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Biophysical and Biochemical Changes and Flavor Development in Mixed Sabah Hybrid Cocoa Beans Fermentation

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Abstract – Lipase specific activity in cocoa beans varied from 70 to 40 μmol/min/mg protein during six days of fermentation. At the end of this period most parts of the cotyledon has turned to brown color which would be more distinguishable after drying. The beans were slightly swollen thus causing its testa to disintegrate. During fermentation there was a decrease in pH from 6.4 to 5.8. Whereas the percentage of acetic acid was increased by 0.04% of wet weight beans on the third day but decreased progressively with time.

Key words – Cocoa beans, fermentation, lipase, products.

Introduction

The evidence for the presence of lipase (triacylglycerol hydrolase EC 3.1.1.3) in cocoa beans was first reported by Ciferrin in 1931 (cited by Forsyth and Quesnel, 1963). This enzyme plays an important role in fatty acid metabolism. Highly active lipases in plants were found to catalyze the hydrolysis of reserve triacylglycerols (Tavener and Laidman, 1972). Many of these studies are restricted to castor bean, soybean, barley, wheat, oat, corn, cotton seed and ground nut which mainly dealt in seed germination (Huang and Moreau, 1978). Previous studies on lipase activity in fermenting beans are rare. The present study was undertaken to elucidate lipase specific activity, temperature, pH, flavor profile, and fermentation products of raw cocoa beans at different stages of fermentation.

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Experimental

Cocoa beans of mixed Sabah hybrid (this hybrid which is widely grown in Malaysia is known for its disease resistance and is found suitably grown on low and hilly ground) were fermented in a sweat box (54 cm × 54 cm × 54 cm) for six days (Abdul Samah, *et al.*, 1992). The bean mass was turned over at every 24 h intervals soon after sampling. The temperature of the beans was measured at a depth of 12 cm from the top surface layer and the fermentation index was determined by the method of Gourieva and Tserevitinov (1979). Cut test studies on beans texture and color changes were conducted by visual inspection.

Enzyme Assay – Crude enzyme was extracted from 5 g samples of cotyledon using phosphate buffer (pH 7.8) containing 5% (w/v) polyvinyl pyrolidine to remove the polyphenolic substances. The procedure was car-

ried out in triplicates. Lipase activity was determined in accordance to the method of Moskowitz *et al.*, 1977.

Chemical Analysis – For the determination of fermentation products, bean cotyledon (5 g) was crushed mechanically in 50 ml of distilled water for 3 min. The suspension was filtered and the pH was then measured before centrifuging at 18,000×g for 20 min at 4°C. Acetic acid was determined by gas-liquid chromatography using a Pye Unicam 204 series chromatograph, USA fitted with a flame-ionization detector and a glass column (25 cm×4.6 mm) packed with Porapak Q (80 to 100 mesh) with argon as carrier gas. The temperature was programmed isothermally at 220°C. The non-volatile acids were determined by HPLC (Shimadzu LC-6A, Chromatopac, Japan) fitted with a variable detector and injector. The detector was programmed at 230 nm and the oven temperature was set at 40°C. The acids were separated using a Hibar RP-18 column (25×4.8 mm) with a mobile phase of methanol-water (10:90 v/v) which was acidi- fied to pH 2.50 and the flow rate adjusted to 0.8 ml min^{-1}. Volatile and non-volatile acids were quantified from the peak areas by reference to a standard of pure acids. All samples were measured in triplicates.

Flavor Analysis – Five hundred grams of dry cocoa beans (moisture content appro- ximately 7.5%) was heated in an oven at 146°C for 30 min. The testa was then separated and 10 g of bean cotyledon were mechanically crushed to fine particles (cocoa powder) to which 100 ml of boiling distilled water was then added to dissolve the cocoa powder. Sensory evaluation test was then conducted in accordance with the procedures establ- ished by MARDI (Malaysian Agricultural Research and Development Institute-Said *et al.*, 1988).

Results and Discussion

The present study shows that fresh beans (cotyledon) were compact and have pale purple colour mostly at the central portion whilst the periphery region was reddish purple. After six days of fermentation most parts of the cotyledon has turned to patches of brown color which would be more distinguishable after drying. The bulk density of the beans was found to be slightly over 1 g per bean in weight. It was observed that the beans were slightly swollen probably due to the heat developed by the action of microbial activity during fermentation and loss of water from the cotyledon during drying. The swelling of the bean caused the testa to disintegrate. However, a few of them still remained attached to the cotyledon even after drying, indicating the presence of the remaining pulp sugar in the bean.

The highest level of lipase specific activity recorded was at 70 μmol/min/mg protein found in the fresh beans. The ANOVA showing the difference in enzyme activity gives an F value 5.25 which is slightly significant. On investigating the means over the fermentation period fresh beans were found to show the highest activity. The enzyme activity appeared to decrease from 70 to 40 μmmol/min/mg protein during the six days of fermentation (Table 1).

Although there was a substantial rise in the temperature till the third day of fermentation, the lipase activity was not affected or stimulated indicating that the enzyme was unable to catalyze the hydrolysis of reserve triacylglycerol further. It may be possible that at low pH level the enzyme activity was inactived or unsuitable under such condition.

Four major acids were subsequently indentified in the cotyledon, acetate, lactate, oxalate and succinate. Acetic acid was distinguished by an early increase to a maximum level of 0.3% of wet weight at 72 h and has the tendency to decrease thereafter. The development of acetic acid, possibly by acetic acid bacteria, may progressively cause

Table 1. Temperature, pH, fermentation index, lipase specific activity and organic acids at different stages of fermentation.

Day	temperature (°C)	pH	lipase specific activity (μmol/min /mg/prot)	fermention index	organic acids (% per gram wet weight cotyledon) acetate, lactate, oxalate, succinate			
0	27	6.4	70	0.56	0.01	0.04	0.04	0.04
1	31	6.3	61	0.62	0.02	0.04	0.01	0.01
2	37	6.1	69	0.71	0.03	0.04	0.02	0.01
3	42	6.0	67	0.82	0.04	0.13	0.16	0.02
4	38	5.8	42	0.97	0.02	0.03	0.03	0.01
5	37	5.7	39	1.00	0.01	0.07	0.14	-
6	31	5.2	40	1.19	0.01	-	0.27	0.01

Foot Note:- Not detected.

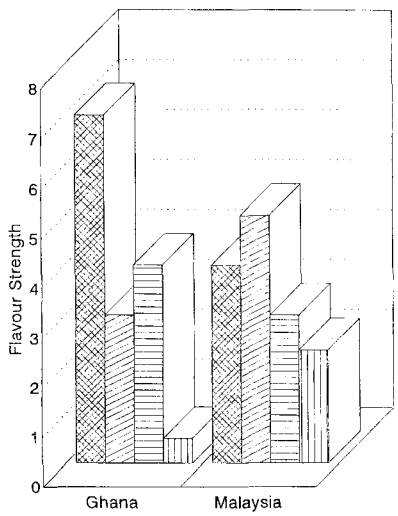

Fig. 1. ▩ Cacao, ▤ Astringent, ▨ Bitter, ▥ Acidic.

the pH to decline. The oxalate and lactate seem to decrease towards the final day of fermentation period and are opposite to the rise in fermentation index values which indicates that the beans were sufficiently fermented.

Malaysian beans are slightly acidic. Fig. 1 shows a comparative study on the flavour profile of the Malaysian and the Ghanaian beans. The flavour assessment indicated that the Ghanaian beans (pH 5.80) had a relatively higher chocolate flavour strength over the Malaysian beans (pH 5.24). Lopez and Quesnel (1973) stated that at low concentrations, acids can be regarded as a contributing factor to normal chocolate flavour but at higher concentrations can result in off flavours. It appears that there are other factors apart from acidity which can contribute to the normal chocolate flavour. This includes the nature of hybrid and the species of microorganisms involved during the fermentation process.

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