Journal of Agricultural Technology 2012 Vol. 8(5): 1613-1623
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Using ultrazyme (Novozyme) for improving cocoa fermentation process and cocoa bean quality in Vietnam

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Binh, P.T., HoaiTram, Tr.T., HoangAnh, T.T., Thuong, N.V., Thoa, P.T., Thao, P.V. and ThamHa, T.T. (2012) Using ultrazyme (Novozyme) for improving cocoa fermentation and cocoa bean quality in Vietnam. Journal of Agricultural Technology (8)5: 1613-1623.

Fermentation of fresh cocoa beans is a process with catalyzing several enzymes and microorganisms to increase the speed of biochemical reactions in pulp and cotyledon. There are two main stages during cocoa fermentation. The initial stage of fermentation (usually two days) is an anaerobic phase of fermentation. In which, the cocoa pulp is broken up, sugar is transformed into alcohol and lactic acid; the following stage (4 days next) is an aerobic phase. Increasing speed cocoa pulp broken up and sorting anaerobic phase time is one of the ways to reduce alcohol and the lactic acid in cocoa bean. Beside, acetic acid is evaporated by high temperature and that is lower in cocoa beans after drying or roasting. Using Ultrazyme 100G (with pectinase) could improve the cocoa bean quality by sorting the anaerobic phase. Enzyme dosages and time adding are tested. Temperature, bean colour, CTS (Cut Test Score), pH, TA, cup testing were collected and evaluated. Results showed in the same condition experiment that using of 80 mg Ultrazyme/kg fresh cocoa bean was good fermentation and bean quality. The temperature increased faster and higher (50.3^0C was peak) than others. The ratio of fully fermentation beans (brown beans) was high (74.75%); purple beans was less (4%); CTS: 853.75 scores; pH = 5:32, TA = 1.15mg NaOH 0.1N/g and cup testing was the best. Enzyme (Ultrazyme 100G) adding at the beginning of fermentation was the best: Temperature increased faster and higher (48.9C was a peak), the percentage of fully fermented beans (brown beans) was 71.0% (higher than others), purple beans was 6.33%; CTS: 823.33 scores; pH = 5.27, TA = 1.16mg NaOH 0.1N/g; cup testing gave better than other.

Key words: quality, pulp, fermentation, cocoa, Ultrazyme.

Introduction

Vietnam is one of the cocoa export countries, but the quality of cocoa beans in Vietnam was not good and stable (Vietnam National extension center,

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2010). Improving cocoa beans quality, stability has to require further researches. Vietnam cocoa beans quality are bad fermentation, not good cup testing, not clear cocoa flavor, high acid content and high smoke (Workshop in "post-harvest technology and quality management of cocoa beans" 4/2010). So improving the stage of cocoa fermentation is one of the ways to change cocoa quality being better. There are two main stages in the cocoa fermentation. The first is anaerobic fermentation in 2 days and the second is aerobic in 4 next days (Wood and Lass, 2001; Arthur, 2006). Temperature in mass beans increases faster in second stage because of the several biochemical-reactions with enzymes catalyzing. The biochemical reactions impact on the precursors-flavor and quality in cocoa beans (Alex *et al*., 1998). Using ultrazyme could catalyze the reactions and improve cocoa quality. Broken up the pulp of fresh cocoa beans using Ultrazyme is one way to increase the temperature in mass beans. Because the aerobic stage was started earlier than normal, so the lactic acid was less and acetic acid was more. The main objective was to investigate the bean quality to improve acetic acid which evaporated with increasing temperature for drying and roasting of cocoa.

Materials and methods

The Ultrazyme from Novozyme Company with 10,000 PECTU/ml was used and storage less than 2 months, d=1.12g/ml. Cocoa bean was the forastero clone in 2011 season from Eakmat Company, Buonmethuot City, Daklak Province, Vietnam.

There were two experiments:- the first was Ultrazyme doses, which treatments were performed as follows:- treatment 1 was 60 mg/kg fresh cocoa bean weight, treatment 2 was 80 mg/kg fresh cocoa bean weight, treatment 3 was 100 mg/kg fresh cocoa bean weight and treatment 4 served as control which did not add enzyme. The second experiment was time of adding Ultrazyme. Treatments were performed as follows:-treatment 1 was add enzyme at the beginning of fermentation (0 h), treatment 2 was 24 hours after fermentation, treatment 3 was 48 hours after fermentation and treatment 4 served as control which did not add enzyme. Both experiments were arranged in Completely Randomized Design with three replications. All treatments were done with six days of fermentation and two times of turning (at 2^{th} day and 4^{th} day) in wooden box. Data were statistically computed analysis of variance and means were compared using Duncan Multiple Range Test at P =0.05.

***Temperature*:** The Ultrazyme catalyses the biochemical reactions and heating cocoa bean mass. The temperature is one of the factors for evaluating cocoa fermentation. Hana electronic thermometer (HI-145, 2009) was used to check the temperature of cocoa in box of each treatment. The 5 points in box at

four corner and central were checked by 12h/time in 6 fermentation days (at 10cm deep from surface of cocoa mass).

***Ratio pulp of cocoa bean*:** Ultrazymes break the pectin to methanol, pectic acid and some cellulose, so the pulp of cocoa beans was released. The ratio of cocoa pulp shows the Ultrazyme catalytic capacity. A 200 g Cocoa bean was collected at 5 points in 10cm deep from surface cocoa mass of each treatment during 6 fermentation days (12h/time) for calculating the ratio pulp in comparison with cocoa bean weight.

***Cotyledon colour*:** The cotyledon colour is showed the quality of dry cocoa bean. Brown cocoa bean (or fully brown) was very good bean (very good fermentation); part purple-brown was good bean (good fermentation); purple was fair bean (a few fermentation); slate bean was not good (not fermentation). Three hundred dried cocoa beans were cut the lengthwise through the middle by using a penknife and checking the colour of cotyledon under the bright light. The ratio of 4 colours were checked and calculated as brown, part purple-brown, purple, slate bean (Anon, 1995).

CTS (cutest score) = *(*10 × % fully brown*)* + *(*5 ×% partly purple - brown*)* + *(*0 ×% fully purple and slate*)* (Anon, 1995).

Shell content: Shell content is one of the qualitative norms of dry cocoa bean. All of chocolate factories preferring the sell content was less. A 100 dry cocoa beans of each treatment was tested by calculating the ratio of shell (AusAID, 2006).

pH: Both pH and TA were made acidity taste which made good or bad product in cup testing. The pH in dry nib was determined according to the AOAC.5. Ground nib (5 g) was homogenized in 45ml boiled distilled water. The homogenate was filtered with No. 4 filter of Whatman paper and cooled to 20–25 ◦C. pH was determined using a pH meter (HI-1221, 2009). This measurement was taken in triplicate.

Titratable acidity (TA): The dry nib TA was determined according to the AuSAID, 2006. About 25mL of the aliquot collected for pH determination was titrated drop by dropping with 0.1N NaOH to pH 7.1, using the pH meter (Hana meter HI-1221, 2009).

Cup testing: Cup testing is one of the evaluated methods for testing cocoa bean quality. In this method the cocoa flavour, acidity, bitterness, astringency and fruit flavour are tested. Cocoa beans (170 g) were roasted in an oven (SANYO MOV-112, 2009) at 145^0C during 30 min and cooled at room temperature. The roasted cocoa beans were broken and winnowed by a laboratory winnower and breaker (John Gordon, UK 1999) to obtain the small cocoa roasted nibs. The roasted nib was ground in a laboratory mill (Capco test equipment, N^0 1193-PA) during 3h to obtain the cocoa liquor for testing.

Ghanaian cocoa liquor was used as reference (AusAID, 2006). The sensory evaluation was conducted by 7 trained panels from WASI.

Statistical analysis: The data obtained from the physical and chemical analyses were analyzed for one-way ANOVA and Duncan's Multiple Range Test using SAS statistical software (Version 9.3, SAS Institute, Cary, NC, USA) at 95% level.

Results and discussions

Experiment 1: Ultrazyme investigation

Mass temperature

Mass temperature is one of the based factors for evaluating of cocoa fermentation. The high and stable temperature showed the good fermentation process as stated by Wood and Lass (2001). The results showed that the temperature in mass of all enzyme treatments was higher than control 1. The temperature reached to 50.3^0C after 78 hours of fermentation in treatment 2 (Fig 1), which showed that the treated enzyme was in good fermentation.

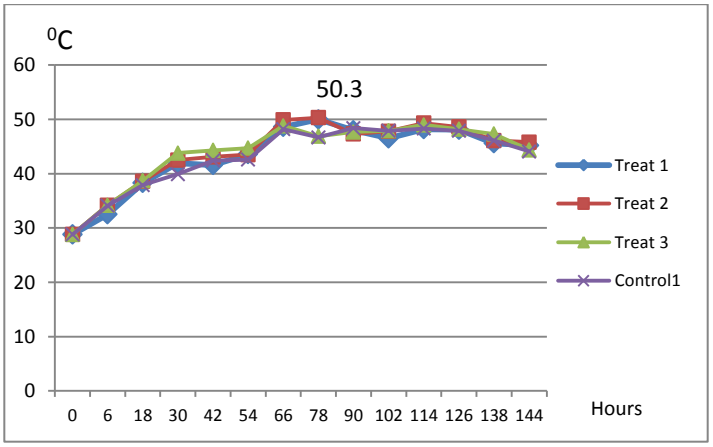

Fig. 1. The temperature in the cocoa mass beans during fermentation

Shell content

The cocoa pulp was broken faster than in general when using Ultrazyme, therefore, the bean shell was very thin. Results showed that treated ones were significantly difference when compared to the control 1 which was the highest shell content (14.92%), and enzyme treatments were not much difference as statistical significance (from 12.53% to 13.32%wb) (Table 1), that were equal

in cocoa shell contents of Ghana and Malaysia (Wood and Lass, 2001). Reducing cocoa pulp in fermentation made a good condition for acid evaporation in drying, then pH increased and TA decreased. However, there was not advantaged when storage bean under thin shell, because the shell would be broken in high column bags (Wood and Lass, 2001).

Table 1. Shell content in cocoa beans

enzyme/fresh cocoa bean weight (mg/kg)	Shell content (%wb)
60	12.53 b
80	12.81 b
100	13.32 b
no enzyme (control 1)	14.92a

Cotyledon color (by cutest)

Beans colors (cotyledon color) are always changed in the aerobic stage (Wood and Lass, 2001; Beckett, 2009) from slate to purple; purple to part brown; part brown to brown and if the fermentation is over time, the brown bean would change to dark brown bean, which is not good for tasting. The bean color is the signs of fermented bean level. The more fermentations are good, the more beans are brown, and these products would make the good chocolate as stated by Wood and Lass (2001) and Beckett (2009). The Ultrazyme catalyzed on biochemical reactions and changed the colour beans, the ratio brown beans in treatment 2 was highest (74.75%); treatments 1, 3 and control were not statistically significant difference at $p \geq 0.05$; the purple brown was lowest (21.25%) in treatment 2, and highest in control (28.25%). The ratio of purple was highest in treatment 1 (9.75%) but was not statistically significant difference with treatment 3 and control 1. The ratio of purple beans of treatment 2 was lowest (4%). Applying ultrazyme at 80 mg/kg fresh cocoa beans was the best and the cut test score was the highest, about 853.75 scores (Table 2).

Table 2. Beans color and cut test score

enzyme/fresh cocoa bean weight (mg/kg)	Bean color (%)			CTS (scores)
	brown	Purple-brown	purple	
60	65.67 b	24.58ab	9.75a	779.58 b
80	74.75a	21.25 b	4.00 b	853.75a
100	66.67 b	26.83a	6.50ab	800.83 b
no enzyme	63.00 b	28.25a	8.33ab	771.25 b

pH and TA (titrable acidity)

pH and TA in dry beans is one of the cocoa bean quality norms. If pH is low and TA is high that creating the higher acid content (Wood and Lass, 2001, Beckett, 2009). When pH was higher, then TA was lower. The treatments were added enzyme in fermentation gave the higher pH than the pH in control 1. The pH in treatment 2 (5.32) was significantly highest at 0.05, the lowest was control 1 (5.12). On the contrary with pH, TA was the smallest in treatment 2 (1.15 ml NaOH 0,1N/g) and the highest was in control 1 and treatment 1 (1.34 ml NaOH 0,1N/g). The changes were consistent with the temperature increasing in fermentation and ratio of brown cocoa beans in the sample (Table 3).

Table 3. pH and TA of dry cocoa beans

enzyme/fresh cocoa bean weight (mg/kg)	pH	TA (ml NaOH 0,1N/g
	5.15 b	1.34 a
60	5.32 a	1.15 b
80	5.18 b	1.27 a
100	5.12 b	1.34 a
no enzyme		

Cup testing

Result showed that there were not different between treatments and control, especially in cocoa flavor that was from 3.1 points to 3.7 points, and the control was the highest (3.7 points); control bitterness and astringency were highest and different from treatments 1 and 3 which statistically significant difference at p= 0.05. Cocoa flavor and fruit flavor between treatments were not significantly different. (Table 4 and fig 2)

Table 4. Cup testing score

enzyme/fresh cocoa bean weight (mg/kg)	Cup testing scores (score)				
	Cocoa flavor	Acidity	Bitterness	Astringency	Fruit flavor
60	3.20a	5.20a	3.40 b	4.00ab	1.20a
80	3.10a	3.80 b	3.80ab	3.75ab	1.10a
100	3.60a	4.95a	3.80ab	3.60 b	1.20a
no enzyme	3.70a	4.85a	4.35a	4.20a	1.20a

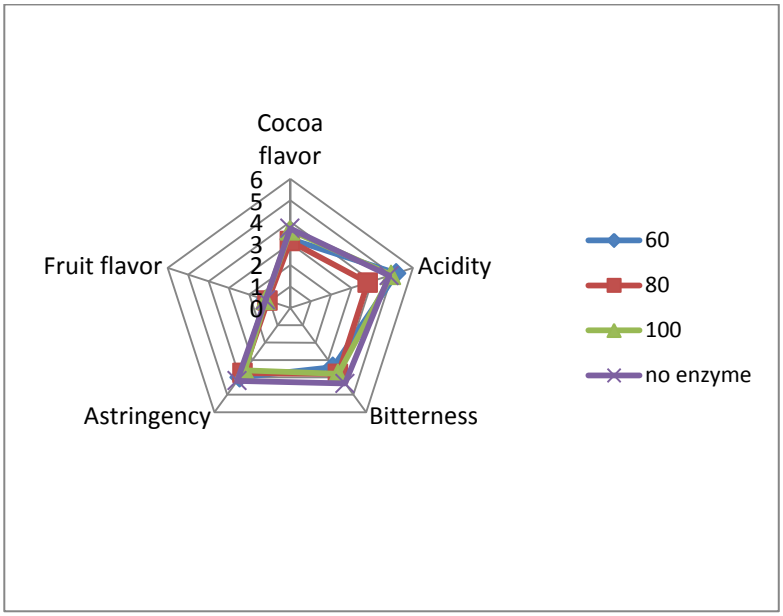

Fig. 2. Cocoa cuptesting scores

Experiment 2: Ultrazyme against time

Ultrazyme adding time was determined and used the treatment of 80 mg Ultrazyme/kg fresh cocoa bean as treatment 2.

Mass temperature

The temperature testing in experiment 2 resulted in the same manner of experiment 1. Result showed that treatments which applied enzyme (treat 4, 5, 6) were significantly differed from the control. The temperature increased very quickly in enzyme treatment. Temperature increased to the highest at 48.9^0C after 96 hours of fermentation (treatment 4, 5 at 24h), at the end of fermentation temperature ranging from $45.1\text{-}46^0$C (Fig 2). This temperature ranging was good fermentation and suitable for any enzyme in cocoa bean and additional Ultrazyme.

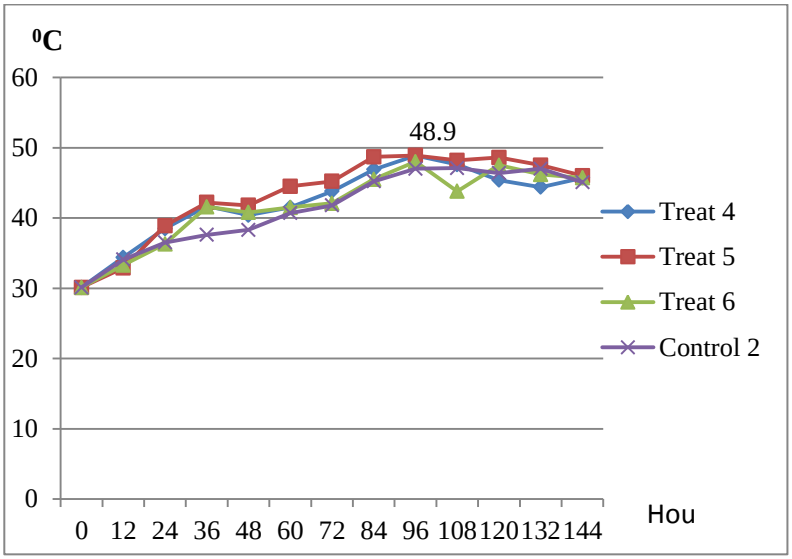

Fig. 3. The temperature in the cocoa mass during fermentation

Shell content

The ratio of cocoa bean shell contents of all treatments was lower than control. The lowest was 12.95%wb in treatment at 24h but that was not different statistically significant at $p \geq 0.05$ with other treats. The highest was control with 14.94%wb, which was different statistically significant at $p \geq 0.05$ (Table 7). This result showed that the pectinase (in Ultrazyme) catalyzed the biochemical reactions in cocoa pulp, broke down pectin and all components ran out. All chocolate plant hope all cocoa beans with low shell content because of the higher incoming but many exporter did not like this because of the lower shell content, the thinner shell content and that was easy broken and not preserved for a long time as stated by Afoakwa (2010).

Table 7. shell content

Enzyme adding time after fermentation(h)	Shell content (%)
0	13.22 b
24	12.95 bc
48	13.42 b
no enzyme	14.94a

Cotyledon colour

The cocoa cotyledon colour was converted into brown during fermentative stage. Brown cocoa bean showed the good fermentation and best product for producing chocolate. The slaty bean showed bad fermentation resulted to be not good products as stated by Wood and Lass (2001). The results showed that the ratio of treated brown beans were higher than control. The ratio of brown beans was highest when treated at beginning fermentation (0 h) with 71% wb and lowest in control with 62.08 % wb. Bean cutest score was highest (823.33 scores) in the treatment at beginning fermentation (0h) and lowest in control (772.08 scores). That was statistically different at $p \geq 0.05$. The ratio of purple bean was not statistically different between treatment and control 2 at $p \geq 0.05$. The ratio of purple bean was highest in treatment 7 (48h) with 8.67% and the lowest in treatment 5 with 6.33 %. Especially, there were not shown the slaty beans in all treatments and control products, which showed that all beans were fermented in boxes (Table 8). Adding preparation enzyme at beginning

Table 8. The cotyledon colour fermentation would be good cocoa colour beans

Enzyme adding time after fermentation(h)	Bean colour (%)			CTS (cutest score)
	Brown	Purple-brown	Purple	
0	71,00a	22.67 b	6.33 b	823.33a
24	66.50ab	26.67ab	6.83 b	789.33ab
48	64.42 b	26.92ab	8.67a	778.75 b
no enzyme (control 2)	62.08 b	30.25a	7.67ab	772.08 b

pH and TA

The pH treatments at 24h, 48h and control were not statistically significant different at $p \geq 0.05$, but the treatment at beginning fermentation was significantly different with others at $p \geq 0.05$. The lowest of pH was 24 h treatment at 5.12 and the highest was 0 h treatment at 5.27, which was the best in chocolate production. TA treatments and control were inversed the proportion to pH. TA treatment at beginning fermentation was the lowest with 1.16mg/g, and the treatment at 24h was highest with 1,3mg/g (Table 9).

Table 9. pH and TA in cocoa bean

Enzyme adding time after fermentation(h)	pH	TA (ml NaOH 0,1N/g)
0	5.27a	1.16 b
24	5.12 b	1.30a
48	5.15 b	1.26ab
no enzyme (control 2)	5.13 b	1.29a

Cup testing

Result showed that cocoa flavor score for treatments after 24 h was lowest and the control was highest, which were statistically significant different at p= 0.05. The acidity score for treatments at beginning fermentation showed lower than other treatments and control, which mean the pectinase activity in Ultrazyme was catalyzed any biochemical reactions and reduced the acid in cocoa bean. Astringency of all treatments was lower than control but only treatment at beginning fermentation was statistically significant different at p = 0.05. Using enzymes at the beginning fermentation would be sour, bitter, and astringency would lower. This was one of the purposes of the additional preparation containing of pectinase (Table 10).

Table 10. Cup testing

Enzyme adding time after fermentation(h)	Cocoa flavor	Acidity	Bitterness	Astringency	Fruit flavor
0	3.40ab	4.40b	4.00b	3.60b	1.30ab
24	3.10b	5.20a	4.70a	3.80ab	0.90b
48	3.65a	4.60ab	4.40ab	3.70ab	1.40ab
no enzyme	3.60a	4.90ab	4.50ab	4.00a	1.60a

Fig. 4. Cup testing scores

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(Published in September 2012)