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THE EFFECT OF THE MATURATION PROCESS ON FERMENTED COCOA BEANS II: ACIDS, SUGARS, XANTHINES, PYRAZINES AND ANTHOCYANINS

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Abstract: This study describes the effect of the maturation process on selected flavour contributing components in the cocoa bean. Non–volatile acids, sugars, xanthines, pyrazines and anthocyanins were studied. The study showed that holding fermented cocoa beans at ambient temperatures with some aeration for 48 hours (the maturation process) had an effect on the levels of flavours and flavour precursors of cocoa. Although pH rises from 4.8 to 5.2 during maturation the content of the main non–volatile carboxylic acids (citric and oxalic acid) did not show any significant change. Maturation causes a loss of sucrose and an increase in reducing sugars (glucose, fructose and galactose). Xanthine content declines. Theobromine losses during maturation is approximately 30% and it is possible that part of this loss is due to migration from the cotyledon to the shell. While pyrazine content increased the profile of pyrazines formed during roasting appears unaffected by the maturation process. Anthocyanin content declined by 40–45% during maturation.

1. Introduction

Maturation is the term used by Liau[6] to describe the process of holding of fermented cocoa beans in thin layers (for aeration) at ambient temperatures with little moisture loss for about two days. This was introduced primarily to reduce acidity of the cocoa bean produced in Malaysia.

The studies of Packiyasothy[5] showed that the maturation process increased the volatile carbonyl content and changed its composition. The authors' previous studies[2] showed that there was an increase in volatile carbonyls and a decline in amino acid content and further that there appeared to be a relationship between the two processes. It was therefore clear that the maturation process had some effect on flavours.

In this study the changes in acids, sugars, xanthines, pyrazines and anthocyanins in cocoa with the maturation process are reported.

2. Experimental

2.1 Sampling, Fermentation and Maturation

Sampling, fermentation (basket method) and maturation were carried out as described previously,[2] samples being drawn during both the major and minor seasons from Wariyapola Estate, Matale. The samples contained a mixed forestero and criollo variety in the ratio of approximately 4:1. The samples

from major (September to December) and minor (April to June) seasons could be taken as indicative of two possible extremes of sampling conditions.

2.2 Preparation of Sample for Analysis

Cocoa beans with the appropriate treatment were dried in the sun for 3—4 days to a moisture content of 10—15%. Analysis of acids and sugars were carried out on the entire bean (with shell) while analyses of xanthines and anthocyanins were carried out on deshelled cocoa bean. Analysis for pyrazines was carried out after roasting (140^0C for 30 min.) and deshelling. Deshelling was carried out in certain instances as in these cases it is the procedure adopted by other workers — for anthocyanins presumably to reduce interference. In the case of xanthines the procedure adopted has added significance since cocoa shell theobromine has also been measured. Pyrazines are measured after roasting as these components develop mainly after roasting.

2.3 pH

PH was measured following the sample preparation procedure of Rohan[10] at 30^0C.

2.4 Non—volatile acids

Non—volatile acids were extracted by the Paper Chromatographic method of Packiyasothy[7] and chromatographed on paper using n—butanol : formic acid : water (15 : 3 : 1) and aniline and furfural as the spraying reagent. Quantification was carried out by the method described by Packiyasothy[7] using standards on the same paper. The coefficient of variation for the determination of citric and oxalic acid were 16.6 and 15.8 respectively.

2.5 Sugars

Sugars were separated by the method of Lato *et al.*[4] and sprayed[3] after chromatography. Quantification was carried out by TLC—UV densitometry (Camag model 76502 equipped with a strip chart recorder model 1107) using a Tungsten lamp at 436 nm (fructose) and 525 nm (glucose, galactose and sucrose) using techniques described previously.[2] Standard curves for glucose, fructose, sucrose and galactose gave gradients of 17.6, 20.0, 17.5 and 22.5 mm^2 peak area per μg respectively and the coefficients of variations for the determination were 6.9, 3.0, 6.2 and 11.0 respectively. Standard curves were only used to confirm linearity of plots and standards on the same plates were employed for purposes of quantification.

2.6 Xanthines

Xanthines were extracted by the method of Senanayake and Wijesekera.[11] Xanthines were separated by TLC as follows —

A slurry of Kieselgel G_{254} (25g) in distilled water (50 ml) was applied on clean glass plates (20 x 20 cm) at a thickness of 500μ. The plates were allowed to stand for one hour at room temperature and dried at 110^{0}C for 1h and determined by TLC—UV densitometry using a Deuterium lamp at 298 nm. Standard curves gave gradients of 11.5 and 21 mm^2 peak area/μg theobromine and caffeine respectively.

Standard curves were used only to ensure the peak area was proportional to the theobromine and caffeine levels. As there was some doubt regarding consistency from plate to plate, standard spots were run along with the samples on the same plate for quantification purposes. The coefficient of variation for the technique was 7.2 and 3.7 for caffeine and theobromine respectively.

2.7 Pyrazines

Pyrazines were extracted by the method described by Reineccius[8] and subject to gas liquid chromatographic analysis using a Varian equipped with a Model 9176 strip chart recorder and a computing integrator.

The stainless steel column (length 3m diameter 3mm) used contained 10% carbowax 20m supported on chromosorb W (80 to 100 mesh). The GLC analysis was carried out using following conditions. Injector temperature, 210^{0}C; detector temperature, 230^{0}C; temperature programme, 60—120^{0}C at 2^{0}C/min; He (carrier gas) flow rate, 25 ml/min; H_2 (detector gas) flow rate, 25ml/min; and O_2 (detector gas) flow rate, 50 ml/min.

Peak area normalization method was used for quantification. Peak identification was carried out by retention time data and peak enrichment only.

2.8 Anthocyanins

Anthocyanins were extracted and determined by the method of Lees and Francis.[5]

3. Results

3.1 Acids

Whereas fermentation over from 4 to 6 days caused a slight decline in pH (from 4.8 to 4.7), the maturation process resulted in an increase of pH to 5.2. However, non—volatile acids which were mainly citric acid and oxalic acid showed no significant change (Table 1) (the differences were well within the standard errors of the estimation techniques).

Table 1. Effect of the maturation process on non—volatile acids and sugars

Sample[a]	Acid Content (%)[b]		Sugar Content (%)			
	Citric	Oxalic	Fructose	Galactose	Glucose	Sucrose
Fermented	0.54(0.46)	0.25(0.22)	0.90(0.87)	0.48(0.35)	0.83(0.81)	0.32(0.22)
Fermented and Matured	0.57(0.52)	0.31(0.24)	1.21(1.05)	0.61(0.52)	0.93(0.94)	0.27(0.17)

Results expressed as a percentage on cocoa bean weight.

a Major and minor season samples : data for minor season sample in parenthesis

b Only trace amounts of tartaric acid was detected in all samples

The coefficient of variation for assay of citric acid and oxalic acid was 16.6 and 15.8 respectively.

The coefficient of variation for assay of fructose, galactose, glucose and sucrose was 3.0, 11.0, 6.9 and 6.2 respectively.

3.2 Sugars

The reducing sugars identified were glucose, galactose and fructose. It was observed that reducing sugar content increased during the maturation process (Table 1).

3.3 Xanthines

There were two main xanthines in cocoa bean (deshelled)— theobromine and caffeine. Maturation caused a decline in xanthine content (Table 2) while theobromine content declined in the cotyledon, its levels in the shell of cocoa bean increased.

Table 2. Effect of the maturation process on xanthines

Sample[a]	Xanthine Content (%)	
	Theobromine	Caffeine
DESHELLED COCOA BEAN		
Fermented	3.29(2.19)	0.07(0.13)
Fermented and Matured	2.22(1.87)	0.04(0.06)
COCOA BEAN SHELL		
Fermented	0.65(0.54)	– (–)
Fermented and Matured	0.88(0.75)	– (–)

Results expressed as a percentage of cocoa bean weight

[a] Major and minor season samples : data for minor season sample in parenthesis

– Not detected

Coefficient of variation of assay technique 3.7 and 7.2 for theobromine and caffeine respectively.

3.4 Pyrazines

Pyrazines content increased from 5.7 μg/g by ∿60% to 9.3 μg/g on fermentation and roasting. Maturation process had no great effect on the profile of different pyrazines after roasting (Table 3).

Table 3. **Effect of the maturation process on pyrazines**

Sample[a]	%				
	2- methyl	2,3–dimethyl	2,5–dimethyl plus 2,6–dimethyl	2,3,5–trimethyl	Tetramethyl
Fermented	0.9(0.7)	10.7(9.2)	3.5(3.7)	13.2(13.5)	6.3(5.2)
Fermented and Matured	0.9(0.8)	12.9(10.3)	4.1(4.0)	14.2(14.0)	8.5(6.7)

All samples have been roasted (140^0C, 30 min.) and deshelled.

Assay by Gas Liquid Chromatography – Individual pyrazines results expressed as a percentage of total basic volatiles

(Note : pyrazines have not been expressed on the basis of cocoa bean weight due to problems encountered with the internal standard).

[a] Major and minor season samples : data for minor season sample in parenthesis

3.5 Anthocyanins

Maturation had a marked effect on anthocyanin content (Table 4).

Table 4. Effect of maturation process on the anthocyanin content

Treatment	Fermentation time (days)	Anthocyanin Content[a] [b]
Fermented	2	10.9(8.5)
Fermented	4	7.0(6.6)
Fermented and matured	2	5.5(4.5)
Fermented and matured	4	3.9(3.7)

[a] Anthocyanin content determined after drying by method of Lees and Francis and expressed as μ moles/100g cocoa

[b] Major and minor season samples : data for minor season in parenthesis.

4. Discussion

The original concept of maturation process of Liau[6] was to reduce acidity. This reduction of acidity also encountered with Sri Lankan cocoa where acidity rose from pH 4.8 to 5.2 with maturation. However, these studies reveal that non—volatile acids do not change significantly with maturation. This is not unexpected as Liau's[6] original hypothesis was that increased aeration would cause loss of acetic acid by oxidation via the Krebs cycle. This is also borne out by the results of Abeygunasekera[1] which showed that the percentage acetic acid in total acid volatiles declined from 55 to 45% while the percentage of the other major volatile acids (propionic and butyric) remain relatively unchanged.

The results also showed that although sucrose content declined the increase in reducing sugar far outweighed this decline. This indicated that reducing sugars were also being formed from other sources, possibly through the hydrolysis of other glycosides. The increase in reducing sugar content is significant as it is one of the important flavour precursors which subsequently undergo reaction during roasting of cocoa.

The decline in xanthines appears to be due in part to the migration of theobromine from the cotyledons to the shell (although this may not have a marked effect on cocoa flavour it could be significant in the process for extracting theobromine from cocoa shell waste). The migration itself is not surprising as during fermentation the cellular organization of the cocoa cotyledon is disrupted and permeability enhanced. Therefore, diffusion along a concentration gradient is plausible especially as theobromine is moderately soluble in acid medium.

The increase in pyrazines with maturation is expected as maturation causes an increase in reducing sugars which reacts with nitrogenous compounds to form pyrazines[1 2] during the roasting process. The pyrazine profile does not appear to change much with maturation. More detailed studies on this aspect using reliable internal standard for quantification will need to be conducted if further conclusions are to be drawn.

Anthocyanin content declines much more in maturation than in extended fermentation. This is understandable as conditions in maturation are more aerobic. The reduction in anthocyanin content is of importance as it would result in a reduction in astringency and development of the characteristic colour of chocolate.

Conclusion

The study indicates that maturation, in addition to the well known effect in reducing acidity, can also play a part in flavour development by affecting the level of flavour precursors in cocoa bean.

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