Study of *Tape* Yeast and Sucrose Addition to COCOA Beans Fermentation (*Theobroma* cocoa L.) on Small Scale

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***Abstract*—The objective of this research is to study the effect of yeast *tape* concentration with sucrose on small-scale cocoa fermentation process. The research was conducted in the form of Completely Random Design of Factorial Pattern, two Factors. The first factor is *tape* yeast concentration consisted of: without yeast (control), yeast 0.5%, and 1%, The second factor is sucrose consisted of 1%, 2%, and 3% sucrose. Observations performed on dried cocoa beans include: fermentation index, pH, and reducing sugar content. The results showed that the addition of yeast and sucrose had a very significant effect on fermentation index, and pH, but no significant effect on reducing sugar content. The concentration of 1% *tape* yeast and 2% sucrose is the best treatment against fermentation index and pH as well as reducing sugar with fermentation index value 1.17, pH 5.89 and reducing sugar 1.48%.**

***Index Terms*—*tape* yeast, sucrose, fermentation index, reduction sugar, fermentation, cocoa**

I. Introduction

Cocoa production in Indonesia is mostly produced by farmers, 95% ownership of cocoa plantations is a smallholder plantation that accounts for 92% of the production of dried cocoa beans. The production of cocoa beans from smallholder plantations generally still faces post-harvest problems. Most farmers do not perform the fermentation process to harvested cocoa beans causing low quality of cocoa beans [1]. Most farmers are reluctant to carry out the fermentation process on harvested cocoa beans. One reason is the fermentation time that farmers consider too long because it takes 5 to 7 days. Meanwhile, the fermentation time determines the success of fermentation because it is related to the characteristics of dried cocoa beans produced. This is due to the biochemical processes and microbial activity that lasts during the fermentation. If the fermentation is performed in a very short time then it has not reached the fermentation goal, namely the death of beans and the formation of flavor precursor compounds. Fermentation also can not be performed in excessive time because it can cause damage to dried cocoa beans produced, such as bland and high acidity.

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During fermentation, cocoa beans will undergo biochemical and physical changes that lead to improved quality of dried cocoa beans. According to that case, several studies for optimization of fermentation have been performed, including: addition of yeast starter [2], addition of lactic acid bacteria starter [3], selection of some yeast isolates suitable for cocoa beans starter fermentation [4], and the addition of mixed inoculum [5]. Generally, the study used pure isolates as starter on fermentation. The starter requires special treatment on preparation, application, and storage. On the other hand, the situations on the field is those who do the fermentation process are farmers, while they generally not familiar with stages of that special treatment. Therefore, more applicable yeast is needed so that the optimization efforts can be implemented mainly by farmers.

One type of yeast that can be used is *tape* yeast. *Tape* yeast contains *Saccharomyces cerevisiae* which has perfect growth at temperatures around 30 °C and pH 4.8. Furthermore in the *tape* yeast there are microorganisms that in anaerobic conditions will produce amylase enzymes and amyloglukosidase enzymes, both enzymes are responsible for the decomposition of carbohydrates into glucose and maltose. *Tape* yeast is a mixed population composed of species of the genus *Aspergilius*, *Saccharomyces*, *Candida*, *Hansenulla*, and *Acetobacter* bacteria [6]. The increased fermentation process that occurred due to inoculation of *Saccharomyces cerevisiae* microorganisms and some other bacterial cultures can improve the performance of cocoa bean fermentation. Additionally, the addition of sugar cane molasses and culture increases the activity of yeast and ethanol content, because the provision of molasses increases the amount of substrate that can be converted to ethanol [7]. During the fermentation process, microorganisms actively involved include yeasts, lactic acid bacteria and acetic acid bacteria.

The addition of yeast as a type of mixed cultures had the opportunity to perform the fermentation process to cocoa beans. The addition of yeast to cocoa fermentation with a range of 1.0% had been tested by [8], which can shorten the fermentation time to 4 days from 6 days in natural fermentation, with the number one quality yield of dried cocoa beans. Therefore, it is necessary to research

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about the use of *tape* yeast and sucrose that is more applicable so that optimization efforts can be implemented mainly by the farmers. Successful fermentation of cocoa beans using *tape* yeast, and sucrose is determined by its concentration. The Research Objective is to study the influence of *tape* yeast with sucrose in optimizing the process of fermentation of small-scale cocoa beans.

II. Research Methodology

A. Materials and Tools

The ingredients used in the study are: Lindak type cocoa, *tape* yeast, sucrose, fermentation box size 40cm x 30cm x 30cm, oven, thermometer, and gunny sack. Chemicals for analysis include: ethanol 70%, N-hexane, methanol 90%, and NaOH 0.1 N.

The tools used are: pH-meter, blender, analytical scale, measuring flask, UV-Vis Type spectrophotometer, measuring cylinder, electric oven, pipette, Petridis plate, magnetic stirrer, Whatman 42 filter paper, test tube, Erlenmeyer, and glassware.

B. Research Design

1) Fermentation implementation method

Cocoa beans fermentation uses *tape* yeast and sucrose. The research uses cocoa Lindak varieties derived from the cocoa garden of the people in North Luwu Regency, South Sulawesi. *Tape* yeast added to cocoa beans, is firstly crushed, then sieved and stirred until it's homogeneous. After it is being homogeneous, yeast is weighed based on the percentage of treatment. The experiment is performed by Group Random Design method (RDM) with two factors of factorial patterns. The first factor of *tape* yeast concentration (R) consisted of: 0.5%, 1%. The second factor is sucrose (S) concentration: 0% sucrose, 1%, 2% sucrose, and 3% sucrose. Fermentation is performed in fermentation box with 12 kg capacity using 10 kg fresh cocoa beans, at room temperature (33-35^{o}C), for 5 days. The fermentation process is continued with the drying stage of the beans (drying under the sun) for approximately 3 days.

C. Observation Parameters

1) Fermentation index [9]

Implementation procedure

a. The cocoa beans that have been crushed (cocoa powder) to 40 mesh are weighed as much as 0.5g then extracted with a mixture of methanol and concentrated HCl with a ratio of 97: 3 as much as 50 ml. The cocoa bean extract was homogenized for 20 seconds using a homogenizer, after that it was stored for 20 hours at 8 ℃. The absorbance of the extract is measured using spectronic at wavelength 460 nm and 530 nm. The value of the fermentation index (FI) is determined by the formula:

$$FI = \frac{abs\ \lambda\ 460\ nm}{abs\ \lambda\ 530\ nm}$$

2) Reduction sugar analysis [10]

Crushed solid material is weighed 25g, dissolved with 100 mL of distilled water into 250 mL cup glass, add Pb Acetate for cleansing, then Na2CO3 is added to remove excess Pb, distilled water is added until exactly 250 mL. 25 mL of solution is taken and put into Erlenmeyer and 25 mL of Luff-Schoorl solution is added. Blank treatment is done, that is, 25 mL of Luff-Schoorl solution is added with 25 mL of distilled water. After adding a few boiling stones, Erlenmeyer is connected to a cooler and boiled for 10 minutes then quickly cooled. 15 mL of KI 20% is added and carefully added 25 mL of H2SO4 26.5%. The liberated iodine is titrated with 0.1N Na-Thiosulfate solution using 1% starch indicator as much as 2-3%. (Titration is terminated after milk cream appears). The formula used to calculate the level of sugar reduction is:

$$X = \frac{(blank\ titration - sample\ titration)\ X\ dilution\ factor}{mg\ Sampel} X100$$

3) pH of dried beans [11]

- Implementation procedure

a. Sample test is taken as many as 12 *beans* up to 20 *beans*, outer skin separated, then milled using a blender

b. The test sample is weighed 10g into the cup glass, add 90 ml of hot distilled water (70 ℃ to 80 ℃), stir slowly until a suspension is formed which must be free of lumps

c. The filtrate is filtered and cooled till the room temperature is (27-29) ℃ and the filtrate pH is determined as soon as possible at that temperature. The results are expressed according to the readings indicated by the pH-meter for the filtrate.

III. Result and Discussion

A. Fermentation Index

Influence of *tape* yeast addition and sucrose in fermented cocoa beans can be identified through Fermentation Index (FI) of dried cocoa beans (Fig. 1). *Tape* yeast and sucrose addition influence in increasing fermentation index of dried cocoa beans. It can be seen at cocoa beans added with 0.5% *tape* yeast and no sucrose performed fermentation index 0.63 and 0.42, meanwhile 1% *tape* yeast addition and 3% sucrose perform the highest fermentation index that is 1.59%. It indicates that *tape* yeast and sucrose addition in fresh cocoa *beans* increase the activity of fermentation process. The higher the yeast and sucrose percentage added, the more the carbohydrate broken down into glucose, alcohol, lactate acid and other substances.

Cocoa beans fermentation with Fermnentation Index less than 1 (FI<1) performs imperfect fermentation. Dried cocoa beans with FI equals to 1 and more than 1 (FI ≥1) can be classified as perfect fermentation [9], [12]. Fermentation index is the brownish level of cocoa beans to define its beans fermentation. Cocoa flavor potential

can be identified from fermentation quality through color index called fermentation index [13], [14].

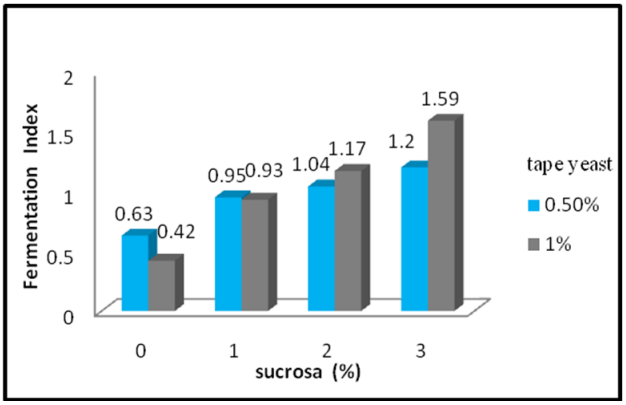

Figure 1. Fermentation index score of dried cocoa beans in sucrose addition and *tape* yeast

Beans color changing from purple to brown applied as thrive indicator of fermentation. Color changing occurs because of fermentation process. In fresh cocoa beans, cotyledon contains a few puple cells which spread among uncolored cell. During fermentation, acid and heat are accumulated then the pH reduce and the temperature increase that lead to a dead cocoa beans then the cells cracked release various enzymes and substrates then react, one of them is antosianin destruction then formed brown liquid (flavonoid complex substances) in the area between cortex and beans pieces. Fermentation index assumed can provide certain information to determine succeed fermentation as well as a determinant indicator of fermentation process [15], [16], [17].

B. pH of Dried Cocoa Beans

pH condition of dried cocoa beans as a result of *tape* yeast and sucrose addition in cocoa beans fermentation can be seen in Fig. 2. Adding 0.5% yeast and 1% sucrose result in 4.76 and 5.87 pH, and pH increasing along with sucrose concentration increasing up to 2% with pH value of 5.87 and 5.89. pH decreasing occurs in 3% concentration of sucrose both at 0.5% yeast and 1% yeast addition respectively 4.6 and 4.58. Cocoa beans with acidity stated in pH as 5.20 – 5.50 or acid titration score 0.12 – 0.15 meq/g accepted as cocoa beans with optimum acid level by cocoa factory. Beans which are classified as acid has pH < 5.0 [18]. Cocoa factory in Europe and America expect dried cocoa beans with pH level around 5.1-5.8 and pH 5.2 is the most favorite one [19], [20].

Adding yeast increase the number of microbe in the beans pulp, thus increase the ability of yeast in breaking down glocise into alcohol. *Tape* yeast contains of *Saccharomyces cerevisiae* which has perfect growing capability in the temperature around 30℃ and pH 4.8. Furthermore, *tape* yeast contains microorganism which in anaerob condition can produce amylase enzyme and amiloglucosidase, both enzymes are responsible for separating carbohydrate into glucose and maltose. Microbe contains in *tape* yeast can be classified into five groups, namely amilolitic mold, amilolitic yeast, non-amilolitic yeast, acid lactit bacteria and amilolitic bacteria. *Tape* yeast is mix population consists of species of genus *Aspergilius*, *Saccharomyces*, *Candida*, *Hansenulla*, dan bakteri *Acetobacter* [6]. In the initial step of fermentation, in low pH pulp (3.0 – 4.0), high sugar content (8–24%) as well as low oxygen pressure are favorable for yeast development. Yeast development is very dominant during 24-36 hours of fermentation. During this step, yeast activity is very intense and more than 90% of total microorganism is yeast. Yeast holds role in breaking glucose into alcohol [21].

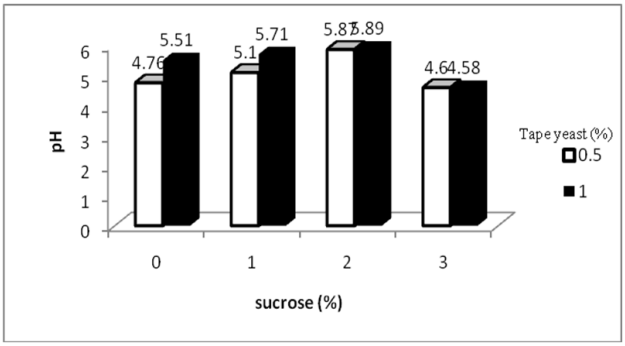

Figure 2. pH of dried cocoa beans after fermentation

Yeast types commonly appear in cocoa beans furing fermentation are *Saccharomyces cerevisiae*, *Saccharomyces theobromae*, *Saccharomyces ellipsoides*, *Saccharomyces apiculatus* dan *Saccharomyces apimulus* [22]. Yeast inoculation increase the number of microbial break glucose reduce pulp into ethanol. The increasing fermentation process occurs because of oculation *Saccharomyces cerevisiae*microorganism and some culture bacteria which induce fermentation process of cocoa beans. The main activity of yeast is metabolized organic acid which contain in relatively a great amount in pulpa of cocoa beans. It makes the reducing organic acid then induce the pH of cocoa beans. Stated by [23], pH changing occur because in the initial process of fermentation acid metabolism accurse in relatively numerous in pulp of cocoa beans.

C. Sugar Reduction

Sugar reduction in cocoa seed is one of the important substance beside amino acid and peptide which role as a taste precursor as well as chocolate flavor [24], [25], [26], [27].

Measurement of sugar reduction level indicate that *tape* yeast addition influence the level of sugar reduction, as well as sucrose addition influence the sugar reduction but there is no interaction between *tape* yeast and sucrose. The concentration of sugar reduction of cocoa seed can be seen in the table below.

TABLE I. THE CONCENTRATION OF SUGAR REDUCTION OF DRIED COCOA SEED

Yeast *tape* (%)	Sucrose concentration (%)				
	0	1	2	3	mean
0.5	0,89	1,31	1,48	1,37	1.26 b
1.0	1,02	1,37	1,43	1,48	1.33 a
mean	0,96 a	1,34a	1,43b	1,46b	

Table I shows the addition of 1% *tape* yeast produces cocoa seed with a reduction level of 1.33% and significantly different from 0.5% yeast concentration. While the addition of 3% sucrose produces cocoa seeds with 1.46% reduction sugar. This is because the activity of inverter enzymes that play a role in the decomposition of sucrose into reducing sugar applied well. According to [28], on the natural fermentation of sugar contained in the seeds of sucrose, during fermentation converted into glucose and fructose by inverter enzymes. [29] states that *Saccharomyces cerevisae* produces enzyme invertase and zimase enzymes. The inverter enzyme serves as a sucrose breaker into glucose and fructose, while the zimase enzyme converts glucose to ethanol. According to [30], yeast can produce invertase, maltase, glycogenase, phosphatase, amylase, oxidoreductase, hexokinase, carboxylase, protease, and peptidase enzymes.

The high content of pulp and sugar encourages the high activity of yeast at the beginning of the fermentation process. This is supported by [31] that the degradation of sucrose is caused by the activity of microorganisms that produce the sucrose degradation enzyme that is the invertase and acid microbe that produce acid. The main source of invertase comes from yeast and other fungi. The higher the enzyme concentration the more active side of the enzyme that binds to sucrose so the higher the reducing sugar is produced. This is in accordance with the theory that the rate of the reaction depends on the concentration of the enzyme acting as a catalyst in a reaction. Increased concentrations of enzymes will generally increase the hydrolysis of substrates into products [32].

Cocoa sugar content is located on the pulp attached to the seed, pulp contains more sucrose, a little glucose and fructose, while if the cocoa seeds have been fermented it contains more glucose and fructose and a little sucrose [33]. Fructose is the main reducing sugar in cocoa seeds and is 92% of the total reducing sugar or 64% of the total sugar [34]. The presence of reducing sugar signifies the degradation of sucrose. The reducing sugar content is affected by the speed of the sucrose invertation reaction and the reduction of reducing sugar to alcohol and acids. The reducing sugar content becomes higher when the sucrose reaction rate is faster than the rate of reaction of reducing sugar degradation into alcohol and acid [33]. During cocoa fermentation, sucrose is almost entirely hydrolyzed to fructose and glucose by invertase present in the seed [35]. The concentrations of fructose and glucose increased significantly during fermentation after seed death [36], [37].

IV. Conclusion

Applying yeast and sucrose influence definitely to the Fermentation Index (FI) and pH but there is no definite influence to the concentration of sugar reduction of dried cocoa seed.

Applying 1% of *tape* yeast concentration and 2% of sucrose is the best application to Fermentation Index (FI) and pH as well as sugar reduction, FI score of 1.17, pH 5.89, and sugar reduction 1.48%.

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