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

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Reducing sugar is one of the important ingredients of chocolate flavour precursors. Using invertase during cocoa fermentation is one of the ways for increasing reducing sugar in cocoa bean. During fermentation, sucrose is hydrolysed into glucose and fructose. This process is catalysed by invertase and other enzymes. Adding invertase (Novozyme) is one of the main ways to increase reducing sugar and flavour quality of dry cocoa beans in Vietnam. Enzyme doses and time of adding enzyme during fermentation were done. Norms of temperature, colour beans (cut test score); pH, TA, and cup testing were collected and evaluated. The results showed that in the same condition experiment, using 60mg/kg fresh cocoa bean enzyme dose was good fermentation, temperature increased faster and higher (49^{o}C), cut test score of 892.5 scores, pH = 5.25, TA = 1.29 mg NaOH 0.1N/g and cup testing were better than others (cocoa flavour: 4.4 scores; fruit flavour: 3.2 scores). In the same condition experiment, invertase adding when fermentation beginning was better than others, temperature increased faster and higher (46.7^{0}C), cut test score of 745 scores, pH = 5.13, TA = 1.15mg NaOH 0.1N/g. Cup testing was better than cocoa flavour (3.0 score) and astringency (3.4 score).

Key words: invertase, fermentation, cocoa, flavour precursors

Introduction

Chocolate flavour is one of the main factors of the chocolate quality in each company. Two main ingredients of chocolate precursors-flavour are acid amine and reducing sugar (Wood and Lass, 2001) so when these are high content leading to good chocolate flavour. On the other hand, the ratio of these substances also affects the flavour of chocolate (Dimik and Hoskin, 2001). Invertase is one of the enzymes catalysing disaccharide into monosaccharides, - sucrose into fructose and glucose (Misnawi *et al.,* 2002). This reducing sugar

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creates chocolate precursors-flavour after roasting through maillard reaction (Wood and Lass, 2001). In addition, the high reducing sugar content also catalyses the reaction to make alcohol faster and more mature in the two first days (in anaerobic fermentation).

Materials and methods

The invertase of Novozyme Company with 600 INVU/g was used and store less than 2 months. Fresh forastero cocoa bean of rip pods was used in 2010 according to Eakmat Company, Buonmethuot City, Daklak Province, Vietnam.

Experiment setting

There were two experiments, experiment 1 (invertase doses) and experience 2 (time of adding invertase). The experiment 1 was arranged in Completely Randomized Design with three replications. Treatments were performed as follows:- treatment 1= 40mg/kg fresh cocoa bean weight, treatment 2= 60mg/kg fresh cocoa bean weight, treatment 3= 80mg/kg fresh cocoa bean weight and treatment 4 served as control which did not add enzyme. The experiment 2 (time of adding invertase) was also was arranged in Completely Randomized Design with three replications. Treatments were performed as follows:-treatment 1 = add enzyme at the beginning of fermentation (0 h), treatment 2 = 24 hours after fermentation, treatment 3 = 48 hours after fermentation and treatment 4 served as control 2 which did not add enzyme. All treatments were done with six days of fermentation and two times of turning (2^{th} day and 4^{th} day) in wooden box. Data were statistically analysed analysis of variance and means were compare using Duncan Multiple Range Test at P =0.05.

Temperature

The invertase catalyses the biochemical reactions and heating cocoa bean mass. So 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. 5 point in box was checked by 12h/time in 6 fermentation days (at 10cm deep from surface of cocoa mass).

Ratio pulp of cocoa bean

Invertases break the sucrose (to glucose and fructose) and some pectin, so the pulp of cocoa beans is released. The ratio of cocoa pulp shows the catalytic invertase. 200g 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 is very good bean (very good fermentation); part purple-brown is good bean (good fermentation); purple is fair bean (a few fermentation); slate bean is not good (not fermentation). Three hundred pieces of dried cocoa beans were cut lengthwise through the middle using a penknife and checking the colour of cotyledon. 4 colours to check and calculate the ratio: brown, part purple-brown, purple, slate bean (Anon, 1995).
CTS = *(*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. 100 dry cocoa beans of each treatment were tested by calculating the ratio of shell (AusAID, 2006).

pH

The dry nib pH 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 drop with 0.1N NaOH to pH 7.1, determined using a pH meter (Hana meter HI-1221, 2009).

Cup testing method is one of the evaluate methods for cocoa quality bean. In this method the cocoa flavour, acidity, bitterness, astringency and fruit flavour were tested. Cocoa beans (170 g) were roasted in an oven (SANYO MOV-112, 2009) at 145^0C for 30 min and cooled at room temperature. The roasted cocoa beans were processed using a laboratory winnower and breaker (John Gordon, UK 1999) to obtain the small cocoa roasted nibs. The roasted nibs were ground in a laboratory mill (Capco test equipment, N^0 1193-PA) for 3h to obtain the cocoa liquor. Ghanaian cocoa liquor was used as reference (AusAID, 2006). The sensory evaluation was conducted by 7 trained panels from WASI.

Results and discussion

Experiment 1

The temperature in fermentation process presented the extreme affect of environment temperature on fermentation. The temperatures between treatments and control 1 were different, especially in treatment 3 (80mg/kg) the temperate increased lower than others, due to the environment and stimulating biochemical reaction by enzymes contained in the cocoa pulp. The maximum temperature in the 6th day of fermentation only reached 49°C, which can often be reached in the 4th day as stated by Allison (1963), Aneani (2006) and Binh Phan Thanh (2003).

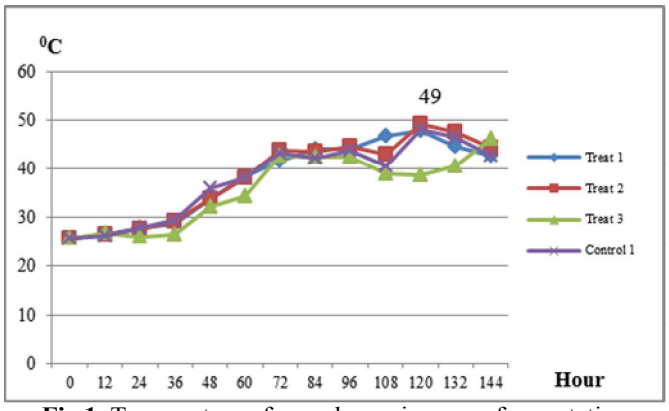

Fig 1. Temperature of mass beans in cocoa fermentation

In fermentation, the speed of releasing cocoa pulp was one of the main factors for changing between two phases: anaerobic and aerobic fermentation phase. When aerobic phase happens early, the process of increasing temperature was faster and better and acid acetic generated more than acid

lactic. From that, there was more evaporation of acid when drying and less acidity in beans as stated by Carr *et al.* (1980) and Lopez (1986).

The difference of the ratio of cocoa pulp was not much, so the affect of invertase in releasing cocoa pulp was not statistical significance difference. However, the ratio of cocoa pulp of treatments during fermentation was fair lower than cocoa pulp of control 2 remaining at the end of the process, from 28% at the beginning to 5,4% (Trea) and 9,7% (control 2), and this incidence tends to reduce gradually according to the increase of enzyme dose (Fig.2). This result was very important to evaluate the quality of cocoa products and cocoa fermentation because the more ratio of cocoa pulp released the less shell content remaining.

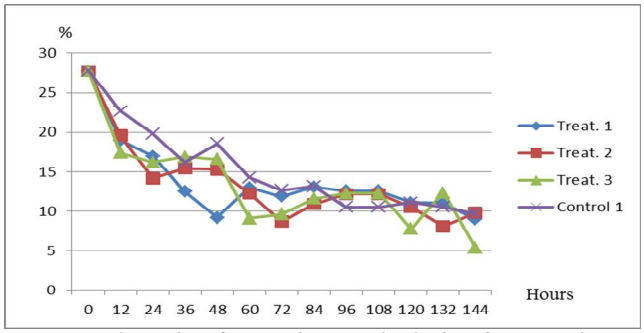

Fig 2. The ratio of cocoa bean pulp during fermentation

Table 1. Shell content and cut test score (CTS) in cocoa beans

Enzyme/fresh cocoa bean weight (mg/kg)	Shell content %	CTS (cut test score)
40	16.64 a	839.17 a
60	15.92 ab	892.50 a
80	15.59 b	872.50 a
No enzyme	16.44 a	872.50 a

The results showed that: 15.59% of the shell cocoa bean content is the lowest at 80mg enzyme/kg fresh cocoa bean weight treatment and 6.64% of the shell cocoa bean content was the highest at 40 mg enzyme/kg fresh cocoa bean weight treatment as seen in Table 1. The difference between 40mg and 80 mg enzyme/kg fresh cocoa bean weight treatment was statistical significance difference at $p \leq 0.05$. From the control proved the effect of enzymes on decomposition and releasing cocoa pulp. These results were higher than some results from any research in Ghana, Indonesia, Malaysia etc. (Carr, 1979; Goto, 2001).

The cut test scores (CTS) in table 1 showed no difference between 3 treatments, but the score of 60 mg enzyme/kg fresh cocoa bean weight treatment was highest and 40 mg enzyme/kg fresh cocoa bean weight treatment was lowest. It is caused by the low temperature and disparity of the beans in fermentation, which leads to biochemistry reactions inside the beans (the oxygenation from anthocyanin to quinonic and anthocyanin) occurring slowly, indefinitely and less differently.

Table 2. pH–TA in dry cocoa beans

Enzyme/fresh cocoa bean weight (mg/kg)	pH	TA (ml NaOH 0.1 N/g)
40	5.05 b	1.40 a
60	5.25 a	1.29 b
80	5.08 b	1.37 a
No enzyme	5.01 b	1.42 a

The pH reflects the acidity of cocoa, which was one of the important quality factors for evaluating the proper degree of cocoa beans to chocolate making process.

Results showed that the pH = 5.25 was the highest at 60mg/kg fresh cocoa bean weight and control was the lowest with pH=5.01, this difference was statistical significance difference at $p \leq 0.05$. Accordingly, TA's was also on the contrary to the pH. The TA=1.29 ml NaOH 0.1N/g was the lowest at 60mg/kg fresh cocoa bean weight and TA= 1.42 ml NaOH 0.1N/g was the highest at no enzyme (as seen in Table 2). The value of pH of our raw cocoa beans was found to be greater than the standard Malaysian estate beans, which is 4.4-4.7 as reported by Nazaruddin *et al*. (2006), and equivalent the research of S. tagro Guehi in West of Africa, pH in dry cocoa beans was from 5.04 to 5.16 in 5 days Without turning (S. Tagro Guehi *et al*., 2010). The pH higher than 5.1 gave the relatively better quality of beans than other kinds of cocoa beans from other countries as: Malaysia, Ghana (Carr, 1979; Goto, 2001).

Table 3. Cup testing score of dry cocoa bean

Enzyme/fresh cocoa bean weight (mg/kg)	Cocoa flavour	Acidity	Bitterness	Astringency	Fruit flavour
40	3.1 b	2.8 a	3.3 a	2.9 a	1.8 b
60	4.4 a	2.9 a	2.9 a	2.6 ab	3.2 a
80	3.6 ab	2.9 a	3.4 a	2.9 a	2.2 b
No enzyme	3.8 ab	3.1 a	2.8 a	3.1 a	2.0 b

Cup testing was one of the important normms for guaranteeing to identify the whole quality of cocoa beans. Result showed that the differences between the treatments, acidity, bitterness and astringency were not much as seen in Table 3. However, there was a difference between the samples in cocoa flavour and fruit flavour, the highest cocoa flavour score was 4.4 but 3.1 score was lowest. The highest fruit flavour score was 3.2 and lowest was 1.8 score, these results were lower than Ghanaian score by the same panels, which statistical significance difference at $p \leq 0.05$. In summary, based on the results of experiment 1, despite the not optimal condition ambience for fermentation, we identified that the most proper dose of enzyme to add on small scale fermentation was 60mg/kg fresh cocoa beans weigh.

Experiment 2

The results showed that the temperature of the mass of cocoa beans in the 2^{nd}, 3^{rd}, 4^{th} day were very low. Because the temperature of the environment was low, the mass of cocoa beans only reached 40.3^{o}C and there was a sharp disparity in temperature between the upper, mid and bottom layers of the same mass of cocoa beans. The lower the layers, the more temperature reduced.

The temperature in the mass of cocoa beans in fermentation increased in the next few days despite the fairly low temperature of the environment (under 20^{o}C). After 72 hours, the temperature in treatments was over 40^{o}C and then reached 45^{o}C. Inspite of the stability, the maximum temperature was only 46.3^{o}C, so the fermentation unfulfilled the need that the temperature should reach 50^{o}C (Fig. 3).

The speed of cocoa pulp releasing depended on the time of adding enzyme that become a question to answer. Results showed that there was no sharp differences between the samples used in treatments and control, which meant that the enzyme did not affect the ratio of cocoa pulp during fermentation and all treatments in different time were not affected. Therefore, the speed of releasing cocoa pulp did not which based on the time of adding enzyme as seen in Fig. 4.

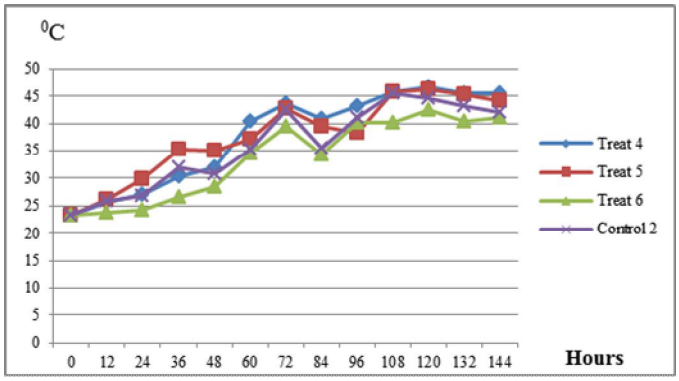

Fig 3. Temperature of mass beans in cocoa fermentation

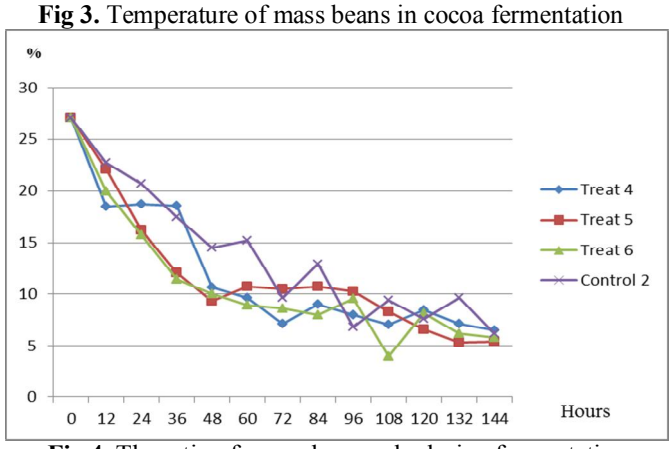

Fig 4. The ratio of cocoa bean pulp during fermentation

Table 4. Shell content - cut test score (CTS) cocoa bean

Enzyme adding time after fermentation(h)	Shell content %	CTS (score)
0	14.34 ab	766 ab
24	14.36 ab	745 b
48	14.48 a	776 a
No enzyme	14.50 a	747 b

The high or low shell content after fermentation reflected partly the decomposition of cocoa pulp in cocoa beans or the strong or weak biochemistry reaction happening outside the shell. Result showed not significantly different of the shell content between all treatments and no enzyme, in which the lowest shell content was 14.34% in 0 h, followed by 14.36% in 24 h and the highest was 14.5% in no enzyme as seen in table 4, which were higher than the Ghana cocoa shells content about 11-11.8%, and the same as Ivory Coast 13.5 – 15.5% (Wood and Lass, 2001). On the other hand, the low cut test scores (CTS) of the treatments and control due to the low temperature of fermentation which led to

the indefinite biochemistry reaction inside the pod. The highest score was 776 scores in treatment 6, followed by 766 scores in treatment 4, and the similarity results between 24 h (745 scores) and no enzyme (747 scores).

The results showed that despite the little difference between the treatments, there was a difference from control, which statistically significance difference. Therefore, enzyme was an obvious effect on fermentation and acidity. All of treats have pH > 5.13, so all of samples were not sourer which more acidity than the sample without adding enzyme.

Table 5. pH-TA in cocoa bean

Enzyme adding time after fermentation(h)	pH	TA (ml NaOH 0.1N/g)
0	5.17 a	1.14 b
24	5.13 a	1.15 b
48	5.16 a	1.11 b
No enzyme	5.06 b	1.40 a

Table 6. Cup testing cocoa bean

Enzyme adding time after fermentation(h)	Cocoa flavour	Acidity	Bitterness	Astringency	Fruit flavour
0	3.0a	3.6ab	3.5ab	3.4 b	2.5ab
24	2.8a	3.9a	3.8a	3.6ab	2.0 b
48	2.9a	3.9a	3.6ab	3.9a	2.5ab
No enzyme	2.8a	4.0a	3.9a	3.8ab	2.7a

The cup testing results showed significantly different in acidity, cocoa flavour and bitterness and between astringency and fruit flavour. The lowest astringency score was 3.4 in 0 h (added at the beginning of fermentation), the lowest fruit flavour was 2.0 scores at 24h which statistical significance difference, and the others were not different. Those results were suitable the results of table 5. From the result, we also identify that the most proper time of adding enzyme invertase was the beginning of cocoa fermentation at 0 h.

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