentation (0 hours), and additional inoculation of amylolytic starter culture at 16 hours of fermentation; (2) AC2, similar to AC1 with added amylolytic starter culture at 16 and 32 hours of fermentation; (3) NTS, inoculated non-toxic microbes (*Rhizopus stolonifer, Rhizopus oryzae, Acremonium strictum, Candida famata*, *Kodamaea ohmeri,* and *Candida krusei/incospicua*) at the beginning of fermentation (0 hours) and additional inoculation of amylolytic starter culture at 16 and 32 hours of fermentation. Observations were done on flour made from corn grits after 0, 36, 48, 60, and 72 hours fermentation (modification [8]).

The research steps included: first, the corn was made into grits. After that the grits was soaked in water and added with mold and yeast according to the treatment (AC1, AC2, NTS). Then, the observation was done according to the fermentation time. Furthermore, the grits was dried and made into flour. Last, the corn flour was analyzed.

2.5 White Corn flour Analysis

The chemical properties of white corn being analysed included water, ash, protein, fat, and carbohydrate contain [9]; amylose contain (Takeda *et al.* in [10]); and pH value [11]. In addition, the particles shape and size were analyzed by an electron microscope.

2.6 Statistical Analysis

The result data was calculated using average value and standard deviation.

3.0 RESULTS AND DISCUSSION

The properties of white corn flour fermented with three types of starters and 4 hours of fermentation included moisture, ash, fat, protein and carbohydrates. The test results are presented in Table 1. Besides that, the amylose content and pH value were also tested.

3.1 Water Content

Generally, in the fermented corn flour with three types of culture starters and 4 hours of fermentation time, the water content was seen to decrease from 9.15 to 12.72 percent, indicating lower than the control corn flour (13.22%). The water content relatively decreased as the fermentation period was longer, except in the treatment of NTS, in which it was relatively stable.

During fermentation, enzyme hydrolyzed the components of flour. Amylase enzyme attacked the bond of a-1,4-D-glycosidic starch [6] so that the granular structure became weak and formed pores in the starch granules that facilitated the absorption of water into the granule. The longer the fermentation, the water absorption was higher, so when the grits were dried, the evaporated water level was higher. It caused the water content of the flour lower. Lower water level in flour would be advantageous because it would be difficult for microorganisms to live and it may extend the shelf life. The overall moisture content of the corn flour produced still met the criteria of SNI 01-3727 1995, stating the maximum water content of 15% [12].

3.2 Ash Content

The ash content of corn flour fermented with three types of starters and 4 hours of fermentation time generally ranged from 0.13 to 0.43 percent, indicating lower than the control corn flour (1.39%). The ash content of the three types of corn flour fermented generally decreased as the fermentation time increased. Ref [13] reported that the white corn Srikandi variety contained minerals Ca 41 mg / 100 g, Mg 212 mg / 100 g, P 123 mg / 100 g and K 276 mg / 100 g. Generally, maize contained minerals P, K, Ca, Mg, Na, Fe, Cu, Mn, and Zn [14]. The ash content decreased because the mineral had a high degree of solubility in water and low affinity, as it was widely available as a free ion [15] so these minerals were dissolved during soaking. Ash decreased when the grits were soaking. The decrease of ash in 36-72-hour fermentation was relatively smaller than that of 0-36-hour fermentation. This is presumably due to the mineral that forms a complex shape. Phosphorus in corn partly presented as a phytic acid potassium-magnesium salts, as an ester form of hexa phosphate inositol. Minerals in the complex forms did not dissolve when soaking [15] so the decrease in ash content after 36 hours was relatively low

Table 1 Proximate analysis test of corn flour with 3 types of culture and various fermentation time

Types	Fermentation time (h)	Moisture Contents (%)	Ash Contents (%)	Lipid Contents (%)	Protein Contents (%)	Carbohydrate Contents (%)
Control		13,22±0,22	1,39±0,01	5,02±0,07	5,27±0,02	75,10±0,14
AC1	36	11,99±0,06	0,20±0,02	2,43±0,05	4,66±0,07	80,71±0,07
	48	9,63±0,13	0,26±0,01	3,90±0,03	4,82±0,14	80,78±0,25
	60	9,66±0,11	0,18±0,00	3,38±0,10	5,43±0,19	81,35±0,18
	72	9,15±0,02	0,13±0,01	1,79±0,03	4,92±0,03	84,01±0,01
AC2	36	9,63±0,07	0,26±0,01	2,84±0,11	2,92±0,08	84,34±0,28
	48	9,82±0,07	0,21±0,01	2,50±0,11	2,96±0,08	84,51±0,28
	60	10,30±0,50	0,20±0,01	2,63±0,39	4,38±0,17	82,48±1,08
	72	9,72±0,01	0,15±0,00	2,50±0,06	4,33±0,24	83,29±0,19
NTS	36	12,46±0,02	0,41±0,04	1,95±0,11	5,00±0,11	80,18±0,20
	48	9,62±0,36	0,27±0,01	3,35±0,08	5,10±0,12	81,66±0,16
	60	11,99±0,23	0,28±0,01	2,34±0,07	5,05±0,20	80,34±0,49
	72	12,72±0,39	0,43±0,04	4,07±0,07	5,50±0,03	77,28±0,33

3.3 Fat Content

The fat content of maize flour fermented with three types of starters and 4 hours of fermentation time generally ranged from 1.79 to 4.07 percent, indicating lower than the control corn flour (5.02%). In general, fat content decreased as the fermentation time was longer.

The decreasing of fat during soaking was a result of lipase activities originating from R. oryzae [16], Kodamae ohmeri [17], Candida famata [18], and Candida krusei [19] as starters. The decrease of fat content in AC1 corn flour was higher than that of AC2. The AC2 starter contained amylolytic microorganisms more than AC1 did. So, the activity of the microorganisms was higher, and it made the competition in AC2 fermentation was also higher, so it caused the microorganisms activities to decline, likewise in the NTS flour.

Generally, the decreased levels of fat are beneficial for human because it becomes simpler component (fatty acids), making it easier to digest. Another benefit from the decreased fat is it reduces the bond of amylose-fat that will soften the resulting gel.

3.4 Protein Content

The protein content of corn flour fermented with three types of starters and 4 hours of fermentation time generally ranged from 2.92 to 5.50 percent, indicating lower than the control corn flour (5.27%). In general, protein content decreased as the fermentation time was longer.

The decrease of protein during soaking was caused by proteolytic activities originating from microbes as the starter. According to [16], Rhizopus oryzae produced the enzyme cellulase, hemicellulase, pectinase, tannase, phytase, lipase, and protease. Besides, Candida famata produced enzymes glucoamylase [20], lipase and protease [18]. In addition to microbial activity, a decrease in protein content was caused by the dissolution of albumin during soaking for 72 hours. The pH value affected the protein solubility in water. At pH outside the isoelectric point, the protein had a high solubility in water. The isoelectric point of proteins, in general, is at pH of 4.5 - 4.8 [21]. During immersion for 0-72 hours the pH of the soaking liquid was above the isoelectric point. So, it presumably affects the decrease in protein.

Similar to the fat content, the decreased level of the protein is beneficial for human because it becomes simpler components (amino acids), making it easier to digest. Another benefit from the decreased protein is it reduces the bond of amylose-protein that will soften the resulting gel.

3.5 Carbohydrate Content

The carbohydrate content of corn flour fermented with three types of starters and 4 hours of fermentation time generally ranged from 77.28 to 84.51 percent, indicating higher than the control corn flour (75.10%). The increase of carbohydrates level was associated with the decrease of water, ash, protein and fat level as a result of the activities of microorganisms.

3.6 Amylose Content

The amylose content of corn flour fermented with three types of starters and 4 hours of fermentation time generally ranged from 24.07 to 27.83 percent, indicating higher than the control corn flour (23.15%).

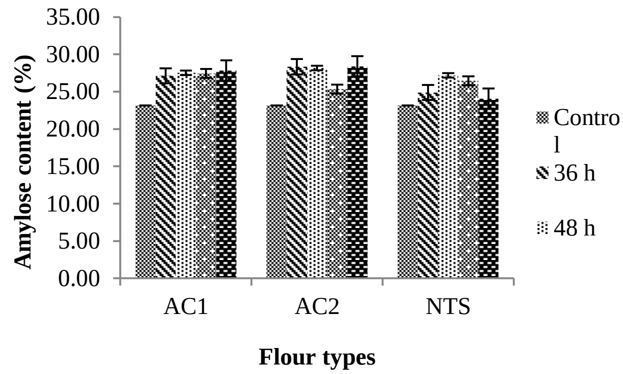


Figure 1 Amylose contents in corn flour with 3 type cultures and various fermentation times

Figure 1 shows that the AC1 corn flour contained amylose that tended to increase in line with longer fermentation time, as well as the AC2 corn flour. Meanwhile, the amylose content in the NTS corn flour tended to increase up to 48 hours of fermentation and then decrease.

Amylose is one fraction from starch that has a linear chain. The increase of amylose content is related to the activities of the amylolytic microorganisms added. According to [20], Candida famata has produced glucoamylase that could break the the outer branch chain of amylopectin [22]. This led to an increase of straight chain, which increased the amylose content. The amylose content of the AC2 corn flour was slightly higher than that of AC1. It was suspectedly related to the addition of amylolytic microbial more than that in the AC2 corn flour, in which the microorganism showed more activities in cutting the outer branch chain of amylopectin. Meanwhile, the NTS corn flour contained the lowest amylose. It was presumably related to the fewer number of microbes in use. The NTS starter did not use Penicillium chrysogenum, Penicillium citrinum, Aspergillus niger and Fusarium oxysporum, as they were suspected as potential toxic. Ref [4] showed that Penicillium citrinum, Aspergillus niger has had amylolytic activity. Aspergillus niger has had not only amylolytic activity, but also ectinolytic activity [23] and [24] it has been reported that Fusarium oxysporum has had cellulolytic and xylanolytic activities.

The amylose content in the NTS corn flour decreased after 48 hours of fermentation. It was presumably associated with the activity of amylase enzyme, which attacks the bond of α-1,4-D-glycosidic in starch granules [25]. Glucose contains many hydroxyl groups so that amylose is hydrophilic and soluble in water during immersion up to 72 hours.

3.7 pH Value

The pH value of corn flour fermented with three types of starters and 4 hours of fermentation time ranged from 5.53 to 6.23, while the pH value of the control corn flour was higher (pH 6.86). Generally, the pH value of corn flour decreased, in line with the increase of fermentation time, as seen in Figure 2.

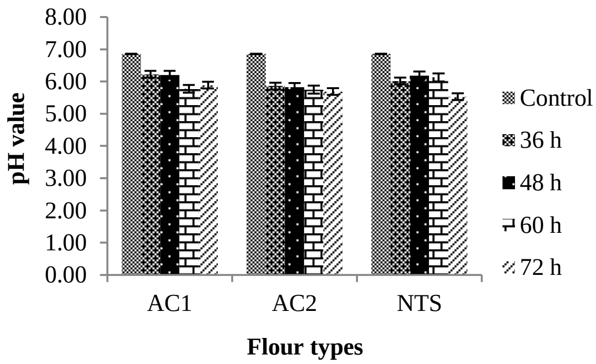


Figure 2 pH value in corn flour with 3 type cultures and various fermentation times

The pH value of corn flour decreased from 0-36 hours of fermentation, and thereafter it was relatively stable (Figure 2). The decrease in the pH value was due to the acids produced by lactic acid bacteria (LAB), mainly lactic acid. Although LAB was not added in this study, [8] showed that the indigenous BAL could live well during the fermentation process. Ref [26] reports that yeast has had a high tolerance to lactic acid, as Candida krusei found on the fermentation of corn for the Ogi production might stimulate the growth of Lactobacillus plantarum so that the pH value remained low. Because LAB was not used as a starter, the pH value of the AC1, AC2 and NTS corn flour was relatively higher than the corn flour observed by Rahmawati [5]. The addition of LAB in fermentation process produced a sour flavor that is less favored [5].

3.8 Particle Shape and Size

The shape and size of the granules of white corn flour without fermentation are presented in Figure 3. Figure 3 shows that the white corn flour granules have spherical polygonal shape (polygons) with varying sizes, ranging between 14.18 µm - 21.84 µm. The shape and size of the flour granules are generally similar to the shape and size of other corn granules. The shape and size of the corn starch granules AC1, AC2, and NTS are presented in Figure 4, 5, 6.

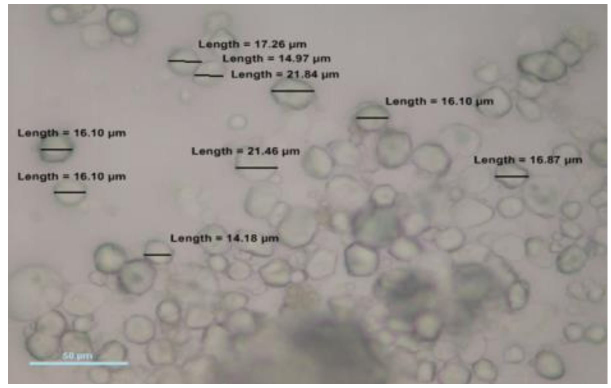


Figure 3 The shape and size of the granules of white corn flour without fermentation (control) using a light microscope at a magnification of 40x

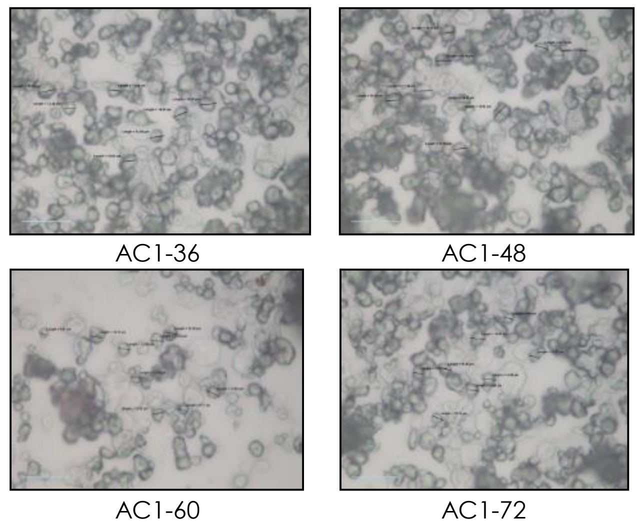


Figure 4 The shape and size of the corn starch granules with AC1 starter at different fermentation times using a light microscope at a magnification of 40x

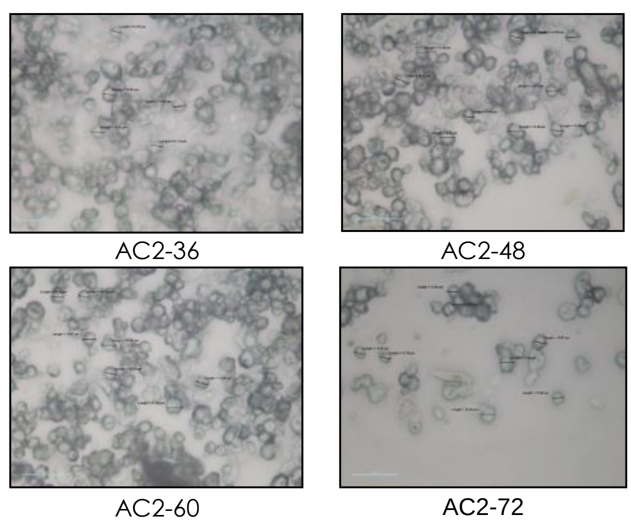


Figure 5 The shape and size of the corn starch granules with AC2 starter at different fermentation times using a light microscope at a magnification of 40x

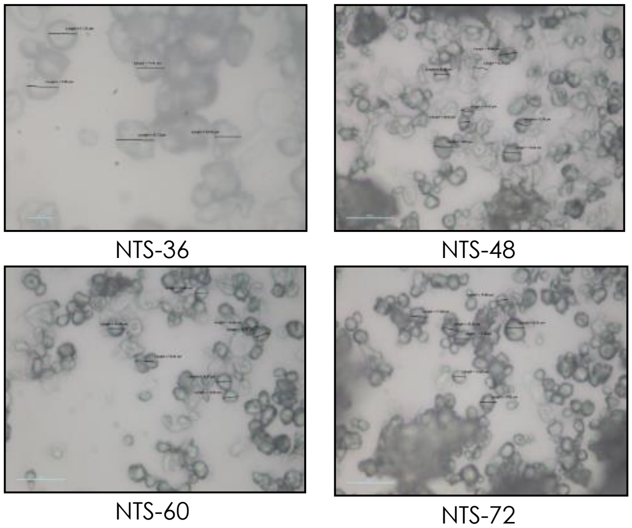


Figure 6 The shape and size of the corn starch granules with NTS starter at different fermentation times using a light microscope at a magnification of 40x

The AC1, AC2, and NTS corn flour granules generally had the same shape with the control, but they were smaller (Figure 4, 5, 6). The AC1 flour had an average size between 11.21 and 14.68 µm, whereas the average granule size of the AC2 ranged from 13.57 to 15.52 µm, and that of NTS ranged from 12.85 to 16.18 µm. The granules size of the AC1 flour tended to be smaller, as the fermentation time got longer. The granule size of the AC2 flour was relatively stable despite longer fermentation time, while the size of the NTS granules tended to be bigger in line with the longer fermentation time. In general, the AC1 granule had the smallest size, followed by the AC2 and NTS flour (Figure 7).

The flour granules became smaller presumably because of the activity of molds and yeasts added. The Rhizopus oryzae produced the cellulase, hemicellulase, pectinase, tannase, phytase, lipase and protease enzymes [16]. Meanwhile, Rhizopus stolonifer produced cellulase enzymes [27]. Aspergillus niger had not only amylolytic activity, but also pectinolytic activity [23] and Fusarium oxysporum had cellulolytic and xylanolytic activities [24]. Not only having mold activity, yeasts used as starter cultures had also been reported to produce various enzymes; for example, Kodamae ohmeri produced the phytase enzyme in grains [28] and lipase enzyme [17]; Candida famata produced glucoamylase [20] and lipase and protease enzymes [18]; while Candida krusei had lipolytic, esterase and amylolytic activities, which contributed to flavor of food products [19]. The activities of the enzymes produced by molds and yeasts were suspected to cause the change of granules size. It cut the complex compounds of the corn starch granules into simpler compounds, causing granule size become smaller than that of the control.

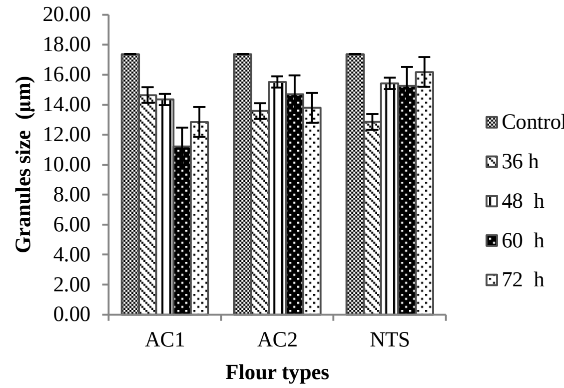


Figure 7 The average size of the granules of the AC1, AC2, and NTS corn flour in a variety of fermentation time

4.0 CONCLUSION

The addition of the AC1, AC2, and NTS as a starter during fermentation generally lowered the chemical content of the corn flour (moisture, ash, protein, fat, amylose and pH value). In general, the decline in the chemical contents of local white corn flour due to the fermentation process was beneficial, both in terms of nutritional content and functional properties. Another advantage of this flour was that it did not smell sour because during the fermentation, lactic acid bacteria were not added. All of the flour had the same shape of granule as the control had, which was spherical polygonal shape (polygons) with varying sizes, ranging between 14.18 µm - 21.84 µm, but the addition of the starter made the granules of AC1, AC2, and NTS corn flours smaller than that of the control.

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