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To cite this article: Andi Sukainah *et al* 2019 *J. Phys.: Conf. Ser.* **1244** 012025

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# Changes in *Lactobacillus fabifermentans* Population and Biochemical Changes during The Process of Controlled Corn Flour Fermentation and The Rheological Properties of Corn Flour Produced

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**Abstract.** This study aims to determine changes in population *Lactobacillus fabifermentans* (*L. fabifermentans*) and biochemical changes during controlled corn flour fermentation. The research is divided into two stages. The first stage was the making of starter by adding 48 hours culture on 50 g corn flour which was added 100 ml distilled water (1: 2), then incubation for 48 hours. The second stage is the application of a starter. The 48-hour starter was applied to cornstarch suspension (1: 2) by controlled method (starter concentrations of 1%, 3%, and 5%) and fermented using the microaerophilic technique at 24 hours and 48 hours intervals. The observed variables were a microbial count of Lactic Acid Bacteria (LAB), pH value and total acid on fermentation liquid, and rheological properties of modified corn flour. The results showed that the best rheological properties of corn flour fermentation resulted from the treatment of starter concentration of 1% with 24 hours fermentation time of 78000 Cps. The rheological properties of the resulting corn flour belong in referred the non-pseudoplastic Newtonian category. Where, during the fermentation process the starter concentration treatment of 1% with 24 hours fermentation time contained the amount of LAB (14.65 Log colony ml<sup>-1</sup>), pH value (5.08), and total acid (0.35%).

**Keywords:** corn flour, *Lactobacillus fabifermentans* concentration, biochemical changes

## 1. Preliminary

Wheat is one source of carbohydrates that have an essential role in Indonesia. The number of processed products made from wheat flour that satisfy almost all of the sales places, both small and large scale, with an ever-increasing number from year to year. Based on data from the Central Bureau of Statistics (BPS), imports of wheat grains in years 2011 reached 4.8 million tons with 1.4 billion US dollars, while wheat imports reached 775 thousand tons. Flour demand is expected to increase to 10 million tons per year, according to the Indonesian Flour Producers Association. Food problems in



domestic not loose from flour. Therefore, to reduce dependence on wheat flour is needed effort by looking for replacement materials. Indonesian's agricultural, domestic products such as dried cassava, rice, corn, sago or sweet potatoes are particularly potential for the development of wheat flour-based products. Indonesian domestic food crops such as cassava, rice, maize, sweet potato, corn especially high potential for the development of based non-flour.

Corn is one of the staple foods in Indonesia that has an important position after rice. Maize production has continued to increase recent years, with amount production from years 2008 to 2012 being 16,317,252; 17,629,748; 18,327,636; 17,643,250 and 19,387,022 tons. Therefore, further needed to processing for increasing the added value of corn, one of which becomes corn flour. However, Natural corn starch produced has the characteristics of pasta loud and clear, and it requires a long time in cooking. The nature of the other cornstarch is unable to form a rigid which gel except at high concentrations so that starch cannot produce a strong and compact product [1][2]. Based on that, then it is necessary to improve the characteristics of corn flour one of them by modification of fermentation.

There are three types of corn flour (Corn BISI-2, Pop, and Srikandi) which is modified using spontaneous fermentation, pregelatinization and a combination of spontaneous fermentation and pregelatinization showed fermentation treatment capable of decreasing peak viscosity value reverse viscosity, the temperature the beginning and the peak temperature and the value of corn flour enthalpic. Corn flour that has been through the process of modification has the potential to be applied in the manufacture of the product. Identification of indigenous microbes on spontaneous flour fermentation by using Corn Bisi-16 corn involved in molds, yeasts, and some types of lactic acid bacteria. One of the lactic acid bacteria produced from spontaneous corn flour fermentation is *L. fabifermentans*. This type of lactic acid bacteria is amylolytic that is capable of producing an amylase enzyme to degrade amylose into a simpler compound [3].

Modification using spontaneous fermentation method still has the disadvantage of types of microbes that live still ordinary and depend on the environmental conditions are difficult to control. Also, the initial population of lactic acid bacteria numbers is low causing spoilage bacteria, and pathogenic bacteria overgrow the growth of lactic acid bacteria. Therefore, this study focused on changes in the population of *L. fabifermentans* and biochemical changes during controlled corn flour fermentation. This study aims to determine changes in population *L. fabifermentans* and biochemical changes during controlled corn flour fermentation.

## 2. Research method

The type of this research is quantitative research with an experimental approach. The study used ANOVA of analysis is Randomized Complete Design (RAL) with one factor with three treatments and three replications. This research was held in Microbiology Laboratory of FMIPA and testing of rheological properties was analyzed at Universitas Hasanuddin pharmaceutical Laboratory on June to August 2017. The equipment used in this research is the oven, machine flouring, the measuring cup, the petri dish, the drop pipette, the stir bar, the bulb, the Bunsen, the fermentation container (jars), the reaction tube, the cup glass, water bath, the measuring flask, the Erlenmeyer, needle inoculum or Océ, beaker, electric bath, pH meter, plastic spoon, colony counter, laminar air flow, lighter, UC bottle, gloves, volume pipette, burette, stative, autoclave (Tomy autoclave High Pressure Steam Sterilizer ES-315), colony counter, micropipet, volume pipette, measuring pipette, analytical balance, and ATK. While the materials used in this research are; BIS-18 maize flour, culture *L. fabifermentans* pure isolate from the spontaneous fermentation of corn flour Bisi-16, MRSA and MRSB media, distilled water, 95% ethanol, NaCl, cotton, plastic wrap, plastic label, paper, and cotton. Material analysis such as indicator PP and NaOH 1 N.

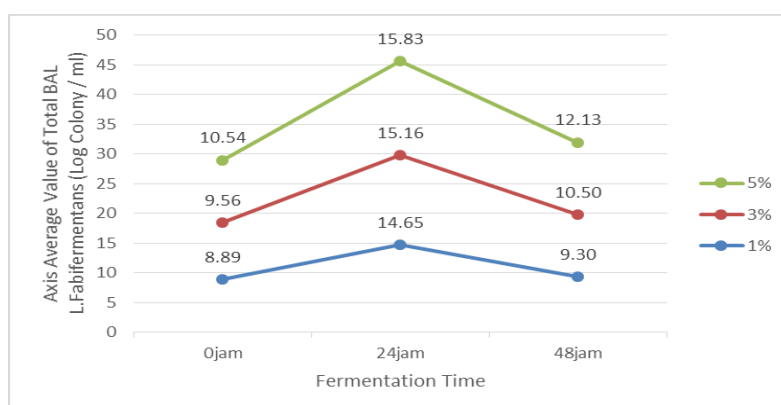
This research is divided into two stages of making a starter and applying starter. The stages of making starter *L. fabifermentans* are as follows: 1) The sterilized corn flour is added aquades with a ratio of 1:2. Then the suspension that has been made added *L. fabifermentans* culture that has been aged 48 hours as much as 10%. Process work is done aseptically in laminar air flow. 2) Furthermore,

fermentation is carried out for 48 hours. The fermentation technique is done by microaerophilic. 3) After the 48-hour starter, the researcher calculated the amount of *L. fabifermentans* by using MRSA media. 4) The starter is ready to be applied to corn flour. The stages of application of starter *L. fabifermentans* are: 1) *L. fabifermentans* starter culture which is 48 hours old and has known the number of microbes applied to cornstarch using controlled fermentation method, fermentation technique used is microaerophilic fermentation. In the fermentation process, the corn flour used was 500 g with the addition of 1000 mL aquades (1:2 ratio). 2) The starter culture concentration of *L. fabifermentans* used in fermentation is considered as treatment variable, consisting of 3 factors, 1%, 3%, and 5%. In addition, the length of fermentation is also considered as a research variable that is 24 hours and 48 hours. 3) During the fermentation process, calculations of *L. fabifermentans*, pH, and total acids in corn flour fermentation were analyzed. The analysis was performed at the time interval of 0 hours, 24 hours and 48 hours. Calculation of BAL using MRSA media. 4) After the fermentation period is 24 and 48 hours, the fermentation liquid is removed and drying on the flour by using the dryer room for 2 days at 50°C. 5) The resulting corn flour analyzed rheological properties.

### 3. Results and Discussion

#### 3.1. A number of *Lactobacillus fabifermentans*.

Analysis of the number of microbes conducted to determine the amount of growth of microorganisms during the fermentation activities take place. The total number of bacteria is the relative amount of some calculations by using a method either the solvent or the calculation plate [4]. The result of calculation of LAB number presented in Figure 1 shows that during the fermentation process all starter concentration treatment causing it to happen the increase in the number of the LAB, but the maximum increase on 24-hour fermentation. However, after 48 hours of fermentation, the amount of LAB decreased. The highest starter concentration during the fermentation process 24 hours contained on at 5% starter concentration with value 15.83 Log colony/ml, while lowest at starter concentration 1% with value 14.65 Log colony ml<sup>-1</sup>. After fermentation 48 hours, the amount of LAB decreased. However, there are similarities with 24-hour fermentation time, where the highest amount LAB of was found at starter concentration of 5% (12.13 Log colony ml<sup>-1</sup>) while the starter concentration of 1% (9.30 Log colony ml<sup>-1</sup>) LAB lowest.



**Figure 1.** Lactic Acid Bacteria (LAB) of corn flour fermentation

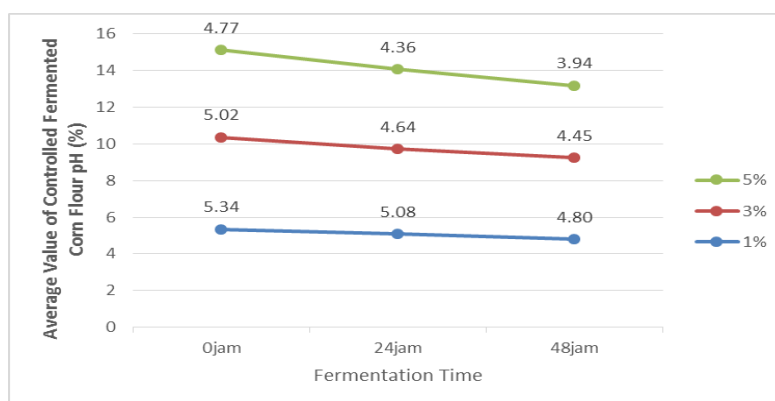
Analysis of variance showed that *L. fabifermentans* concentration treatment had a very significant effect on the amount of LAB. Duncan test results showed that treatment with 5% *L. fabifermentans* concentration had the highest growth of LAB number, whereas 1% starter concentration treatment had the lowest amount of LAB. This shows that the higher the concentration of *L. fabifermentans* added, the higher the LAB number. The amount of LAB in the early fermentation (0 hours) was higher when compared with microbial counts using spontaneous fermentation, where the amount of microbial

growth at the beginning of spontaneous fermentation was still very low since microbes had an adaptation period ranging from 1.9 to 6.2 Cf<sub>u</sub> log ml<sup>-1</sup>. However, at 24-hour fermentation, the addition of 1% starter concentration with controlled fermentation resulted in higher colonies 14.65 Log colony ml<sup>-1</sup> compared to spontaneous fermentation of 10.3 Log colony ml<sup>-1</sup>. This suggests that the use of controlled fermentation can potentially reduce fermentation time.

The time of fermentation affects the microbial activity, the more active bacteria the breed has the most number. The number of colonies that grow more and more if the longer fermentation longer. However, after fermentation reached at 48 hours, the number of LABs decreased. The decrease in the amount of Lactic Acid Bacteria (LAB) after the second day of fermentation is due to the decrease in the number of nutrients available in the fermentation medium, as well as the presence of antimicrobial components from the metabolism of acids produced by the LAB which are bactericidal for other microbes.

### 3.2. The pH value of fermented corn flour

The pH value is the degree of acidity used to state the level of acidity or alkalinity possessed by a solution. PH testing is done by using fermentation fluid to determine the level of acidity contained on corn flour during fermentation. All starter concentration treatments affected decreasing pH value of corn flour during fermentation (Figure 2). After fermentation lasted 24 hours, the resulting pH value decreased, where the starter concentration 5% (4.77) had the most acidic pH while the starter concentration of 1% had a pH of 5.34. After 48 hours of fermentation, the pH value decreased significantly differently on the fermentation of 0 hours with the most acidic pH value at 5% starter concentration with the pH value of 3.94%.



**Figure 2.** pH value of corn flour fermentation

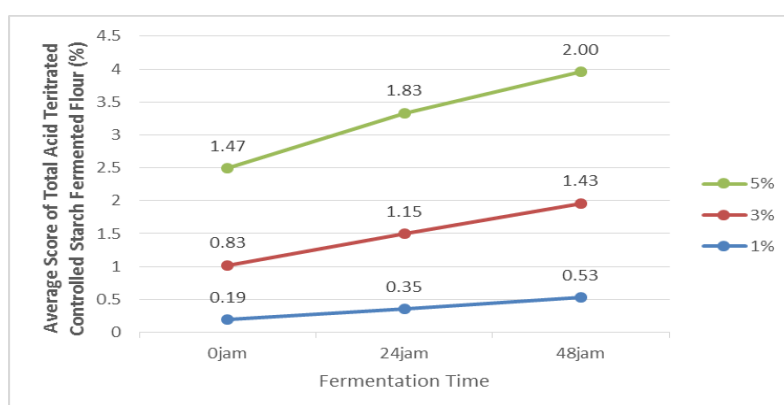
Analysis of variance showed that the addition of *L. fabifermentans* concentration affected pH value. Duncan test results showed that the addition of 5% concentration had the most acidic pH value. The decrease in pH is related to the starter concentration used; the higher microbes produce more acid thereby decreasing the pH value. This is by the theory expressed by Nawaz [5] that the more active and breeding microorganisms on fermentation the ability to break the substrate the better, so resulting in lactic acid in large quantities.

The decrease in pH was positively correlated with an increase in total titration acid (lactic acid) after 48 h hours (Fig. 2). The longer the fermentation, the more the amount of acid produced, so the pH of fermentation fluid at 48 hours fermentation time is lower than 24 hours. This is because the total acid produced at time 48 hours is higher than 24 hours. Many researchers stated that the lower pH of flour indicates that the increasing content of acid compounds contained in the flour. Furthermore, the fermentation process will produce volatile acids such as lactic acid, acetic acid, formic acid, butyric acid, and propionic acid. These acids are produced from the glucose overhaul and alcohol more, so the pH value decreases [6][7].

The pH on 24-hour fermentation at each starter concentration of either 1%, 3%, and 5% ranged from 5.08 to 4.36 (Fig. 2). The pH has been similar to the pH generated during spontaneous fermentation (48 hours), which is about 4.68. This suggests that controlled fermentation potentially reduces fermentation time.

### 3.3. Total fermented corn flour fluid acid

Total acid is the amount of acid formed during the fermentation process, the total acid is a parameter for the durability of a food product, especially in products treated with acid. Total acid testing to determine the number of acid levels produced by bacteria during the fermentation process. The result of data analysis about the effect of concentration and fermentation length on an acid level can be seen. Figure 3. The result shows that there is an of acid levels enhancement fermentation with the average value of increase is 10.27%. During fermentation 24 hours, the highest percentage was found at 5% concentration with 0.36% value while the concentration of 1% had the lowest percentage with value 0.16%. Enhancement in acid levels correlated positively with microbial growth, the higher the concentration and length of fermentation, the acid content as a result of its metabolism will increase. At 48 hours long fermentation the highest total acid enhancement was present at 5% while the lowest was at 1%.



**Figure 3.** Total Acidated Acid (TAT) of corn flour fermentation

Analysis of variance showed that the addition of *L. fabifermentans* concentration gave effect to total acid. The Duncan test results showed that the total acids of corn flour fermented liquors have different mean values. Treatment with 5% *L. fabifermentans* concentration for 48 hours of fermentation had the highest total acid with a value of 2.0%. This shows that the higher the concentration of *L. fabifermentans* and the longer the fermentation time, the higher the total acid obtained. The increasing of total acid is because the increase in LAB population is positively correlated with the increase in total acid, the higher concentration of *L. fabifermentans* added then the bacteria would produce lactic acid as the main metabolism product is higher [8]. Lactic acid bacteria will use simple sugars on the fermentation liquid used as raw material to produce organic acids, especially lactic acid so that the total acid will increase.

The occurrence of the increase in total acid along with the increased length of fermentation, this occurs because the central metabolism produced as microorganism activity by *L. fabifermentans* are organic acids [9]. The longer the fermentation, the potential of metabolic compounds produced will be more. Therefore, the fermentation 48 hour of resulting total acid was higher than 24 hours. The total acid produced from spontaneous fermentation was lower with a range of 0.10% when compared with controlled fermentation with 1% starter addition with 24 hours fermentation time, i.e., 0.35%.

3.4. Rheology of fermented corn flour

Rheology is a science that focuses its attention for studying deformation or changes in shape and flow so that the action that produces styles in deformation and the flow of materials and other mechanical properties can be said to be rheological properties. Other mechanical properties of rheology properties are usually related to the motion of the material which is charged to style. Here are the results of rheological testing on fermented corn flour by using starter concentration of *L. fabifermentans* and fermentation time [10].

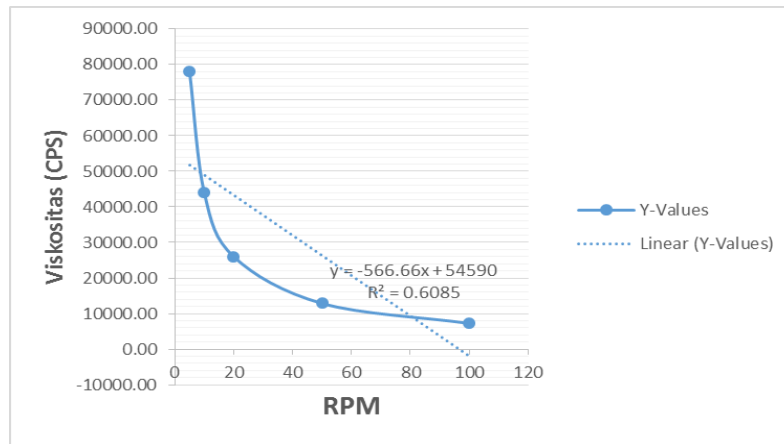


Figure 4. Rheology corn flour of fermentation controlled of concentration 1% on 24 hours fermentation

Rheology with 1% treatment on 24 hour fermentation time showed viscosity of flour decreased with value 78000 Cps. The determination value of 1% treatment on the 24-hour fermentation period decreased with the value  $\gamma = -566.66$  so that the viscosity produced dilute.

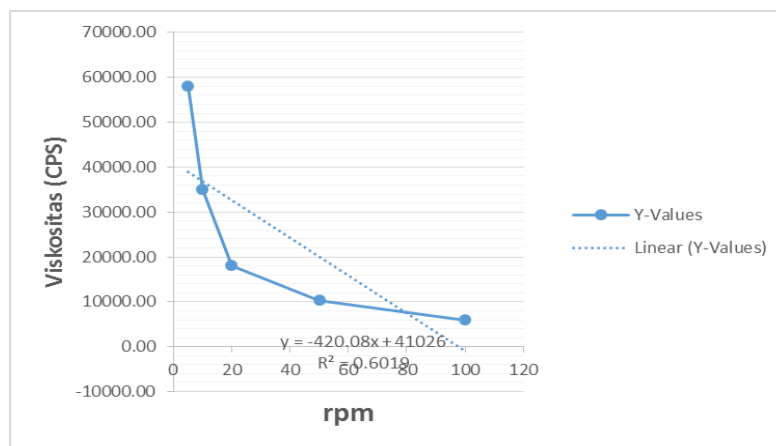
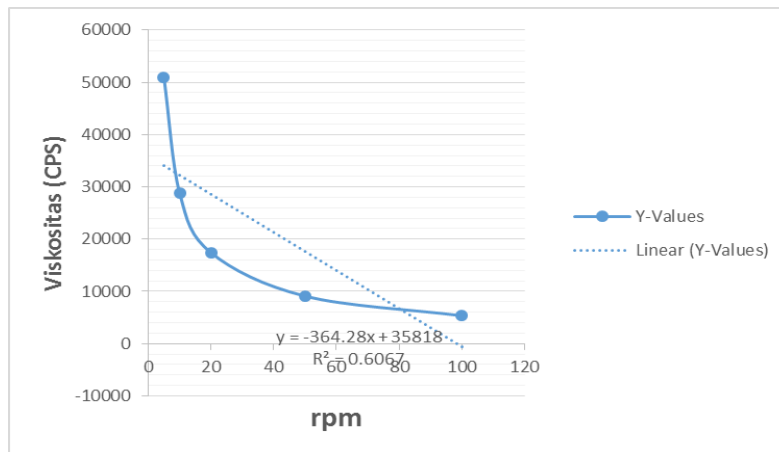


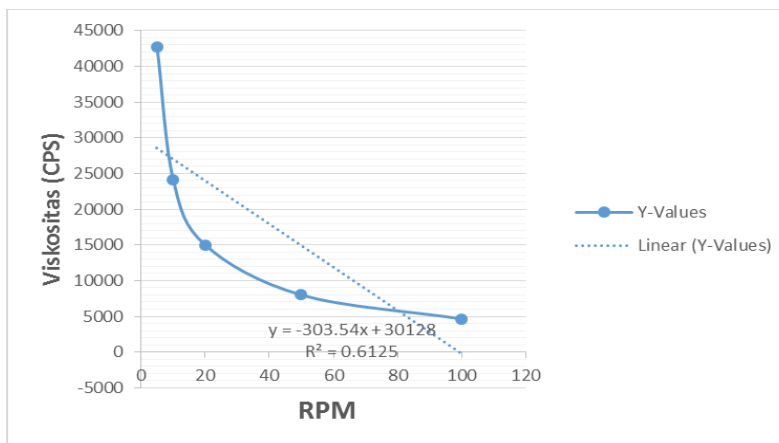
Figure 5. Rheology corn flour of fermentation controlled of concentration 1% on 48 hours fermentation

The results showed that the viscosity of 1% flour treatment in 24 hours fermentation experienced a more significant decrease compared to 48 hours of fermentation time with 58000 Cps. The decrease of corn flour viscosity at starter concentration treatment of 1% with 48 hours 48 hours fermentation time of 20000 Cps.



**Figure 6.** Rheology corn flour of fermentation controlled of concentration 3% on 24 hours fermentation

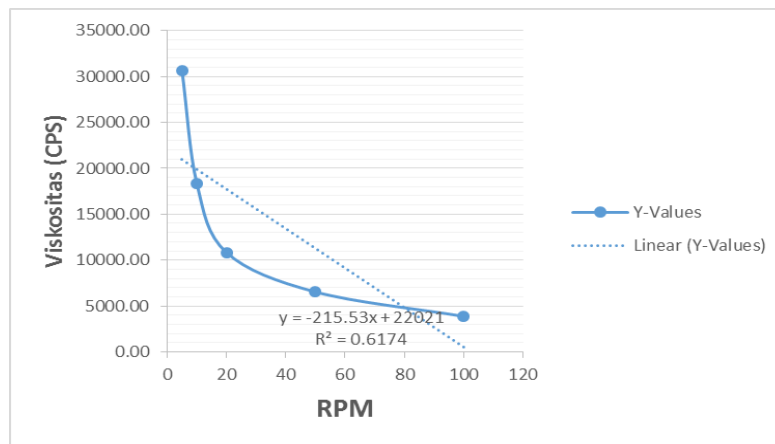
The rheological properties of 3% concentration treatment with 24 hour fermentation time decreased with the value of 51000 Cps. The decrease of the viscosity value on the rheological properties did not vary in the treatment of 1% starter concentration on 48 hours fermentation length with 7000 Cps difference.



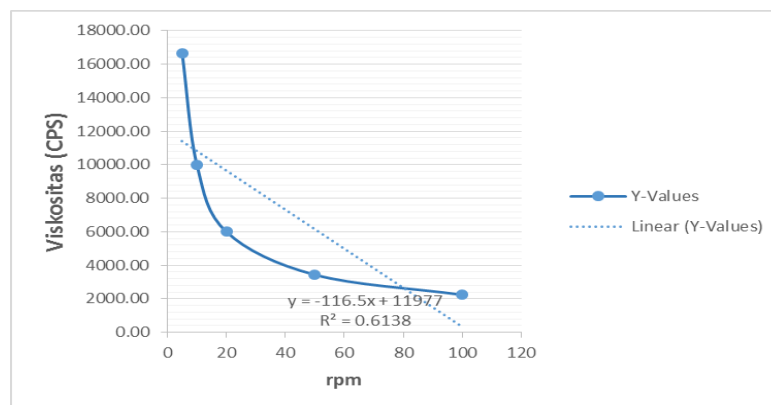
**Figure 7.** Rheology corn flour of fermentation controlled of concentration 3% on 48 hours fermentation

Viscosity value at 3% treatment for 48 hours was 42666.67 Cps, with linear determinants of rheology showed the decrease of flour viscosity with value 42666.67, where value  $\gamma = -303.54$  was lower when compared to 24-hour treatment. Decrease in viscosity due to the lower amylose content so that water absorption will decrease caused by acids and enzymes.





**Figure 8.** Rheology corn flour of fermentation controlled of concentration 5% on 24 hours fermentation



**Figure 9.** Rheology corn flour of fermentation controlled of concentration 5% on 48 hours fermentation

The flow properties presented in Figure 8 show that 5% concentration treatment with 24 hours fermentation time decreased with a value of 30666.67 Cps. Treatment of 5% concentration with fermentation time 24 hour showed lower viscosity of flour compared with the concentration of 1% and 3% at each fermentation time used. It is also illustrated in the linear determination of each treatment.

The results showed the viscosity of corn flour at 5% concentration with 48 hours fermentation time decreased with value 16666.67 Cps. The viscosity value of the treatment is the lowest viscosity value compared to the other treatment fermentation 24 and 48 hours the other (Figure 9) with  $\gamma = -116.5$ . The results showed that each treatment of starter concentration decreased, but *L.fabifermentans*, 5% treatment, decreased viscosity reached 16666.67 Cps lower than the treatment of starter concentration *L.fabifermentans* 1% and 3% (Figure 8 and Figure 9).

The results of rheological testing of corn flour fermentation showed that the concentration and duration of fermentation affect the nature of corn. The higher it is concentration and length of fermentation will decrease the resulting viscosity. The higher it is the concentration produced then the number of enzymes produced more and more so it will decrease the amylose content in starch so that the effect on viscosity. Controlled fermented corn flour is produced in the Pseudoplastic Non-Newtonian stream. This stream non-Newtonian Pseudoplastic occurs because the force to stream it increases so that the viscosity of the material decreases. The larger the force worn, then the fluid flow

more smoothly or more dilute. The flow is caused by a decrease in the starch (amylose) caused by the acid produced by *L. fabifermentans* so that the viscosity decreases when the product is given a style.

The rheological properties of 1% starter concentration with 24 hours fermentation have the highest rheological properties compared to other treatments. The rheological properties are identical to the viscosity of flour, where a determination is strongly related to the degree of flour amylose levels. Therefore, high rheological properties are positively correlated with high amylose levels.

#### 4. Conclusion

The concentration of *L. fabifermentans* during fermentation affects the amount of LAB, the pH value and the total acid as well as the modified rheology properties of corn flour. The treatment of *L. fabifermentans* 1% concentration with 24 hours long fermentation gave the best result with LAB amount 14.65 Log colony ml<sup>-1</sup>, pH 5.08, total acid 0.35%, and rheological properties of corn flour referred to in non-Newtonian category (pseudoplastic flow).

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