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The effect of fermentation period on physicochemical properties of *Lactobacillus casei* fermented sweet corn meal (*Zea may saccharate* Sturt)

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Abstract

The purpose of this research was to study the effect of fermentation on the chemical properties of fermented sweet corn meal using *Lactobacillus casei*. Complete Randomized Design (CRD) was used in this research. Sweet corn meal was fermented with *L. casei* at different fermentation periods, i.e. 24, 48 and 72 hrs. Physicochemical analysis such as moisture content, starch content, amylose content, size of starch granules, crude fibre content and total reducing sugar content was performed. The results showed that the 24-hour fermented sweet corn meal had the lowest water content (5.15%) of all the various fermentation period. The 48-hour fermented sweet corn meal had the lowest amylose content (0.43%) and the lowest crude fiber content (2.32%). Fermented flour would make a significant improvement to the quality of the flour and its products such as bread.

1. Introduction

Corn (*Zea mays* L) are grain crops, and seasonal crops with a life cycle of 80-150 days. In Indonesia, Gorontalo is the province with the best corn commodity and, it is the largest exporter to countries namely Malaysia, Philippines and South Korea. Approximately 91,500 tonnes of corn is produced per year from Gorontalo. In 2014, corn production was 719,787 tonnes with dried corn kernels production increased 7.58% compared in 2013. However, in 2015, corn production reduced to 643, 512 tonnes. (Gorontalo Central Bureau of Statistics, 2015).

Sweet corn (*Zea mays saccharate* Strut) is one of the agricultural commodities favoured by Gorontalo farmers due to shorter harvest age of 70 days compared to the usual corn. Processed into flour (Irawan *et al.,* 2017), sweet corn flour has great characteristics that greatly affects the quality of food products. It can be fermented to obtain the desired flour (Aini *et al.,* 2016) with significant physicochemical properties and functional characteristics of cornstarch, enzymatically. Fermented sweet corn flour was reported to have a finer texture than the original corn flour and, it has different gelatinizing properties. The addition of microbes such as lactic acid bacteria during the fermentation process can improve the quality of flour and bread development in bread production (Gerez *et al*., 2006). In addition, different fermentation period also produces different physicochemical properties which will be investigated in the study. The objective of this research is to evaluate the physicochemical properties of different fermented periods of sweet corn flour using *L. casei.*

2. Materials and methods

The processing of fermented sweet corn flour was conducted in the laboratory of Livestock Production Technique Faculty of Livestock, Sam Ratulangi University of Manado from March to April 2018. Physicochemical analysis of fermented sweet corn flour is conducted at Integrated Laboratory of Sam Ratulangi University of Manado.

2.1. Fermented sweet corn meal production

The production of fermented sweet corn meal is shown in Figure 1. Sweet corn, harvested at the 90th day, was dried in the oven for 48 hrs at 60°C until the water content reached ±17%. Then, the sweet corn kernels were separated from the cobs. The sweet corn kernels were weighed and milled using a corn grain mill to produce sweet corn meal. Sweet corn meal was added with *L. casei* at a ratio of 3 g/kg of sweet corn meal. Aquadest and coconut water were added next at a ratio of

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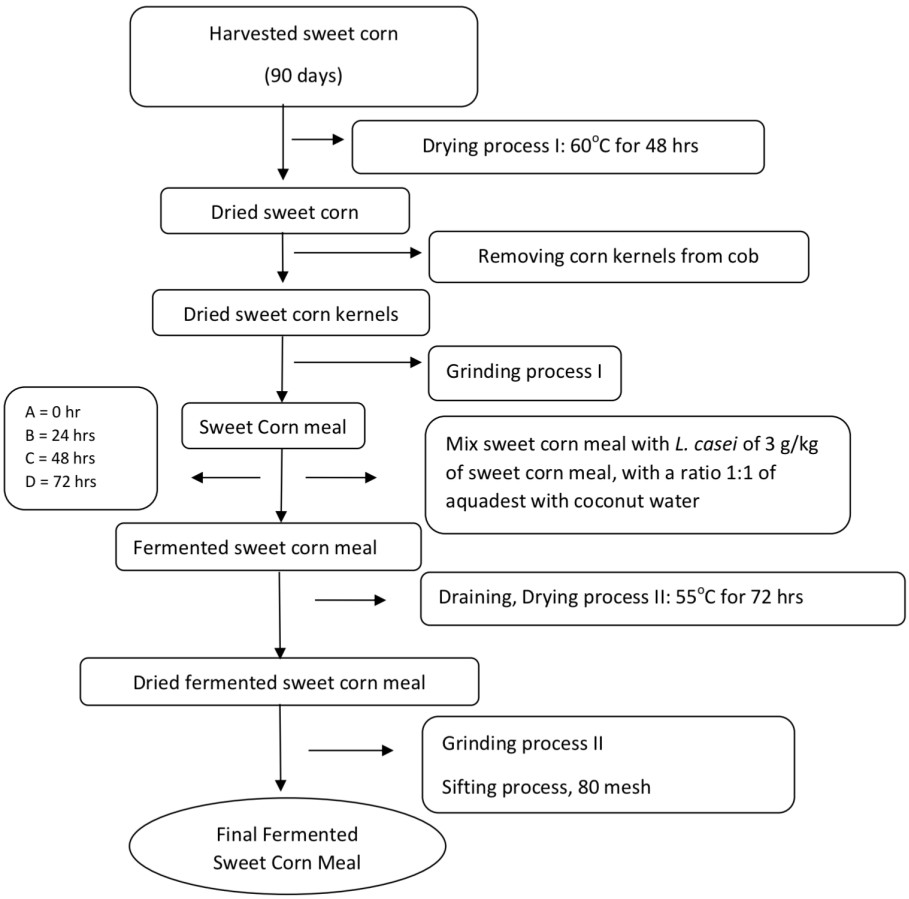

Figure 1. Production of fermented sweet corn meal

1:1 and mixed well. The mixture was separated into three (3) different containers equally and ferment accordingly; 24, 48 and 72 hrs. For control, non-fermented sweet corn meal was used. After the fermentation, the sweet corn meal was re-dried at 55°C for 72 hrs. The dried fermented sweet corn meal was ground and sieved through an 80-mesh sieve to produce a finer flour. The final weight of the fermented sweet corn meal was recorded to calculate the yield.

2.2 Completely Randomized Design (CRD)

The experimental design used was the Completely Randomized Design (CRD). There were four treatments and each treatment with three replicates. The treatments carried out as follows:

A = Sweet corn meal with no addition of *L. casei* and no fermentation.
B = Sweet corn meal fermented for 24 hrs
C = Sweet corn meal fermented for 48 hrs
D= Sweet corn meal fermented for 72 hrs

2.3. Physicochemical analysis

2.3.1 Moisture content

The moisture content analysis was conducted using the oven-drying method (AOAC, 1995). A total of 3 g of fermented sweet corn meal was weighed into a cup and placed into the oven with a temperature of 100°C to be dried until a constant weight was obtained (approximately 6 hrs or more). The moisture content was calculated using Equation (1):

$$\text{moisture content (\%)} = \frac{(\text{initial weight (g)} - \text{final weight (g)})}{\text{initial weight (g)}} \text{ x } 100\% \quad (1)$$

2.3.2 Starch content

Fermented sweet corn meal was weighed 2 to 5 g in a 250 mL glass beaker and added with 50 mL of distilled water. The mixture was stirred for an hour and then filtered and washed with distilled water to a filtrate volume of 250 mL. This filtrate may contain dissolved carbohydrate that was removed. For materials containing fat, the residue on the filter paper was washed five (5) times with 10 mL of ether. The ether was evaporated from the residue and then washed with 150 mL of 10% alcohol to dissolve the carbohydrates. The residue from the filter paper was transferred quantitatively into a 200 mL Erlenmeyer flask by washing with distilled water and then added with 20 mL of HCl±25% (specific gravity 1.125). The Erlenmeyer flask was covered with cooling back and heated in a boiling water bath for 2.5 hrs. It is left to cool and subsequently neutralized with 45% NaOH solution, diluted to a volume of 500 ml, then strained. The glucose obtained from the filtrate was expressed as the sugar content and multiplied by 0.9 to calculate the weight of the starch.

2.5 Amylose content

The amylose content was determined following the IRRI method (AOAC, 1995).

2.5.1 Standard curve

Pure amylose was weighed 40 mg into a test tube and added with 1 mL of 95% ethanol and 9 mL of 1 N NaOH. The mixture was boiled for 10 mins until the gel forms. The formed gel was transferred to a 100 mL measuring flask and topped up with distilled water. The solution was pipetted 1 mL, 2 mL, 3 mL, 4 mL and 5 mL into five different 100 mL measuring flask. Each of the measuring flask was added with 0.1 N acetic with the following volume 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1 mL to 1 mL, 2 mL, 3 mL, 4 mL and 5 mL respectively. Then, 2 mL of iodine solution was added into the measuring flask. The intensity of the blue colour formed was measured using a spectrophotometer at a wavelength of 625 nm. The graph of the concentration against absorbance was plotted.

2.5.2 Preparation of sample

Fermented sweet corn meal was weighed 100 mg and added with 1 ml of 95% ethanol and 9 mL of 1 N NaOH. The mixture was boiled for 10 mins until the gel forms. The formed gel was transferred to a 100 mL measuring flask and topped up with distilled water. The solution was pipetted 5 mL into a 100 mL measuring flask and added with 1 mL 0.1 N acetic Then, 2 mL of iodine solution was added into the measuring flask. The intensity of the blue colour formed was measured using a spectrophotometer at a wavelength of 625 nm. The amylose content was determined from the plotted graph of concentration against absorbance.

2.6 Starch granules

The forms of starch granules were viewed using a Scanning Electron Microscope (SEM). The sample was weighed 5 g and added with distilled water in the ratio 1: 4. The solution was heated in a water bath and stirred until the specific temperature following: 64°C for sample A, 59°C for sample B, 65°C for sample C, and 64°C for sample D. The gelatinized structures were observed with SEM at a magnification 2000 times.

2.7 Crude fiber

Crude fiber content was determined following procedures of Apriyantono *et al*. (1989). A total of 2 g of fermented sweet corn meal was weighed and extracted with petroleum ether solvent using Soxhlet. The fat-free sample was transferred quantitatively into a 600 mL Erlenmeyer flask and added with 200 mL of boiling H_2SO_4. The Erlenmeyer flask was then placed into the cooling back in a closed condition and heated to boiling for 30 mins and occasionally shaken. Once completed, the solution was filtered, and the residues were washed with boiling water. The washing was considered done when the filtrate was tested with litmus paper as not acidic. The residue was transferred into another Erlenmeyer flask and washed using 200 mL of boiled NaOH to completely ensure all residue from the filter paper was transferred. The solution was boiled again for 30 mins with the cooling back, occasionally shaken. Once the boiling process completed, the solution was filtered and washed with 10% K_2SO_4 and then with boiling water followed with 95% alcohol. The filter paper was dried in an oven at 105°C to a constant weight (approximately 1-2 hrs) and weighed after being cooled in a desiccator. The crude fiber content calculated using Equation (2):

$$\text{Crude fiber content (\%)} = \frac{W2-W1}{W} \times 100\% \quad (2)$$

Where W = weight of the sample analyzed (g); W1 = weight of filter paper (g); and W2 = weight of residue and dried filter paper (g).

2.8 Total reducing sugar content

The reduction sugar analysis aimed to determine the amount of reducing sugar contained in fermented sweet corn meal following the procedures of Luff-Schoorl (Sudarmaji *et al*., 1997)

2.8.1 Sample preparation

Fermented sweet corn flour was weighed 5 g into a 250 mL measuring flask and topped up with distilled water. The mixture was mixed well and filtered into a new 250 mL measuring flask. While shaking the solution, 10 mL of half wet Pb acetate solution was added. The adequacy of Pb acetate solution added was tested by dripping 10% Na_2HPO_4. The white precipitation from the reaction indicated sufficient addition of Pb acetate solution. About 15 mL of 10% Na_2HPO_4 to precipitate excess Pb acetate until no precipitation. The solution was topped up with distilled water, mixed well and left to stand for 30 mins. The solution was then filtered.

2.8.2 Determination of sugar content before inversion

The filtrate was pipetted 10 mL into a 500 mL Erlenmeyer flask and added with 15 mL of distilled water, boiling stones and 25 mL of Luff-Schoorl solution. The mixture was heated for 2 mins until boiling and simmered for 10 mins in a water bath. Immediately, the flask was removed and cooled in ice bath. After cooling, 10-15 ml of 30% KI solution and 25 ml of 25%

H_2SO_4 was added. It was titrated immediately with 0.1N $Na_2S_2O_3$ solution and 0.5% starch solution as an indicator. Blank sample was prepared by replacing the sample with distilled water following the similar procedure. The volume of $Na_2S_2O_3$ solution used to titrate sample subtracted from the volume used to titrate blank sample determines the sugar content before inversion obtained from the Luff-Schoorl table. The sugar content before inversion (%) was calculated using Equation (3):

$$\% = \frac{\text{mg} \times \text{FP} \times 100\%}{\text{sample (mg)}} \quad (3)$$

2.8.3 Determination of sugar content after inversion

The filtrate was pipetted 50 mL into a 100 mL measuring flask and added with 5 mL of 25% HCl. The measuring flask was inserted into the water bath heated to 60-70^{0}C and allowed to stand for 10 mins (to invert sugar). The measuring flask was then cooled and added with 30% NaOH until pH reached 7 and topped up with distilled water. The mixture was pipetted 10 mL and added with 15 mL of distilled water, boiling stones and 25 mL of Luff-Schoorl solution in a 500 mL Erlenmeyer flask with cover. The flask was heated about 2 mins to boil and continue to boil in the water bath for 10 mins. The flask was removed and cooled immediately with ice and added with 10-15 mL of 30% KI solution and 25 mL of 25% H_2SO_4 solution. It was titrated immediately with 0.1N $Na_2S_2O_3$ solution and 0.5% starch solution as an indicator. Blank sample was prepared by replacing the sample with distilled water following the similar procedure.

The sugar levels were calculated using Equations (4) and (5):

Sucrose level = (% sugar after inversion of sugar before inversion) x 0.95 (4)

Sugar levels were calculated = % sugar after inverse x 0.95. (5)

2.9 Data analysis

Data of research results were analysed with analysis of variance (ANOVA) and Least Significance Different (LSD) test to determine significant difference.

3. Results and discussion

3.1 Physicochemical properties of fermented sweet corn meal

The results of this research are presented in Table 1.

3.2 Moisture content

From Table 1, the moisture content of the fermented sweet corn meal ($p<0.05$) varied for the different fermentation time. The moisture content of the fermented sweet corn meal ranged from 5.15-6.91%. The 24-hour fermented sweet corn meal had the lowest moisture content (5.15%), followed by 48-hour fermented sweet corn meal (5.86%) and the 72-hour fermented sweet corn meal has the highest moisture content (6.91%). On the other hand, the non-fermented sweet corn meal recorded 8.08% of moisture content. The 24-hour fermented sweet corn meal and 48-hour fermented sweet corn meal were significantly different ($p<0.05$) from the 72-hour fermented sweet corn meal and non-fermented sweet corn meal. This could be due to the drying process of unfermented sweet corn meal which was performed for 48 hrs compared to fermented sweet corn meal, which was performed longer. Drying time affects the moisture content, this is because long drying causes the amount of water that is evaporated more so that the water content in the flour is reduced (Lubis, 2008).

The low moisture content of food products also indicates a better stability and longer shelf life. In addition, water is required by bacteria, fungi and yeast to multiply so that there will be changes in food during the early stages of fermentation. Water is also needed for ongoing biochemical reactions that occur in food, such as enzyme-catalyst reactions (Winarno, 2004). Therefore, the 24-hour fermented with the lowest moisture content has a longer shelf life.

The requirement of moisture content of a quality flour based on SNI 01-3727-1995 is maximum 10%. It

Table 1. Average results of physicochemical properties of fermented sweet corn meal

Observation parameter	Treatment				5% LSD
	A	B	C	D	
Moisture content (%)	8.08^{b}	5.15^{a}	5.86^{a}	6.91^{b}	1.22
Starch content (%)	2.09^{a}	1.14^{a}	2.04^{a}	0.94^{a}	-
Amylose content (%)	0.82^{bc}	0.62^{ab}	0.43^{a}	0.88^{cd}	0.21
Crude fiber content (%)	3.97^{c}	3.65^{b}	2.32^{a}	3.93^{c}	0.26
Total reducing sugar (%)	28.50^{b}	19.00 ^{a}	19.00^{a}	22.40^{a}	4.75

A = non-fermented sweet corn meal; B = 24-hour fermented sweet corn meal; C = 48-hour fermented sweet corn meal; and D = 72-hour fermented sweet corn meal. Values with different alphabet superscript are significantly different ($p<0.05$)

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can be concluded that the moisture content of the fermented sweet corn flour satisfied the requirements of a quality flour based on SNI 01-3727-1995.

3.2 Starch content

There was no significant difference ($p>0.05$) in the starch content, expressed in percentage, of the fermented sweet corn meals. Non-fermented sweet corn meal had 2.09% of starch content. The 72-hour fermented sweet corn meal recorded the lowest starch content of 0.94% while the 48-hour fermented sweet corn meal produced the highest starch content of 2.04%. The 24-hour fermented sweet corn meal contained 1.14% of starch content.

Fermented corn flour produces a relatively low starch content compared to corn flour without fermentation. The starch content in corn flour is 68.20% (Juniwati, 2003) while sweet corn flour had 64.09% of starch content (Irawan *et al.,* 2017). Herawati and Widowati (2009) revealed that the high content of starch in foodstuffs is influenced by plants, soil fertility, climate, and storage time.

The allegedly low starch content in fermented sweet corn meal was affected by the length of storage and the presence of bacteria. To further preserve the starch content, the sweet corn meal that has passed through the process of milling stage I is not immediately done fermentation process.

Starch is a type of polysaccharide widely used in food processing. The starch polymer consists of amylose and amylopectin, although both are made up of the same molecule of glucopyranose, the characteristics of the two polymers are different. The function of starch in the food is to form a unique texture, flavour, and aroma, in the use of some types of starch can be modified to produce food products according to the desired product by considering the characteristics and content of both starch polymers (Harijono *et al.*, 2016). However, foods with high starch content easily absorb water due to the presence of reactive amylopectin (Herawati and Widowati, 2009)

3.3 Amylose content

The amylose content of each fermented sweet corn meal at different fermentation period was significantly different ($p<0.05$). The amylose content for 24-hour, 48-hour, and 72-hour fermented sweet corn meal were 0.62%, 0.43% and 0.88% respectively. The amylose content in non-fermented sweet corn meal was 0.82%. A further test of BNT showed significant difference ($p <0.05$) in each treatment of fermented corn flour.

3.4 Starch granules

Figure 2 illustrates the starch granules of non-fermented sweet corn meal, 24-hour fermented sweet corn meal, 48-hour fermented sweet corn meal, and 72-hour fermented sweet corn meal viewed under SEM.

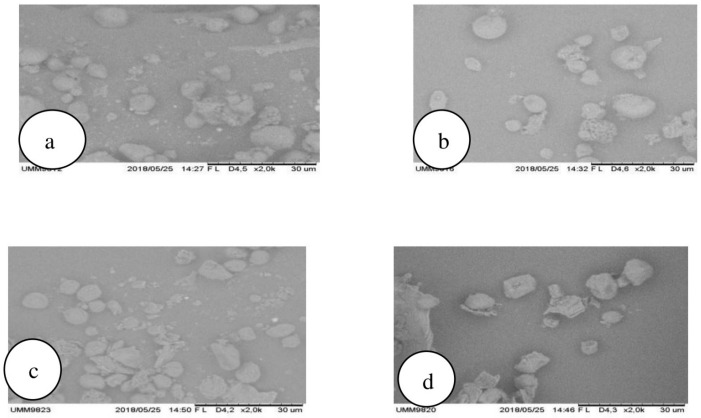

Figure 2. Starch granules. a: non-fermented sweet corn meal; b: 24-hour fermented sweet corn meal; c: 48-hour fermented sweet corn meal; and d: 72-hour fermented sweet corn meal.

It was observed from Figure 2 that the starch granules had no difference. This is due to the sweet corn starch granules for each treatment produced non-flat rounded granules during the gelatinization process. Non-flat rounded granules indicate that amylose could have been released.

3.5 Crude fiber

The crude fiber content of the fermented sweet corn meal was expressed in percentage. The results in Table 1 showed significant difference ($p<0.05$) for each treatment of the sweet corn meal. The 72-hour fermented sweet corn meal had the highest crude fiber content of 3.93% compared to 24-hour and 48-hour fermented sweet corn meal.

The result of crude fiber obtained in the fermented corn flour did not meet the requirements specified in SNI for corn flour, which is the maximum of 1.5%. In this study, the lowest crude fiber was 2.32% which was from the 48-hour fermented sweet corn meal.

Fiber is an integral part of the plant's natural structure composed of several components such as lignin, cellulose, hemicellulose and pectic substances, gums, waxes and undigested oligosaccharides, hemicellulose and pectic substances capable of binding water and inflate are called soluble fibers. Corn fiber consists of 70% hemicellulose and 23% cellulose and 0.1% lignin (Boyer, 2003). The high-fiber corn portion is found on the skin (pericarp) and tip cap (Watson, 2003). In the processing of sweet corn flour, allegedly part of the skin was carried on the grinding process, causing the different fiber content in each treatment. Irawan *et al*. (2017) reported that the fiber content of sweet corn

starch ranged from 11.27 to 23.71%. However, the crude fiber content in the fermented sweet corn flour (2.32-3.97%) may be due to the fermentation process of the sweet corn meal as the skin part dissolves in the fermentation process.

3.6 Sugar

The total sugar on the fermented sweet corn flour was significantly different ($p<0.05$) from non-fermented sweet corn meal. The 24-hour and 48-hour fermented sweet corn meal had 19.00% total sugar content while the 72-hour fermented sweet corn had 22.40% total sugar content. Non-fermented sweet corn meal had the highest total sugar content of 28.50%.

The increase of the total sugar content when the sweet corn meal was fermented for 72 hrs could be due to the *L. casei* ability to degrade the cell wall of maize into simple sugars and amino acids during fermentation (Aini *et al*., 2016).

4. Conclusion

Fermented sweet corn meal with *L. casei* had slightly different physicochemical properties compared to non-fermented sweet corn meal. Fermented sweet corn meal had lower moisture content, starch content, crude fiber and total sugar content compared to non-fermented sweet corn meal. The starch granules of both fermented and non-fermented sweet corn meal were similar. Overall, 48-hour fermented sweet corn meal produced the best quality flour for bread production.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgments

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