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RELATIONSHIP OF FERMENTATION TIME TO THE CUT TEST AND ANTHOCYANIN CONTENT IN COCOA *(THEOBROMA CACAO)*

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Abstract: Cocoa collected from Wariyapola Estate, Matale, was fermented by the basket method. It was found that the "cut test" can be applied to determine fermentation times of upto two days provided the batch is a mixture of the criollo and forestero varieties. Although anthocyanin content declined by nearly 70% within six days fermentation this parameter cannot be generally used to determine fermentation time.

1. Introduction

Fermentation, which is an important step in the processing of cocoa in Sri Lanka has received insufficient attention in the past and the process is largely uncontrolled. The duration of fermentation is a crucial aspect of primary processing of raw cocoa as it results in the formation of precursors of essential aroma compounds while bringing about a decline of astringency and bitterness. Therefore it is essential that cocoa beans are fermented for an optimum period in order to produce an acceptable flavour.

Fermentation trials on Sri Lankan cocoa have been reported by Ogutuga[4] who monitored pH changes and temperature variation of the fermenting cocoa. However, this study does not report a specific period for fermentation and highlighted the tray method of fermentation for mixed varieties of beans.

The present study was conducted on cocoa beans obtained from Wariyapola Estate, Matale, Sri Lanka, where there is a mixed variety of forestero and criollo beans. While it is known criollo beans generally require 2—3 days of fermentation[10] and forestero beans 5—7 days, optimum fermentation time would also depend on the method of fermentation adopted.

Of the several methods of fermentation[4] available the basket method was used during this study as the target group of processors were the smallholders. It is also noted that at the present time most smallholders limit fermentation time to 1—1½ days.

Raw material from the estate selected was subject to a basic study on selected fermentation parameters after which an attempt was made to correlate fermentation and storage time with the cut test and anthocyanin content with a view to aiding in the grading of cocoa. This study is considered important as the present practice[9] of grading cocoa on external appearance is considered undesirable.

2. Experimental

2.1 Plant Material

Mature pods of *Theobroma cacao* were collected from different divisions of Wariyapola Estate, Matale, Sri Lanka, pooled and a representive sample drawn. These pods were a mixture of the criollo and forestero varieties in the ratio of approximately 7:3.

2.2 Fermentation

2.2.1 Method

The basket fermentation technique[4] was employed. Total fermentation time was 6 days and samples were drawn daily. The beans were turned after 48 h fermentation. Fermented cocoa beans thus produced were sun—dried for 3 days after spreading on coir matting.

2.2.2 Routine Measurements

The following parameters were measured throughout fermentation:—

(i) pH of water extract of beans and pulp[6]
(ii) Temperature
(iii) Acetic acid content[7]
(iv) Reducing and total sugar content[1,8]
(v) Fat content (by Soxhlet extraction with diethyl ether)

2.3 Storage

Cocoa beans (2 kg) were stored in small jute—hessian (gunny) bags upto three months.

2.4 Cut Test

The cut test was performed by cutting the cocoa beans (of varying fermentation and storage time) lengthwise and counting the number of lilac, cinnamon—brown, purple, purple—brown and brown beans which are easily distinguishable to the naked eye (modified from method of Wood[10]). Approximately 1 kg. of cocoa beans was used for each experimental point.

2.5 Assay of Anthocyanin

Anthocyanins were extracted and assayed by two methods — that of Pettipher[5] and Lees and Francis[2] where absorbance is measured and converted to anthocyanin content by formulae given in the respective methods.

2.5.1 Method of Pettipher

Cocoa beans (100g) were deshelled and blended in 1% v/v HCl (1 litre) using a Waring blender. The suspension was allowed to stand for 5 min. An aliquot (10 ml) was centrifuged for 10 min.

A Sep Pak C18 cartridge (Waters Associates, London) was prepared for extraction by passing methanol (2 ml) followed by (1% v/v) HCl (5 ml) through the cartridge using a plastic syringe.

The supernatant (2 ml) of the acid extract was passed through the cartridge and the eluent discarded. The cartridge was then rinsed by passing through 1% v/v HCl (4 ml) and the eluent discarded. Air was passed through the cartridge to expel any remaining liquid.

The anthocyanins were eluted from the cartridge by passing through 50% (v/v) methanol (4.5 ml), followed by air, collecting the total eluent. The absorbance of the eluent was measured at 520 nm using a Perkin Elmer model 552 spectrophotometer.

2.5.2 Method Lees and Francis

Dried deshelled cocoa beans (100 g) were blended 0.1N HCl in ethanol, 15:85 (1 litre). The macerate was stored overnight at 4^0C in a stoppered flask.

An aliquot (10 ml) was removed and centrifuged. The absorbance of the supernatant was measured at 535 nm using a Perkin Elmer model 552 spectrophotometer.

3. Results

3.1 Preliminary Results

As expected the pH of cocoa bean pulp increased (from 3.2) and finally stabilized at about 4.5 and that of the bean decreased from pH 6.0 to 4.7 in 5—6 days. A final acetic acid content of 0.7% w/v was attained in the bean after 6 days of fermentation. A maximum temperature (41 — 42^0C) of the fermenting mass was attained around 4—5 days after fermentation began — this was followed by a decline in temperature to 38^0C on the sixth day.

Total sugar content declined by about 15% during 6 days fermentation to 1.7% w/w. While initially approximately 50% of the sugar was non—reducing at the end of 6 days fermentation approximately 95% of the sugar was reducing in nature. Cocoa fat content remained relatively steady between 40 and 44% w/w upto six days fermentation.

3.2 Effect of Fermentation Time

3.2.1 Cut Test

Results of the cut test are shown in Table 1. There was a tendency for the criollo bean to turn from lilac to cinnamon–brown and the forestero bean from purple to brown with fermentation.

Table 1. The cut test with varying fermentation time

Fermentation Time (days)	Cotyledons (Number)				
	Lilac	Cinnamon Brown	Purple	Purple/Brown	Brown
0	15–20	0–5	65–70	0–5	–
1	5–10	10–15	45–50	25–30	10–15
2	<5	15–20	40–45	30–35	15–20
3	–	15–20	20–25	30–35	25–30
4	–	15–20	10–15	15–20	35–40
5	–	15–20	<5	10–15	50–55
6	–	15–20	–	<5	>65

3.2.2 Anthocyanin Content

Anthocyanin levels paralleled the results of the cut test (Table 2).

Table 2. Anthocyanin content of fermented cocoa beans

Fermentation Time (days)	Anthocyanins (%)	
	A	B
0	100	100
1	92.4	91.7
2	72.0	71.4
3	62.5	62.4
4	50.2	49.6
5	35.5	35.3
6	31.2	31.5

Moisture 6 – 8%

Duration of drying – 3 days (sun drying)

A – Method of Pettipher[5]

B – Method of Lees and Francis[4]

100% anthocyanin represents approximately 13.3μ moles per 100 g cocoa

3.3 Effect of Storage

Results of the cut test are shown in Table 3 and the corresponding results of anthocyanin assays are shown in Table 4.

Table 3. Variation of storage time on the "cut" test

Storage Period (months)	Fermentation Time (days)	Cotyledons (Number)				
		Lilac	Cinnamon brown	Purple	Purple brown	Brown
0	0	15–20	0– 5	65–70	0– 5	0
	1	5–10	10–15	45–50	25–30	10–15
	2	<5	15–20	40–45	30–35	15–20
	3	0	15–20	20–25	30–35	25–30
	4	0	15–20	10–15	15–20	35–40
1	0	15–20	0– 5	65–70	5–10	0
	1	5–10	10–15	45–50	25–30	10–15
	2	<5	15–20	25–30	35–40	15–20
	3	0	15–20	10–15	35–40	25–30
	4	0	15–20	5–10	20–25	35–40
2	0	15–20	0– 5	65–70	5–10	0
	1	5–10	10–15	45–50	25–30	10–15
	2	<5	15–20	25–30	35–40	15–20
	3	0	15–20	10–15	35–40	25–30
	4	0	15–20	5–10	20–25	35–40
3	0	15–20	0– 5	65–70	10–15	0
	1	5–10	10–15	40–45	30–35	10–15
	2	<5	15–20	20–25	35–40	25–30
	3	0	15–20	10–15	35–40	30–35
	4	0	15–20	5–10	20–25	35–40

Table 4. Assay of cocoa bean during three months of storage for anthocynin content

Fermentation Time	Storage (Months)			
	0	1	2	3
(days)	Anthocyanins (%)			
0	100	92.2	90.9	90.6
1	92.0	88.6	88.6	87.5
2	79.5	74.2	68.2	68.0
3	72.9	70.6	67.0	66.7
4	68.9	68.2	66.3	64.3

Absorbance was measured by the Method of Pettipher[5]

100% anthocyanins represent approximately 19.5 μ moles anthocyanins per 100 g cocoa

4. Discussion

The study defined the basic fermentation characteristics of mixed Sri Lankan criollo and forestero cocoa when fermented by the basket method.

Although the acidity of the fermented cocoa bean is low it is not as low as that reported by Liau in Malaysia.[3]

The study revealed that fermentation time can be determined by the cut test soon after fermentation provided the plantation is a mixed forestero and criollo. The more complicated anthocyanin test is of less use especially as initial anthocyanin content may not be known.

Storage of beans results in a complex situation but still using the criollo beans one can distinguish between 0,1 and 2 days fermentation. The cut test is of less use at 3 or 4 days fermentation as for example, 3 day fermented beans after three months are difficult to distinguish from a more fermented bean stored for a lesser time.

The anthocyanin test is of still less use as it does not have the advantage of evaluating the criollo and forestero beans separately. Further it does not monitor the development of the brown pigment.

As cocoa beans in Sri Lanka are almost never fermented beyond 2 days, the cut test has considerable value provided there is a significant proportion of criollo beans in the particular batch of cocoa.

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