Intl. J. Food. Ferment. 5(1): 11-16, June 2016

DOI: 10.5958/2321-712X.2016.00002.8

Changes in Chemical Properties of Dreid Cocoa (*Theobroma cacao*) Beans during Fermentation

Mulono Apriyanto

Department of Food Technology, Faculty of Agriculture, Universitas Islam Indragiri, Tembilahan, Indragiri Hilir, Riau. Jl. Propinsi parit 1 Tembilahan. INHIL-Riau, Indonesia

*Corresponding author: mulonoapriyanto71@gmail.com

Abstract

Changes in acidification and sugars of cocoa pulp during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans were investigated using a 3 × 3 full factorial experimental design with tree level treatment (control (TI), inoculum added in begin fermentation (IA), inoculum added in step (IB) and fermentation time as principal factors. pH, non-volatile (titratable) acidity, reducing sugars, and fermentation indexes of cocoa beans were studied using standard analytical methods. pH of the cocoa nibs increased with inoculum added and fermentation with consequential decrease in non-volatile acidity. Contrary, inoculum added and fermentation decreased the reducing sugars in cocoa nib. The most abundant fermentation indexes in unfermented cocoa bean with values of (TI) 0.31 – 0.88, (IA) 0.32 – 0.99, (IB) 0.33 – 1.03).

Keywords: cocoa beans, drying, fermentation, non-fermented

Post–harvested cocoa beans are handled by farmers into two forms: dry fermented and dry non-fermented beans. According to National Statistic Board (Anonym, 2013), annual production in 2013 was approximately 545,000 tons for dry cocoa and 38,5000 tons for the non fermented. Non fermented dry cocoa beans alone was classified into dried (farmer production) and half-fermented dry beans. Around 93% of cocoa produced in Indonesia handled only by washing and sun-drying without fermentation, the remaining 7% produced otherwise by both private and state plantation (Anonym, 2013).

Cocoa fermentation is basically conversion of pulp's sugar and citric acid into organic acids by microorganism (Camu *et al.,* 2008; Ardhana and Fleet, 2003). The organic acids will induce enzymatic reaction inside the beans resulted biochemical changes generating several compounds critical for aroma, taste, and color formation (Biehl *et al.,* 1985; Afoakwa *et al.,* 2014), This process was done by heaping the cocoa beans inside closed container or basket for 5-7 hari with turning once every 2 days. Without fermentation, cocoa beans will taste bitter, astringent, and without distinctive cocoa aroma after processing (Schwan and Wheals, 2004).

Dry cocoa beans lost most of their moisture content and substrate. Around 35% water is needed during fermentation, used for enzymatic enzymatic reaction inside the beans and microbial growth in pulp (Schwan and Wheals, 2004) as the media for enzyme-substrate interaction to mediate hydrolysis and oxidation to generate precursors for cocoa taste, color, and aroma. Substrate is any substances converted by microorganism during fermentation, such as pulp's sugar and citric acid, which get metabolized into organic acids. Acids will then diffused into the beans and induce enzymatic reaction for taste, aroma and color (Afoakwa *et al.,* 2014). Considering this critical

role, rehydration is done for cocoa beans prior to fermentation. Experiment on non-fermented dry cocoa beans was conducted to measure chemical composition changes during the beans fermentation.

Material and Methods

Cocoa beans drying

Cocoa beans were taken from yellowish-orange ripe pods identified with tapped hollow sound, then dried inside cabinet dryer at 40°C until moisture content was 15%. A hundred gram dry beans wetted with 60 ml water, then fermented for 5 days (120 hours) spontaneously at room temperature. Reducing sugar, pH, acidity, fermentation index, and microbia succession were observed, compared to the same parameter taken from fresh cocoa beans fermentation.

Pulp preparation

Pulp was manually separated by peeling the cotyledons using sharp knife.

Fermentation of dried cocoa beans

Fermentation of dried beans was done in glass jar; each contained 100 gram. Beans were pre-rehydrated using 60ml of distilled water, then incubated at room temperature for 5 days. The treatment consists of the fermentation: 1. Fermentation without the addition of inoculum (control) , 2. Fermentation with the addition of inoculum beginning (IA) and 3. Fermentation with addition of inoculum stages (*Saccharomyces cerevisiae* beginning, *Lactobacillus laktis* on the hour to 36 and *Acetobacter aceti* in hours to 48) (IB).

Preliminary analysis

This stage was done to measure moisture content using gravimetric method and reducing sugar in fresh and dry pulp using the method by Nelson-Somogy (Sudarmadji *et al.*, 1997).

Experimental design

A 3 × 3 full factorial experimental design was used for the study. The principal factors investigated were inoculum added (control (TI), inoculum added in begin (IA), inoculum added in step (IB) and fermentation time (0, 24, 48 days). The pH, non-volatile (titratable) acidity, reducing sugars, total solids and mineral composition of the pulp were studied.

pH and non-volatile (titratable) acidity

pH and non-volatile (titratable) acidity of the pulp were determined using the method described by Nazaruddin *et al.* (2006) with slight modifications. Ten grams of the pulp was homogenized in 90 ml of hot distilled water, stirred manually for 30 s and filtered using Whatman No. 4 filter paper and cooled to 20–25°C. Twenty five (25) ml aliquot of the resulting filtrate was pipetted into a beaker and the pH was measured using a pH meter (model MP230 Mettler Toledo MP 230, Mettler Company Limited, Geneva, Switzerland) calibrated with buffers at pH 4.01, 7.00 and 9.21. A further 10 ml aliquot was used to determine acidity by titration to an end point pH of 8.1with 0.1 N NaOH solution and the values reported as moles of sodium hydroxide per 100 g sample. The analysis was conducted in triplicates and the mean values reported.

Fermentation index determination

Five hundred milligrammes of defatted cocoa powder were weighed into a 125 ml conical flask before a mixture of 50 ml of methanol: hydrochloric acid (97:3) solutionwas added, the mixture was cooled at 8 ± 2°C in a refrigerator for 16–18 h. A clear extract was obtained by filtration through a Whatman No. 1 filter paper. Fermentation index was calculated, based on the ratio of the absorbance at 460 nm to the absorbance at 530 nm.

Determination of reducing sugars

Reducing sugars of the pulp was determined using the phenol sulphuric acid method as described by Brummer and Cui (2005) with slight modifications. About 0.5 g of the pulp was boiled in 30 ml 80% ethanol under reflux for 30 minutes. The supernatant decanted into another round bottom flask. The

collected supernatant was concentrated (not to dryness) under reduced pressure using the rotary evaporator. After the removal of ethanol, the extract was then clarified using 7.2 ml of 5% $ZnSO_4$ and 10 ml of 0.3 N barium hydroxide octahydrate [$Ba(OH)_2$. $8H_2O$] to precipitate proteins, colour, and other organic substances out of the solution and allowed to stand for about 5 minutes and then filtered.

Statistical analyses

SPSS software IBM version 22 was used to analyzed the data for analysis of variance (ANOVA). Least significant difference (LSD) was used to separate and compare the means, and significance was accepted at 5% level ($p < 0.05$).

Results and Discussion

Changes in pH profile of cocoa pulp

The quality of cocoa beans resulted from fermentation is determined by pH and acidity during fermentation. Microorganism degraded pulp's sugar into alcohol and organic acids diffused onto the beans. Since the diffusion is critical to start biochemical reaction inside the beans, pulp degradation during fermentation determined beans quality. There is inverse correlation between pH and acidity; lower pH indicated higher acidity.

The pH tree level experiment in begin fermentation decreased after 3 days slightly to increased, which has been reported to reduce the pulp volume per seed leading to the decrease in citric acid concentrations (Biehl *et al*., 1989; Sanagi *et al*., 1997). The pH of unfermented cocoa ben has been reported to range between 5.7 – 6.0 (Schwan and Wheals, 2004). The pH of unfermented cocoa bean (TI) was (6.0) which decreased gradually to 3.5 after 3 days during fermemtation. After 3 days increased gradually to 4.7. (Fig. 1).

During initial period of fermentation, beans acidity increased for 3 days then subsequently decrease until the final period. Initially, acids were produced but diffusion was still limited, thus pH was low but increased in the range of 5-6 in the third day (Schwan *et al*., 2003).

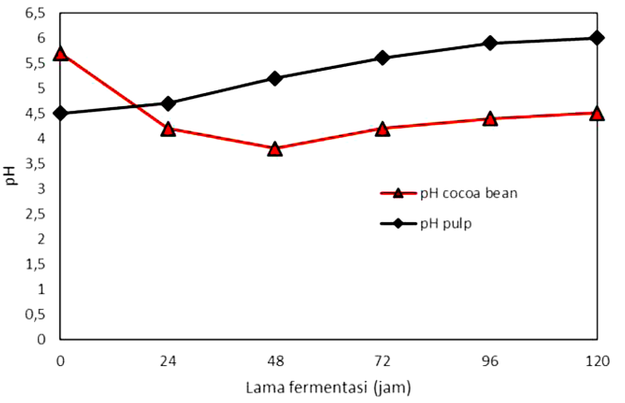

Fig. 1: The Changes in pH pulp during cocoa beans fermentation

Changes in non-volatile (titratable) acidity of cocoa pulp

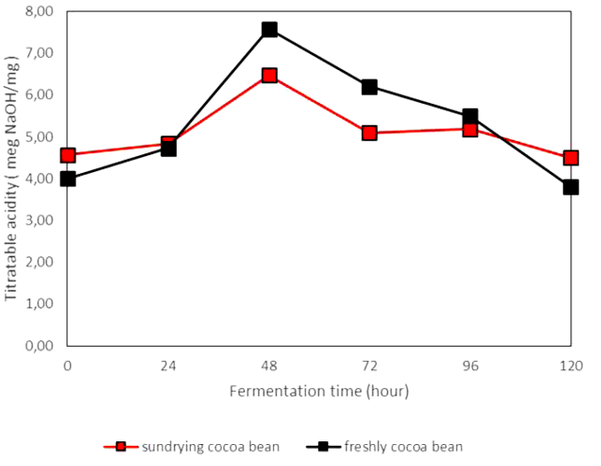

Fig. 2: The Changes in titratable acidity of cocoa beans during fermentation

During fermentation of cocoa beans, microorganisms breakdown the sugars in the pulp resulting in the production of alcohols and organic acids, predominantly acetic acid which then diffuse into the beans. Production of acids in the pulp is important in cocoa fermentation as these acids diffuse into the beans and subsequently induce the important

biochemical reactions leading to well fermented cocoa beans. However, high acid production in the pulp is detrimental as it leads to excessive acid diffusing into the beans resulting in the production of acidic beans.

Acidity was then slightly decreased in following days due to part of organic acids diffusion or evaporation, as presented in Fig. 2.

The interaction between fermentation time and preparation cocoa beans significant ($p < 0.05$) effect acidity level of pulp. At the figure 2 noticeable decrease in acidity occurs after hours to 48 as reported also Rossane and Shcwan, (1998), This might be due to the fact that reduced pulp volume per seed, reduced pulp sugar content and thus, increased micro-aeration within the pulp. This decreased the sugar metabolized by yeasts during subsequent fermentation and eventually reduced alcohol fermentation and acetic acid formation in the pulp (Biehl *et al*., 1989). This suggests of cocoa beans preconditioning could be effectively employed to reduce acidity levels in cocoa beans during fermentation.

Reduction sugar

Reducing sugar in each treatment increased from 4.5% - 4.69% to 11.45% - 11.54% in 24 hours and then declined slowly to 10.5% at the end of fermentation it is in line with research Afoakwa *et al.*, (2014) that reduced sugar increased during fermentation, fermentation of natural sugars initial reduction 3:57 mg/g then rose to 10.69 mg/g at the end of fermentation. In the fermentation of cocoa beans in the pod, reducing sugar also increased from 3.89 be 11.94 mg/g, 4:29 to 13:34 mg/g, and 4:52 to 13:56 mg/g on a long fermentation 3, 7, and 10 days, and the results of this study did not in line with Ardana research and Fleet, (2003) found decreased levels of glucose in the frutosa and cocoa beans fructose decreased from 0.8 mg/g to 0.3 mg/g and glucose of 0.6 mg/g to 0.1 mg/g during fermentation.

If you look at the treatment of pure inoculum addition it appears that the addition of pure inoculum increase the sugar content reduction, it is suspected because it degrades inokulummurni sugar in an amount more than those who did not plus pure inoculum as presented in Fig. 3.

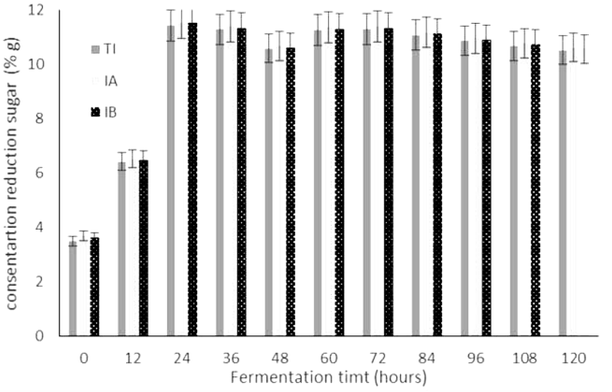

Fig. 3: The changes reduction sugar during fermentation

Quality Seed Fermentation

pH and acidity of cocoa beans

Quality fermented cocoa beans is determined primarily by the acidity (pH) and non-volatile (titratable) acidity of cocoa *beans* during fermentation. Microbial during fermentation degrading of sugar in the pulp to produce alcohols and organic acids are diffused kedalaam seeds.

Acid production from the degradation of the pulp is very important in the fermentation, the beans become tedifusinya acid into the initial reaction in the seed begins bikimia that will produce good fermented cocoa beans.

Early in the seed fermentation acidity increased until the third day of fermentation and then decreased until the end fermentasai. This is caused by fermentation has not yet occurred at the beginning of the diffusion of acid into the seed so that the value of acidity lower seed with a high cocoa beans pH between 5 - 6. After the third diffusion lead acid acidity seed value decreased because some of the acid evaporates diffused so low acidity seed. Changes in the acidity of seed shown in Fig. 4.

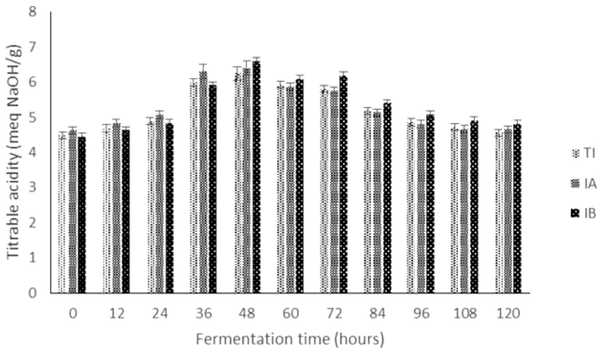

Fig. 4: The Changes in acidity during fermentation of cocoa beans

Between pH and acidity interconnected seed where the pH indicates a low value then the value increases acidity seed. Processing cocoa beans require a pH between 5.2 to 5.8 to produce high quality cocoa butter (Wood and Lass, 2001). Observational data of pH of seeds during fermentation is presented in Figure 5

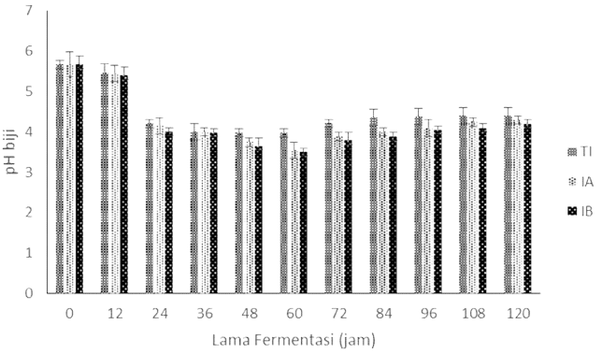

Fig. 5: The changes in pH during cocoa beans fermentation

Dry beans drying initial pH 5.7 and then decreased to 3.8 in the hours to 48. This is caused by the fermentation of organic acids undergo diffusion into the seed then increased to pH 4.4 due to some organic acid begins to evaporate and partly left behind in the seed. The quality of fermentation quality is also measured fermentation indexs.

Fermentation indexes

Fermentation index is an index that compares the absorbance at a wavelength of 460 nm and a wavelength of 540 nm. A good fermentation, the fermentation of the resulting index close to 1. Results of fermented dry beans dry in the sun and fresh cocoa beans is presented in Fig. 6.

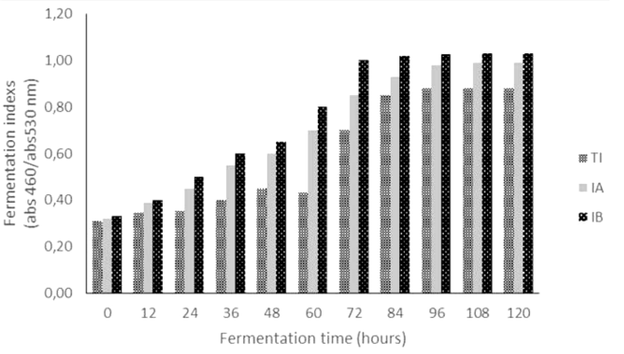

Fig. 6: Change fermentation indexs cocoa bean during fermentation

In Figure 6 shows that the index lower control fermentation at the end of fermentation, this is possible because the amount of microbial fermentation less, it is characterized by the fermentation temperature is not reached as reported also cempaka *et al.* (2014).

Conclusion

From the results of this study concluded that during drying the microbe is not dying, so when rehydrated. This can be seen in the succession of microbes that occur with yeast population, bales, acetic acid bacteria during fermentation. The quality of dried cocoa beans fermented drying when compared with fresh fermented cocoa beans have in common. Index results fermented dry cocoa beans drying the highest is 0.95. From whole fermentation quality data it can be said that the quality of dried cocoa beans drying can be improved through fermentation. To speed up the fermentation process can be done adding inoculum in order to get a good fermentation index.

References

Afoakwa, E.O., Budu, A.S., Mensah-brown, H. and dan Felix, J. 2014. Changes in Biochemical and Physico-chemical Qualities during Drying of Pulp Preconditioned and Fermented Cocoa (*Theobroma cacao*) Beans. *Internasional Food Research Journal*.

Afoakwa, Emmanuel Ohene., Kongor, J.E., Takrama, J. and Badudu, A.S. 2013. Changes in nib acidification and biochemical composition during fermentation of pulp preconditioned cocoa (*Theobroma cacao*) beans. *Internasional Food Research Journal*, **20**(4): 1843–1853.

Anonim. 2013. Produksi Perkebunan Besar menurut Jenis Tanaman, Indonesia. *Http://www.bps.go.id. Diakses Tanggal 29/03/014*.

Ardhana, M. and Fleet, G. 2003. The microbial ecology of cocoa bean fermentations in Indonesia. *International Journal of Food Microbiology*, **86**(1-2): 87–99. http://doi.org/10.1016/S0168-1605(03)00081-3

Biehl, B., Brunner, E., Passern, D., Quesnelh, V.C. and Adomako, D. 1985. Acidification, Proteolysis and Flavour Potential in Fermenting Cocoa Beans, 583–598.

Camu, Nicholas Camu, N., Winter, T. De, Addo, S.K., Takrama, J.S., Bernaert, H. and Vuyst, L. De. 2008. Fermentation of cocoa beans : influence of microbial activities and polyphenol concentrations on the flavour of chocolate, 2297(May), 2288–2297. http://doi.org/10.1002/jsfa, Winter, T. De, Addo,

Erna, M. and Setyani, S. 2008. *Pengaruh penambahan inokulum campuran terhadap perubahan kimia dan mikrobiologi selama fermentasi coklat*, **13**(2): 73–84.

Moreira, I.M.D.V., Miguel, M.G.D.C.P., Duarte, W.F., Dias, D.R. and Schwan, R.F. 2013. Microbial succession and the dynamics of metabolites and sugars during the fermentation of three different cocoa (*Theobroma cacao* L.) hybrids. *Food Research International*, **54**(1): 9–17. http://doi.org/10.1016/j.foodres. 2013.06.001

Nazaruddin, R., Seng, L.K., Hassan, O. and Said, M. 2006. Effect of pulp preconditioning on the content of polyphenols in cocoa beans (*Theobroma cacao*) during fermentation. *Industrial Crops and Products*, **24**(1): 87–94. http://doi.org/10.1016/j.indcrop.2 006.03.013

Schwan, R.F. and dan Wheals, A.E. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Critical Reviews in Food Science and Nutrition*, **44**(4): 205–221. http://doi.org/10.1080/10408690490464104

Schwab, R.F. 1998. Cocoa fermentations conducted with a defined microbial cocktail inoculum. *Applied and Environmental Microbiolgy*, **64**(4): 1477-1483.

Takrama, S.K., Bernaert, H. and Vuyst, L. De. 2008. Fermentation of cocoa beans : influence of microbial activities and polyphenol concentrations on the flavour of chocolate, *2297*(May), 2288–2297. http://doi.org/10.1002/jsfa