ATLANTIS PRESS
Advances in Social Science, Education and Humanities Research (ASSEHR), volume 201
International Conference on Indonesian Technical Vocational Education and Association (APTEKINDO 2018)

The Changes in Aspergillus Sp 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 Aspergillus sp population and biochemical changes during the process of controlled corn flour fermentation. The research is divided into two stages. The first stage was the making of starter by adding aquades as much as 100 ml into 50 g of corn flour. Furthermore, Aspergillus sp culture which has been incubated for 5 days was added to the corn flour batter and then, incubated for 48 hours. The second stage was the starter applied to the batter of corn flour 500 grams that was added water as many as 1000 ml (1: 2) with starter concentrations of 1%, 3% and 5%. The cornflour was fermented for 24 hours and 48 hours. The variables observed were the amount of molds, pH value, and total acid at fermentation liquid and the rheological properties of corn flour produced. The results showed rheological properties of the best fermented corn flour were produced from 1% treatment with 24 hours fermentation length of 4533.33 Cps. The rheological properties of the corn flour results are included in the non-newtonian category (pseudoplastic flow). During fermentation process, starter concentration treatment of 1 % with fermentation of 24 hours has amounted fungi (9.22 logs / ml), pH value 4.92) and total acid (0.36%)**

Keywords*— *Corn Flour, Aspergillus sp, biochemical changes

I. INTRODUCTION

Corn (Zea mays L.) is one of the most important food crops in the world, besides wheat and paddy. As a major source of carbohydrates in Central and South America, corn is as an alternative source of food in the United States. Inhabitant of several regions in Indonesia (for example in Madura and Nusa Tenggara) also use maize as a staple food. South Sulawesi's maize production in 2014 was 1.49 million tonnes of dry kernels obtained from 291,11 thousand hectares harvested area and productivity level of 51.47 quintal per hectare. The comparison between 2013 and 2014 shows that maize production in South Sulawesi province increased by 248.28 thousand tons of dry kernels or increased by 19.86%. The increase of maize production was caused by the added harvested area by 17.06 thousand tons (up 6.23 percent) and the productivity increased a many as 5.85 quintal per hectare (up 12.86%) [1].

Increasing the functional properties and characteristics of starch can be done in a way of modification. The modification of corn flour characteristics can be done by fermentation and pragelatinization. These methods have the potential to be developed because the operational costs are cheap. The fermentation process is defined as the decipherment of the starch enzyme by microorganisms on the substrate. Therefore, corn starch fermentation is the decipherment of corn starch which is conducted by amylase enzyme and amyloglucosidase produced by lactic acid bacteria [2]. The research results of Sukainah et al. on the identification of indigenous microbes in spontaneous flour fermentation by using bisi-16 maize showed the microbes involved were lactic acid bacteria, yeast and several types of molds [3].

The modification of corn flour using fermentation method spontaneously showed that at the beginning of fermentation that is 0 to 3 hours, microbes playing a role is mold. After fermentation enters the exponential and stationary phase, which is 6 to 48 hours, the fermentation process is dominated by the growth of yeast, bacteria, and lactic acid bacteria [3]. Mold is used as natural culture because it is capable of producing starch breaker enzymes, namely amylase enzyme.

One type of mold used as mold indigenus is Aspergillus sp. Mold is widely used as a producer of α-amylase because it has advantages, such as α-amylase produced from molds, if more stable when compared with α-amylase produced from bacteria making it more profitable if used for industrial purposes [4]. Mold is widely used as a producer of α-amylase which is a group of Aspergillus [5].

According to Sukainah et al. modification of corn flour using fermentation method spontaneously is considered to still have a weakness that is the type of living microbes can vary and very dependent on the conditions and environment which are so difficult to control. Therefore, this study focused more on changes in Aspergillus sp populations and biochemical changes during controlled corn flour fermentation [3].

This study aims to determine changes in Aspergillus sp population and biochemical changes during the process of controlled corn flour fermentation.

II. METHOD

The type of this research is quantitative research with experimental approach. The study used anova of analysis which is Randomized Complete Design (RAL) with one factor with 3 treatments and 3 replications.

Equipment used in this research was basin, grinding machine (flour mill). Analyzes equipment included, Petri dish, micropipette, Erlenmeyer, beaker, measuring cup, needle of inoculum or ose, stir bar, bunsen burner, hot plate, autoclave, incubator, pH meter, digital scales, plastic spoon, colony counter, jars, laminar air flow, pipette drops, measuring pipettes, glass bottles, test tubes, gloves, volume pipettes, measuring flask, burette, stative, refrigerator. While the materials used in this research are; bisi-18 corn obtained from Jeneponto Regency and Aspergillus sp culture obtained from spontaneous fermentation of cornmeal [3]. The analysis materials for the fermentation liquid are 0.1 N NaOH, spiritus, aquades, water, 70% alcohol, stationery, plastic wrap, plastic, labels, paper, cotton, Potato Dextrose Agar medium (PDA) to calculate the amount of molds.

The implementation of the research was conducted in two stages: preliminary research and main research. Preliminary research was conducted to determine the type of Aspergillus sp concentration to be used in the corn flour fermentation process. The best concentrations resulted from preliminary research will be the starter used in the main research. Aspergillus sp type that will be used is Aspergillus sp (C) with concentration of 1%, 3%, and 5%.

The main research consists of two stages of making starter and starter application. The stages of making starter of Aspergillus are as follows: 1) Aspergillus sp ulture inserted into corn flour that has been sterilized, then added aquades with a ratio of 1: 2. Process work done aseptically in laminar air flow. 2) Fermentation was done for 48 hours. Fermentation techniques performed by microaerophilic. 3) After incubation for 48 hours, the starter counted the number of Aspergillus sp (C, D, and G) using PDA media. The highest number of Aspergillus sp will be used in the study. 4) Starter ready to be applied into corn flour.

The stages of application of starter Aspergillus sp was as follows: 1) Starter Astergillus sp that has been 48 hours old was applied to modified corn flour using controlled fermentation method, fermentation technique used is microaerophilic fermentation. The fermentation process uses 500g of corn flour by adding 1000 ml of water (1: 2 ratio). 2) Aspergillus sp starter culture concentration used in fermentation was considered as treatment variable, consisting of 3 factors, 1%, 3% and 5%. In addition, the duration of fermentation was also considered as a research variable that was 24 hours and 48 hours. After the fermentation process, corn starch fluid was analyzed on control days ie 0 hours, 24 hours and 48 hours using PDA media to calculate the amount of molds, pH test, and total titrated acids. 3) After the fermentation time of 24 and 48 hours the fermentation liquid was thrown away and carried out drying on flour by using room dryer for ± 48 hours. 4) corn flour was analyzed rheological properties.

III. RESULT AND DISCUSSION

A. *Number of Molds*

Aspergillus sp is one type of microorganism that includes fungus, and is included in eukaryotic microorganisms. Aspergillus sp microscopically characterized as a bersepta and branched hyphae, conidiofora appear from the foot cell (miselia swollen and thick-walled) carry stigmata and conidia will grow forming a green, brown or black chain [6]. The results of the research showed the amount of molds in the liquid of fermented flour in general, all starter concentration experienced an increase in the number of molds, but the highest increase is in 24-hour fermentation. After 48 hour fermentation, the amount of molds decreased. The highest amount of molds at 5% treatment with 24 hours fermentation time of 12.71 Log colony/ml, while the lowest at starter concentration of 1% was 9.22 Log colony/ ml. After fermentation 48 of hours, the amount of molds decreased, but the highest amount of molds at starter concentration of 5% (10.89 Log colony/ml) while the lowest treatment at starter concentration of 1% (8.34 Log colony/ml). The analysis results of the number of molds from various treatments can be seen in Figure 1.

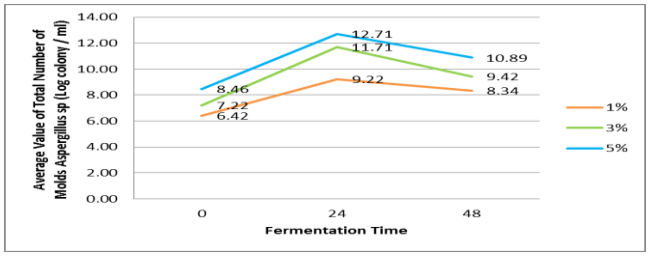

Fig. 1. Amount of Molds Liquids of Corn Flour Fermentation

The analysis of variance showed that the concentration treatment Aspergillus sp had a very significant effect on the amount of molds. Duncan test results showed that the treatment with Aspergillus sp concentration of 5% had the highest amount of molds number, while the starter concentration treatment of 1% had the lowest amount of molds. The higher the Aspergillus sp concentration addition, then microbes have a higher chance of growing, so the amount of molds is increasing. This is because its grow rate is strongly influenced by the starter concentration of the growing medium such as nutrient content and environmental conditions including air temperature and humidity. In the growth phase, microbes require more energy than other phases and culture is most sensitive to the environment so that the pH value decreases and the total acid increases [7]. 24-hour fermentation period is the highest of all treatments. The optimal increase in the amount of molds occurred on 24 hour fermentation and decreased at 48 hour fermentation. The longer the fermentation caused the amount of molds to decrease, but is different from the acid total content that continues to increase during fermentation. Molds utilizes starch as a source of energy, so the longer the fermentation time, the more starch is utilized by molds and converted to acid. Increasing the total acid is one of the causes of the decline in the number of microorganisms that are sensitive to acid [8]. In addition, after 48 hour fermentation, the amount of molds also decreased due to the build up of toxins due to

cell metabolism and nutrient content that began to run out resulting in competition. Some cells will die because they cannot compete to get the remaining nutrients, while those able to compete get the nutrients alive, so the number of bacterial cells tends to be constant [9]. The research results of fermentation of corn flour spontaneously showed dominant molds growing at 0 hours until fermentation time of 3 hours. The utilization of Aspergillus sp as a starter can optimize the performance of BAL indigenous, so that with the addition of Aspergillus sp concentration, the complex starch substrate during more fermentation is converted into simpler form, so that BAL is easier to grow.

B. pH Value of Fermented Corn Flour Powder

The pH value is a symbol for the degree of acidity or alkalinity of a solution. The pH value is very important for the growth of microorganisms, because the enzyme work is greatly influenced by pH [10]. The effect of the addition of starter concentration and during the fermentation process on pH testing can be seen in Figure. 3

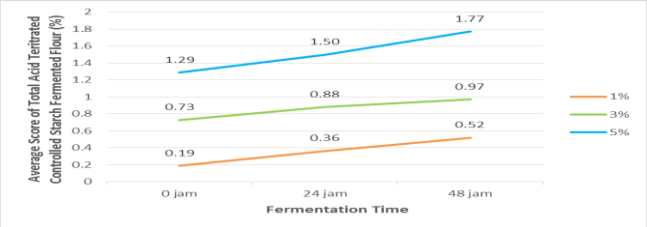

Fig. 2. pH Value of Corn Flour Fermentation

The result of the average analysis of pH test of fermented corn flour from the addition of starter concentration during fermentation ranged from 5.04-4.13. In general, there was a difference of acidity level against flour. The mean value of the pH test decreased during fermentation. The 24-hour treatment with 5% starter concentration (4.25) had the most acidic pH compared to the treatment concentrations of 1% and 3%. After 48 hours of fermentation, the pH value decreased. The most acid treatment was obtained from a 5% starter concentration with a value of 4.13.

Analysis of variance showed that the addition of a concentration of Aspergillus sp influence on the 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. The longer the fermentation, then more microbial making use of carbohydrates in flour for metabolic processes, so the ability of microbes to produce lactic acid is increasing. Increased lactic acid can be measured by decreasing pH. The decrease in pH causes the taste to become acid due to the formation of lactic acid as the main product of metabolism of lactic acid bacteria [11]. The decrease in pH occurs because during the fermentation process, microbes (molds and BAL) utilizes carbohydrates as a source of energy, the more (carbohydrates or source sugars) that can be metabolized, the more acids organic produced, especially lactic acid which causes a decrease in the pH value of liquids of fermented flour [12].

The pH values decreased during the fermentation that is ranging from 5.04-4.13. The 48 hour fermentation time produces a higher acid than the 24-hour fermentation. The decrease in pH is related to an increase in total titrated acids, the amount of mold, and the number of BALs. The longer the fermentation time, the amount of fungus, the amount of BAL, and the total of the titrated acids increases so the pH value decreases. The main product of results metabolism during fermentation is organic acids. The results fermentation which is an acid or base produced during the growth of microorganisms and organic components in the medium [10].

The pH test values on spontaneous fermentation ranged from 5.04-4.13. The pH value has been similar to the pH value generated during spontaneous fermentation (48 hours) which is ranged 4.68. This suggests that controlled fermentation potentially reduces fermentation time. The decrease in pH values occurs after 24 hours of fermentation due to the presence of lactic acid produced by BAL.

C. Total Acidated Acid (TAT)

TAT measurement is the determination of the total acid concentration. TAT is related to the total measurement of the acid contained in the food. TAT is a estimators influence of acidity to taste and have aroma better. The result of calculating the TAT number can be seen in Figure. 4.

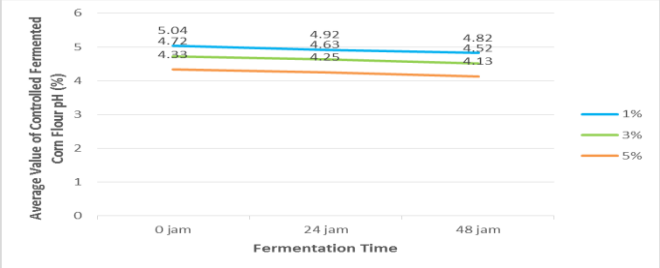

Fig. 3. Total Acidated Acid (TAT) of Corn Flour Fermentation

The total value of titrated acids produced on the fermentation fluid of 0 hours, 24 hours, and 48 hours with each addition of starter concentration of 1%, 3%, and 5% increased. After fermentation lasted for 48 hours, the highest concentration of TAT of fermented flour was obtained by 5% starter concentration treatment which was 1.77%, while the lowest TAT was obtained from the treatment of starter concentration of 1% (0.52%). Increases in acid levels correlated positively with growth in the number of molds, number of BAL, and pH values. The more the number of microbes (mold and BAL) the acid levels as a result of metabolism will increase and the pH value decreases. The analysis of variance showed that the addition of concentration sp Aspergillus give effect to the total acid. The Duncan test results showed that the total acids of corn flour fermented liquors have different mean values. Treatment with Aspergillus sp concentration of 5% for 48 hours of fermentation had the highest total acid with a value of 1.77%. The increased in total acid is due to higher starter concentration and fermentation time. The more number of growing microbes will produce an increasingly higher acid as a result of metabolism. In general, the addition of starter

concentration causes an increase in acid total . However, treatment the addition of Aspergillus sp 5% concentration was the treatment that had the highest acid total compared to the treatment of concentration of 1% and 3%. The higher the concentration of yeast, then more the amount of acid produced [13]. 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 reversal of glucose and alcohol. The higher it is of Aspergillus sp concentration then the growth of BAL number of indigenus of more and more. This is caused by the more and more complex carbohydrate compounds are converted to simple forms, then the more indigenus bacteria are mutually supportive and synergistic in cell propagation. BAL produces pyruvic acid, formic acid and CO2, and foalic acid that stimulates the growth of BAL which will release amino acid valine, glycine and histidine [14]. The total value of lactic acid in corn flour increased significantly after fermentation for 0 hours, 24 hours to 48 hours. Time 48 hours fermentation yields the highest total acid compared to 24 hours. The enhancement in total acid is related to the amount of BAL, especially the decrease in pH value. The longer the fermentation time, then lactic acid produced by BAL is higher. This is in line with Zaini's study, that the total acid increases with the length of fermentation because many substrates are able to be overhauled by starter and the presence of organic acids produced by microbes, including lactic acid [15]. The total value of corn flour fermented acid was spontaneously lower than the addition of Aspergillus sp starter concentration with values ranging from 0.03-0.38% [3]. This is caused because on spontaneous fermentation process is still many other types of microbes are actively working on the fermentation process.

D. Rheology of Fermented Corn Flour

The result of rheologic R2 al test of corn flour fermentation resulting from in addition of starter concentration of 1% with time 24 hours fermentation time decrease that is viscosity 4533.33 Cps, while time 48 hours of fermentation time that is 3200 Cps viscosity value. Decrease in viscosity due to the getting lower amylose content so that water absorptioned will decrease. The result of rheological measurement at 3% concentration treatment with fermentation time 24 hours and 48 hours has a similar viscosity value that is 2000 Cps. However, on the determinant coefficient value R2 has a difference that is 24 hours fermentation with R2 value 0.6768 and 48 hours fermentation with value R2 0,6282. The longer the fermentation the lower the R2 value so that the resulting corn flour is more dilute than 24-hour fermentation.

The result of fermented corn starch rheology test showed that starter concentration and fermentation duration influenced rheological properties. The higher starter concentration and fermentation time will decrease the viscosity . The higher the concentration produced, the more the number of enzymes produced, 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 non-Newtonian Pseudoplastic stream occurs because the force to flowing it increases so that the viscosity of the material decline. The larger the force worn, the fluid flow the more smoothly or the more dilute. This is caused by a decrease in the starch (amylose) caused by the acid produced by Aspergillus sp so that the viscosity decreases when the product is styled, the resulting viscosity will decrease. Foodstuffs with Pseudoplastic flow properties are well suited for thickeners, especially additional ingredients in cream making, bread making and pastries [16].

IV. CONCLUSION

The concentration of Aspergillus sp during fermentation gave effect to the amount of mold, pH value and total acid, as well as the rheological properties of modified corn flour. Concentration treatment of Aspergillus sp 1% with fermentation time of 24 hours gave the best result with the amount of fungus of 9.22 Log colony / ml, pH value of 4.92, total acid of 0.36%. The rheological properties of the resulting corn flour are included in the non-newtonian category (pseudoplastic flow).

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