

The Correlation between Lipase Activity and the Production of Fatty Acids of PBC Cocoa Clone

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ABSTRAK

Kajian untuk menentukan hubungan antara aktiviti lipase dalam biji koko dengan kehadiran asid-asid lemak yang boleh mempengaruhi aroma dan citarasa koko telah dijalankan. Aktiviti lipase didapati menurun daripada 0.46 kepada 0.15 $\mu\text{mol}/\text{min}/\text{mg}$ protein semasa enam hari penapaian. Penurunan pH kotiledon daripada 6.6 kepada 5.1 dan peningkatan suhu semasa penapaian mungkin telah menyumbang kepada penurunan dalam aktiviti lipase, walaupun korelasi adalah tidak konsisten. Penghasilan asid asetik yang maksimum pada hari ketiga adalah selari dengan pencapaian suhu maksimum dalam biji koko. Walau bagaimanapun asid-asid lemak yang lain meningkat sehingga hari kelima dan menurun selepas itu. Peningkatan ini selari dengan peningkatan Indeks Penapaian. Oleh kerana tiada hubungan antara aktiviti lipase dengan penghasilan asid lemak maka kajian ini mencadangkan bahawa lipase di dalam biji koko tidak memainkan peranan yang penting dalam menentukan aroma dan citarasa koko.

ABSTRACT

A study was carried out to determine the correlation between lipase activity in cocoa beans and the presence of free fatty acids which could affect the aroma and flavour of cocoa. Lipase activity fell from 0.46 to 0.15 $\mu\text{mol}/\text{min}/\text{mg}$ protein during six days of fermentation. Changes in the pH from 6.6 to 5.1 in the cotyledon and the increase in temperature seems to have contributed to the decrease in lipase activity, although no consistent correlation was observed. Maximum acetic acid production was observed on the third day of fermentation, when the maximum temperature was attained by the cocoa beans. However, other fatty acids increased continuously until the fifth day of fermentation and declined thereafter.

Keywords: cocoa beans, fermentation, lipase, products

INTRODUCTION

Lipase (triacylglycerol hydrolase EC 3.1.1.3) is known to hydrolyze esters of long chain aliphatic acids from glycerol at oil or water interfaces. Lipases play an important physiological role of preparing the fatty acids of water-insoluble triacylglycerols for absorbance and transport through membranes by converting

them to the more polar diacylglycerols, monoacylglycerols, free fatty acids and glycerols (Jensen 1986).

The role of lipase in cocoa fermentation is not understood. Since this enzyme has the properties of hydrolyzing lipids the end product are usually affected by fermenting and drying practices. The genetic variation of cocoa hybrids and the microorganisms involved during the fermentation period can also contribute to the nature of the end products.

The characteristics flavour and aroma of chocolate arise as a result of two principal processes, curing and roasting. In the absence of either process, no cocoa aroma is observed (Knapp and Churchman 1937). The present study was undertaken to elucidate lipase specific activity and the production of some of the volatile fermentation products and to correlate them with the flavour at different stages of fermentation.

MATERIALS AND METHODS

Cocoa beans of PBC 113 clone weighing approximately 40 kg were fermented by the conventional method in a sweat box (54 × 54 × 54 cm) for six days. The bean mass was turned over (for aeration purposes) 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 Tsevitinov (1979).

The pulp and cotyledon of the beans were separated and the crude enzyme was extracted from cotyledon (5 g) using phosphate buffer (0.2M, pH 7.8) containing 5% (w/v) polyvinyl pyrrolidone for removing the polyphenolic substances. The procedure was carried out in triplicate. Lipase activity was determined in accordance with the method of Moskowitz *et al.* (1977). Specific activity of lipase is expressed as mole free fatty acid released/min/mg protein.

For the determination of volatile fermentation products, samples of cotyledon (5 g) were crushed mechanically in 50 ml distilled water at room temperature for 3 min. The suspension was filtered and the pH measured before being centrifuged at 18,000 × g for 20 min at 40°C. Similar procedures were carried out for the pH determination of the pulp.

Each sample of cotyledon (1 ml) was mixed vigorously with 0.06 g of oxalic acid and 1- μ l samples were then analysed by GLC (Shimadzu) using a flame-ionization detector on a Thermon 3000 (5%) column (25 cm × 4.6 mm), Shincarbon 60/80 held isothermally at 200°C. Volatile acids were quantified from the peak areas by reference to standards of pure acids. All samples were measured in triplicate.

RESULTS AND DISCUSSION

The present study showed that the highest level of lipase specific activity in the cocoa beans (cotyledon) was 0.46 μ mol/min/mg protein. The ANOVA showing the difference in enzyme activity gives an F value of 183, which is highly significant. Fresh beans (i.e. at zero time fermentation) showed the highest activity. However, the enzyme activity appeared to decrease from 0.40 to 0.15 μ mol/min/mg protein during the six days of fermentation (Table 1).

TABLE 1
Temperature, pH, fermentation index and lipase specific activity
at different stages of fermentation

Day	Temperature (°C)	pH		Fermentation Index	Lipase Specific Activity ($\mu\text{mol}/\text{min}/\text{mg prot}$)
		Cotyledon	Pulp		
0	24.5	6.6	4.7	0.45	0.46 ± 0.02
1	27.5	6.4	4.3	0.49	0.22 ± 0.05
2	38.0	5.4	4.2	0.61	0.11 ± 0.03
3	43.5	4.7	4.3	0.76	0.10 ± 0.03
4	41.0	4.7	4.7	0.98	0.15 ± 0.01
5	30.0	4.9	5.2	1.01	0.05 ± 0.00
6	29.0	5.1	6.4	1.06	0.15 ± 0.01

Despite the continuous rise in the temperature up to the third day, there was no concomitant increase in the lipase activity. The specific activity dropped significantly from 0.46 to 0.10 $\mu\text{mol}/\text{min}/\text{mg}$ protein. Probably this can be accounted for by the instability of lipase activity at high temperature. The decrease in pH of the cotyledon from 6.6 to 5.1 may also contribute to the decrease in lipase activity. However, the pH of the pulp increased after the third day, probably because less sugar was being metabolized. A lower sugar content would decrease the ethanol production and as a result the pH values become higher (Abdul Samah *et al.* 1992). Changes in the temperature of the beans during fermentation seem to affect the lipase activity although there was inconsistent correlation. This is also true for the pH. The combination of the two factors could probably explain why there was inconsistent correlation of temperature and pH with the lipase activity in the cocoa beans. The heat produced could arise from the breakdown of sugars in the pulp through microbial activities.

The volatile acids identified in the cotyledon were acetic, butyric, isobutyric, valeric and isovaleric acids. The various acids present in the cotyledon could be derived from the pulp by diffusion. Acetic acid increased to a maximum level of 169 mg/10g beans at 72 h and decreased thereafter (Table 2). The production of acetic acid, possibly by acetic acid bacteria, and other acids from hydrolysis by lipase in the beans may cause the pH to progressively decline. These results confirmed the findings on bean acidity problems by Carr *et al.* (1980) using shallow box fermentation techniques.

All acids in this study seemed to decrease towards the final day of the fermentation period, parallel to the rise in fermentation index. Values of 1.0 and above in the fermentation index indicate that the beans were sufficiently fermented (Gourieva and Tserevitinov 1979). The overall results did not show any close relationship between lipase activity and that of fatty acids production. With the exception of acetic acid which was distinguished by an early increase to a maximum level at 72 h, the isovaleric acid seemed to continually increase towards the fifth day of fermentation.

TABLE 2
Volatile organic acids concentration at different stages of fermentation.

Day	Volatile Organic Acids (mg/g beans)				
	Acetate	Butyrate	Isobutyrate	Isovalerate	Valerate
0	0.7 ± 0.01	-	-	-	20.30 ± 0.14
1	0.59 ± 0.01	-	-	-	19.30 ± 0.14
2	74.30 ± 0.14	-	-	3.86 ± 0.14	16.80 ± 0.14
3	169.00 ± 0.14	-	-	4.26 ± 0.14	14.10 ± 0.14
4	98.80 ± 0.14	-	2.11 ± 0.14	7.32 ± 0.14	17.90 ± 0.14
5	82.10 ± 0.14	4.57 ± 0.14	2.21 ± 0.14	8.16 ± 0.14	17.10 ± 0.14
6	34.10 ± 0.14	0.29 ± 0.14	0.76 ± 0.14	0.20 ± 0.14	1.00 ± 0.14

Foot Note: ± Standard deviation

- Not detected

CONCLUSION

It can be concluded that since there was no close relationship between lipase activity and that of fatty acid production, the present results suggest that lipase activity in the cocoa beans does not play a significant role in the development of aroma and flavour of cocoa.

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