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RESEARCH DEVELOPMENT OF PROCESSING COCOA BEANS

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ABSTRACT

The purpose of this study is to get a fresh way of processing cocoa beans or cocoa beans are not fermented into (1) dry cocoa bean with traits such as fermented, (2) powder-paste dry beans with traits such as fermented, through the removal of the pulp and seed coat cocoa, as well as pH and temperature control as well as certain long. Stages of the study consisted of : stages of treatment research 1 Introduction the determination of the cocoa beans before incubation, 2. Determination of treatment introduction to cocoa beans before incubation and incubation media solution pH determination, 3. Determination of treatment introduction to cocoa beans before incubation and incubation media concentration, 4. Incubation process conditions and incubation media concentration. The results showed that the fermentation cocoa with formic acid buffer immersion obtained results with the highest nitrogen index and the highest acidity at a concentration of 48 to 175 mM in incubation buffer using formic acid, are the best conditions for dry cocoa beans. The results of the color index during incubation at 12, 24, 36 and 48 hours of incubation media with 175 mM concentration 0.9440; 0.9952; 1.0961, and 1.1623. So the application of the cocoa beans using Formic acid as a medium 175 mm marinade for non pulp cocoa beans for 36 to 48 hours. Fermented cocoa with acid immersion liquor acetate gives the expected product form acceptable to the consumer. The use of acetate acid is a material that is very cheap and easily available in the market.

Key Words : Cacao, cocoa bean, fermentation, processing.

INTRODUCTION

This research was conducted within the scope of 'technique development of cacao processing under the absence of microbiological fermentatationto produce good quality of raw materials and roasting product'. It has never been done previously and based on new recent research with which the objective of the research was achieved. It is facts that cacao bean exports are important non gas-oil foreign exchange revenue although most of the export is low in quality particularly if the bean comes from small holder plantation. It is also a fact that processed cacao enter domestic market through import. The main challenge to improve the quality of cacao bean is to find easy and quick ways to process the cacao bean to become dried qualified cacao beans and downstream products. Low quality cacao beans are produced by either in the absence or lack of fermentation, and even very acid cacao bean. Farmers' reluctance to cacao beans being fermented is due to large heaps of cacao beans needed and necessity to stirring the beans for several times to achieve a required temperature, as well as tool requirements such large boxes to contain the beans. Very acid cacao beans are generated from UAH clone cacao plant culture which results in thick fruit meat or pulp. One of fermentation functions is to remove the pulp from the cacao beans. Thick pulp with high sugar content creates lots of acid. The pulp can be reduced or removed using *depulper* with which the pulp is mechanically peeled away. Soaking in an acid solution at controlled temperature and incubation period can replace the

fermentation process. The soaking technique is expected to have better result within a shorter period of process. The treatment of acid dissipation into cacao bean can be accelerated by way of pressurization within an airtight container until appropriate homogenous pH condition is met in any part of the bean. Temperature and incubation period are further controlled in order to obtain fermented-like cacao bean. Un-soaking bean fermentation allows better polyphenol oxidation because higher air exposure of the beans creates more contacts to oxygen. If fresh cacao beans in a container is the pulping and the husking with acid further added into the container under pressurization, acid dissipation will be more rapid allowing more intensive polyphenol oxidation. Crushed the pulping and the husking beans controlled under pH, temperature and incubation period will lead to more rapid and homogenous enzymatic reaction creating precursor taste similar to that under fermentation. The cacao beans might be roasted in paste form. The beans which characteristics similar to that the fermented ones can be crushed and roasted at certain temperature and period to achieved the required taste. Roasting creates homogenous contacts among reactants allowing *Strecker* degradation and *Maillard* reaction to take place well and rapidly. These kinds of processing similar to fermentation can be implemented at large scale industry. The aims of the research was to find new ways for unfermented cacao bean processing to become (1) dried cacao beans which characteristics similar to fermented beans, (2) pasta-powder of dried cacao bean which characteristics like those treated with fermentation (un-pulped and un-skinned cacao bean), as well as controlled pH and temperature for a certain period.

RESEARCH METHODS

The research was carried out in several stages as follows:

1. First Stage. Fresh cacao beans with their pulp attached were directly incubated in 20, 40 and 60 mM formic acid buffer and acetate acid buffer solutions. Their pH was analyzed after 3, 4 and 5 days of the incubation.
2. Second Stage. Fresh cacao beans with their pulp removed using sands were washed. The un-pulped cacao beans were then incubated in formic acid buffer and acetate acid buffer solutions at pH 5. After 1, 2 and 3 days of the incubation, their pH was analyzed.
3. Third Stage. Fresh cacao beans which pulp mostly removed using *depulper* to produce reduced pulp fresh cacao beans. The beans then were washed and incubated in10 mM formic acid buffer and acetate acid buffer solutions at pH 5 and 5.5, respectively. The incubation lasted for 3 days within which the acidity was analyzed every 48 hours.
4. Fourth Stage. Fresh cacao beans were *depulper*. Unwashed depulped cacao beans were then incubated with 35 mM citric acid solution at pH 4.5 for 20 hours. The beans then were drained and incubated in 200 mM Citric acid buffer formic acid buffer solutions at pH 5.5. The beans were then sampled at 0, 24, 48, and 60 hours after incubation and dried for pH and nitrogen index analysis.
5. Fifth Stage. This stage was a continuation of the fourth stage within which the cacao beans were sampled at 48, 60, and 72 hours after the incubation. The bean samples were dried and analyzed for acidity.

The research was conducted in Agroindustri Laboratory of Agriculture Faculty of Tadulako University, Palu, Agricultural Product Processing Engineering Laboratory, Food Chemistry and Biochemistry Laboratory, Processing Engineering Laboratory, Center for Food and Nutrition Studies of Gadjah Mada University during September 2012 to January 2013. Chemical analysis done on the samples included:

a) Analysis of soluble and total nitrogen contents using Rohan and Stewart method (1967).
b) Analysis of dried bean acidity (pH) (Yusianto and Teguh, 1991).

A completely Randomized Design with two-factorial experiment was employed with variables observed were bean pH, total nitrogen, soluble nitrogen, nitrogen index and color index. There were 3 controls including fresh cacao beans, farmers' conventional fermented cacao beans and citric incubated cacao bean (0 hour). The factors were incubation period variations and formic acid concentration variations.

Each treatment was replicated twice with thrice replicates for analysis. Data obtained were statistically analyzed using ANOVA to determine the significance of the treatments. The analysis was continued using Duncan's Multiple Range Test (DMRT) at 95% level of significance for differences determination.

RESULTS AND DISCUSSION

Acidity Level. This research seeks an optimal method using formic acid buffer solution. Other methods were also explored from various journal articles. The results of the first stage research which incubation using formic acid buffer solution is depicted in Figure 1.

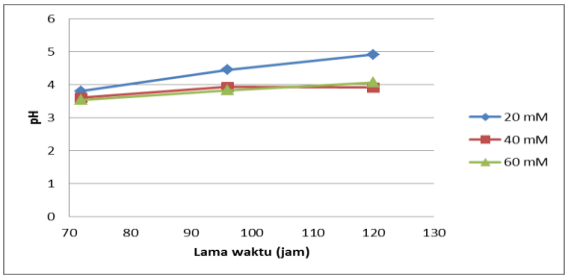

Figure 1.

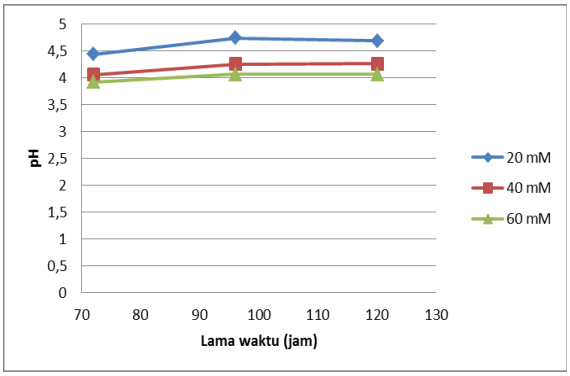

Figure 2.
Cacao Bean pH Under Various Formic Buffer Concentrations at 50^{0}C

Figure 1 shows that the cacao beans pH tends to increase with longer period of incubation when the temperature maintained at 40^{0}C. Similar trends is also occurred when the temperature raised to 50^{0}C although the pH is higher (Figure 2).

The increase of pH in this research is different from previous research. During fermentation pH will decrease from 6.6 to 4.8 and acetic acid formed during this process then enters the beans causing mortality to the seed (Biehl *et al*., *1982*). The pH slowly increases with incubation period and reach 5.5 as shown in Figure 3. In this research, 2 incubation media were used namely acetic acid buffer and formic acid buffer at 50^{o}C and pH 5.

At the second stage, the pH of the beans incubated decreases with longer period of incubation but the pH is still sufficiently high and unstable. This might due to the pulp removal using sands was not optimal yet as many sands still attached to the pulp. Thus, washing was done repeatedly.

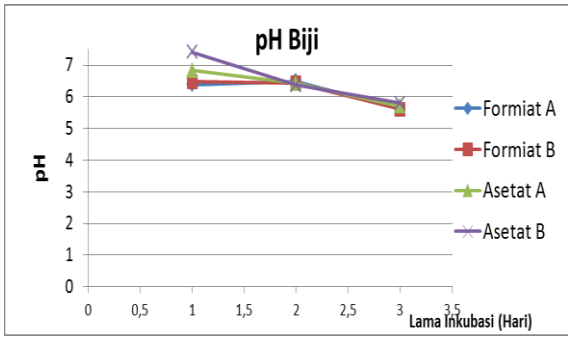

Figure 3.
Cacao Beans pH vs. Incubation Periods at Two Various Acids (Initial Experiment to Determine Treatments)

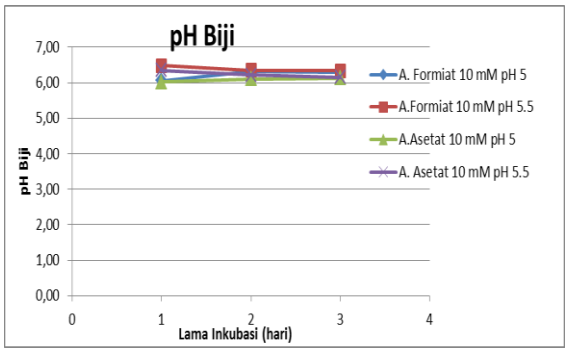

Figure 4.
Cacao Beans pH vs. Incubation Periods Under Various Acids and pH

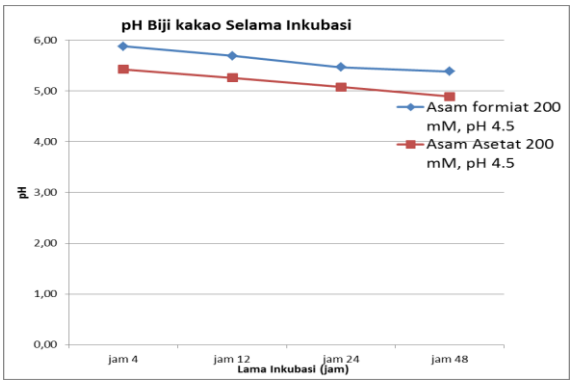

Figure 5.
Beans pH vs. Time (Hour) Under Formic Acid and Acetic Acid

At the third stage, the technique to remove pulp was changed into that using a depulper machine. The use of this machine can save time and the beans produced were better then those using sands. The incubation media used were formic acid buffer and acetic acid buffer at pH 5 and 5.5, respectively and each at 10 mM concentration. These media were developed from the previous stage along with that of Misnawi *et al*. (2003) who used 5 g cacao powder. The cacao powder was defatted using 250 mL of 10 mM acetic acid buffer at pH 5.5.

Figure 4 indicates that the pH of the beans incubated in formic acid is not yet stabile, thus it cannot reflect what the end result is. In general, the incubation in the two acids at different pH reduces the beans pH but the pH is still sufficiently high. This pH range has not met the standard pH of 5 – 5.5 that causes seed mortality (Biehl *et al*., 1982). Masnawi et al. (2003) use a similar concentration but the material used was defatted cacao powder. Therefore, the unclear results generated from the third stage is very likely due to the acid concentration used was very low whereas the material used was the skinned cacao beans. Under low acid concentration and the presence of skins at the fresh beans, it was hard for the acid to enter the beans causing the beans to have sufficiently high pH.

At the fourth stage, Kirchhoff and Biehl (1989) method was used in which formic acid buffer was utilized with additional treatment such as drying after the incubation period. Figure 5 shows that the beans pH decline with increasing the period of incubation in which the formic acid buffer produces higher pH than the acetic acid. It is due to that formic acid is more easily to evaporate than acetic acid. With additional treatment such as drying, the evaporation of acid in the cacao beans becomes more intense. Thus, it is expected that dried cacao bean can reduced their pH better than those generated by conventional methods.

The fifth stage used the same method as in the stage four except the incubation period were extended to 48, 60 and 72 hours with additional treatments i.e. after incubation the beans were soaked for 2 hour and then dried. The results of the fifth stage were similar to that of the previous stage. So it is concluded that the incubation treatment only slightly affected the acidity of the dried cacao beans.

The results of acidity level observations are as follows:

The method of Kirchhoff and Biehl (1989) was adopted using varied incubation periods of 0, 24, 36, and 48 hours with only formic acid buffer used because based on the research results this formic buffer is less acid than acetic acid.

This research is developed by varying the concentration of formic acid based on Biehl (1976) who stated that during fermentation, 70 – 170 mM of acetic acid was produced. Therefore, in this stage the acetic acid concentrations used were 105 mM, 140 mM, and 175 mM. Besides, drying was also implemented before conducting the analysis with expectation that the dried cacao beans would have better acidity level as shown in Figure 6.

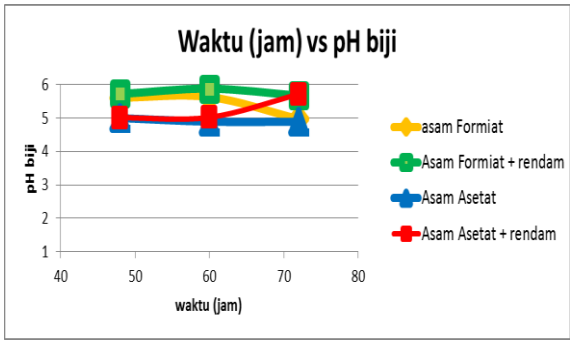

Figure 6.

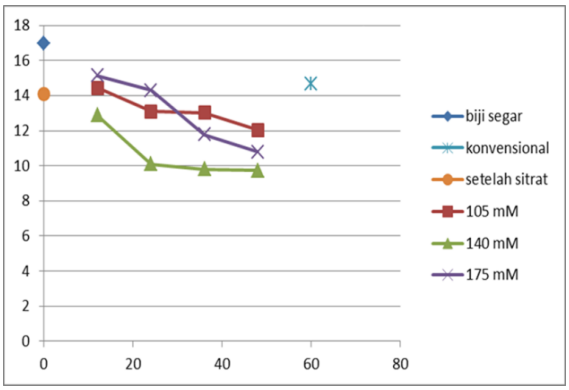

Figure 7.
Total Nitrogen vs. Incubation Period Under Various Control and Formic Acid Buffer Concentrations

Total Nitrogen. Total nitrogen content decline with increasing fermentation period. This is due to some of proteolysis produced was diffused out of the beans (Rohan and Stewart,1967). With reducing pulp by way of the pulp process, it was expected that rapid seed mortality would occur so more nitrogen is diffused out of the seed leading to lesser total nitrogen content.

The dried cacao beans produced through incubating in formic acid buffer affected the amount of total nitrogen (Figure 7). The nitrogen in the fresh cacao bean decreases after incubation in formic acid.

The longer the incubation period and the higher the buffer concentration the lower the total nitrogen concentration. This is due to some proteolysis generated was diffused out from the beans reducing the total nitrogen content within the beans.

Soluble Nitrogen. Soluble nitrogen is one of substances that initiate the taste of chocolate. It is formed by disintegration of protein through proteolysis process by protease enzyme. According to Rohan and Stewart (1976), proteolysis increasingly generates soluble nitrogen substances during fermentation process. The increase of this soluble nitrogen is mainly due to the rate of proteolysis products generated is faster than that the diffusion of the product out of cocoa beans.

Soluble nitrogen of the fresh beans incubated in formic acid increased with increasing time of incubation and formic acid concentration. During fermentation, amino acids are generated as well as soluble nitrogen. This result is in accordance with Zak, Keeney, Biehl *et al.*, (1982) who stated that during fermentation amino acids are produced through proteolysis reaction at the same rate as the development of flavor. In that process, beside amino acids, other soluble nitrogen substances are generated such as peptide.

Nitrogen Index. Based on Gourieva dan Tseravitinov (1979), color index is a comparison between absorbent values of 530 nm wavelength with 460 nm wavelength of cacao powder in a mixture of methanol and concentrated chlorite acid (97:3) after stored in a fridge at 80^0C for 20 hours. Sufficiently fermented cacao beans will have fermentation index equal to or larger than one whilst less fermented cacao beans have index lower than one. The degree of fermentation indicates the amount of protein that has been degraded as free acid amino or the amount of soluble nitrogen generated from protein degradation. Therefore, the degree of fermentation can be determined by measuring the amount of soluble nitrogen formed during fermentation. In this case, the degree of fermentation accounted as nitrogen index which is the ratio of soluble nitrogen content to total nitrogen content. The value of nitrogen index of 40% is acceptable but the best index is 50% (Rohan & Stewart 1967[a]).

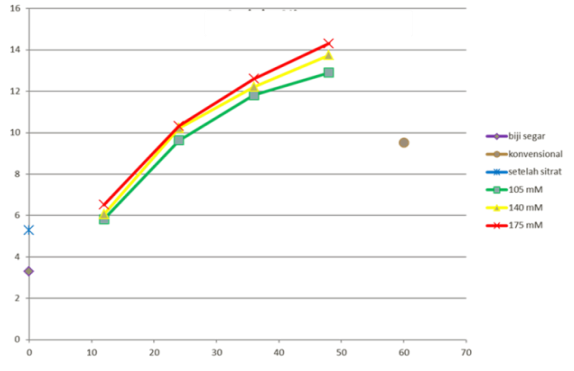

Figure 8.
Nitrogen Index vs. Incubation Periods Under Various Control Treatment at Different Concentrations of Formic Acid Buffer

The nitrogen index of the fresh cacao bean incubated in formic acid increase with increasing incubation period and the acid concentrations (Figure 8).

The Duncan Test indicates that there was no significant difference of the nitrogen index among various concentrations of formic acid buffer at the same incubation period. Similarly, there was no significant difference among various incubation periods at the same formic acid buffer concentration. A comparison between the beans incubated in formic acid buffer and that in conventional incubated showed that the former had larger nitrogen index than the latter. This can be associated with that the conventional fermentation is less controllable leading to over fermentation to occur thus reducing the production of soluble nitrogen. Three days after fermentation soluble nitrogen content declines due to decreasing the rate of proteolysis intensity causing more proteolysis product diffusion out of cacao beans than their formation (Rohan and Stewart, 1967[b]).

Nitrogen index is closely related to the quality of cacao beans because the index reflects the magnitude of soluble nitrogen to total nitrogen ratio. Soluble nitrogen i.e. amino acid and peptide are flavoring precursor which later produce unique chocolate aroma appearing during drying and baking process. Amino acid and peptide are generated under proteolysis process when fermentation takes place. Therefore, the higher the soluble nitrogen the higher the nitrogen index. The amino acid and peptide will react with reduced sugar during baking process resulted in unique chocolate aroma. So increasing higher nitrogen higher index is expected during fermentation process.

Color Index. In general, the index color of the cacao beans incubated in formic acid is higher than that in acetic acid. The anthocyanin index reached 1 under 70 mM acetic acid media and 60 mM under formic acid at 40^0C. During incubation at the first stage, cacao beans post-mortem proteolysis is larger at 40^0C than 50^0C (Biehl, 1982). The result of analysis shows that during the fermentation process, the color index tended to decrease along with increasing concentrations. This is due to the still presence of thick pulp which enclosed the beans hindered the acid to penetrate the beans. According to Biehl (1989), to produce good quality cacao beans, pulp reducing is necessary when its content is 0.6 ml bean^{-1}.

During fermentation, the color index increased with increasing period of fermentation. The change of the color index is shown in Figure 16. Longer period of incubation allows more acid to penetrate the beans creating wider contact between enzyme and substrate. This leads to more production of enzymatic hydrolyzed anthocyanin. The incubation in formic acid at day 3 (72 h) produced the color index of 1 whereas that in acetic not yet showed a good color index. In general the use of formic acid as the incubation media generated higher color index than acetic acid.

The color index of the beans incubated at pH 5.5 is better than at pH 5. However, at both pH 5.5 and 5 under various acids, the color index had not reached 1. This lower color index is likely due to the very low acid concentration of 10 mM. Under this condition, the acid penetrating the beans are very slow compared to that defatted cacao powder used by Misnawi (2002).

The cacao beans incubated in formic acid generated better fermentation index than that in acetic acid under the same concentration and incubation period. Lower molecule weight of formic acid allows the acid to quickly penetrate the beans. The change of fermentation index with various acids is depicted in Figure 18. Figure 18 shows that a longer period of incubation results in increasing anthocyanin index due to more acid penetrates the beans and longer contact time between enzyme and substrate.

Besides, the higher the formic acid concentration the higher the fermentation index. The acid concentration strongly affected the formation of aroma precursor

(Biehl, 1982). This is due to more rapid acid penetration into the beans as the acid concentration increases leading to quick seed mortality. According to Gourieva dan Tseravitinov (1979), sufficiently fermented cacao beans will have index equal to or more than 1. Based on the research results, the index of 1.1623 was found in the cacao beans incubated for 36 h and 68 h at media concentration of 175 mM and for 48 h at 140 mM concentration. This index is larger than that under conventional fermentation. The research result of Fahmi (2013) showed that the husking treated cacao beans previous to bioconversion led to higher end pH and color index of the beans than the pulping treated beans.

CONCLUSION

Fermented cacao beans incubated in formic acid at concentrations ranging from 48 – 175 Mm was the best condition for good quality dried cacao beans which produced the highest nitrogen index and acidity. The color index of cacao beans incubated in formic acid at 175 mM concentration for 12, 24, 36 and 48 hours were 0.9440, 0.9952, 1.0961 and 1.1623, respectively. Therefore, incubating un-pulped cacao beans in 175 mM formic acid for 36 – 48 should be implemented. Fermented cacao soaked in acetic acid resulted in product expected and accepted by consumers. It is recommended to use acetic acid as it is a very cheap and widely available material in market.

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