

Free Fatty Acid Content Levels Decide the Fermentation Strategy for Cocoa Beanse

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ABSTRACT

A study was carried out to develop a protocol for primary processing of cocoa beans based on free fatty acid content (<1.75%) using different types and periods of fermentation. The cocoa beans were subjected to three different types of fermentation (basket, heap and sack method) for different period's 5th, 6th and 7th day of fermentation. The fermented beans were subjected to physico chemical analysis, initially and on 5th, 6th and 7th day of fermentation. Fermentation bean recovery was more in heap method and 83.33% of fully fermented beans were observed at seventh day of fermentation through cut test. Moisture content was decreased in all methods of fermentation and the lowest was 37.83% in heap method. A steady decrease in pH was observed in all three methods of fermentation with no significance difference. The fat content decreased during fermentation periods. The lipase activity and percentage of free fatty acid (%FFA) content also decreased with fermentation periods in all three methods. The lowest free fatty acid content of 0.80% was also observed in heap method at seventh day of fermentation. Hence it can be concluded that heap method of fermentation with duration of seven days and free fatty acid content of 0.80% is the best method for fermenting cocoa beans.

Keywords: Cocoa beans; Bean recovery; Cut test; Fermentation methods; Free fatty acids; Lipase activity

INTRODUCTION

Cocoa is a supporting crop to farmers due to its remunerative income. Chocolate is a product obtained from cocoa beans, the fruit of the cocoa tree. Raw cocoa beans are inedible because of their bitter and astringent flavor and unpalatable and unpleasant taste; they must be cured before they can be processed into good-tasting and full-flavored cocoa and chocolates. The primary processing of cocoa beans consists of two major steps, namely fermentation and drying [1]. The quality of final product depends upon the fermented dried beans.

However, the two widely used methods are the heap and box fermentation [2]. Fermentation takes 6-7 days during which time the cocoa beans are mixed twice to ensure even fermentation. The reduction in bitterness and astringency is the result of diffusion of alkaloids (30% fall) and polyphenols (20% fall) out of the cocoa beans [3]. High free fatty acid content is a serious quality defect and reduces the economic value of the cocoa beans. Recently, the cocoa trade has assumed a more scientific position and a lot of emphasis are placed on the content of free fatty acid. It is expected that the Free Fatty Acids (FFA) content must be less than 1.0% to meet the acceptable level of 1.75% in cocoa butter extracted from

the dry cocoa beans [4]. A reduction in the free fatty acids level will definitely have a positive impact. Food stuffs with high moisture content is easily attacked by molds and these molds could produce lipase. This study was, therefore, conducted to develop a protocol for primary processing based on free fatty acid content of cocoa beans in order to save farmers from the drop on price for their products.

MATERIALS AND METHODS

Standardization of fermentation methods and periods based on free fatty acid content

Matured and fully ripened cocoa pods were harvested from Cocoa Research Centre, Vellanikkara, Kerala Agricultural University. The beans were separated from the pods, and any black or diseased beans and germinated beans were removed. The wet cocoa beans were grouped in to nine samples. The beans were subjected to three different types of fermentation (basket method, heap method and sack method) for 5th, 6th and 7th day separately with three replications. The fermented beans were subjected to physico chemical analysis like bean recovery, cut test, moisture, pH, lipase activity, total fat and free fatty acid content initially and on 5th, 6th and 7th day of fermentation.

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Received: January 18, 2021; **Accepted:** January 28, 2021; **Published:** February 04, 2021

Citation: Panjikkaran TS, (2021) Free Fatty Acid Content Levels Decide the Fermentation Strategy for Cocoa Beans 12:909.

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Bean recovery

Fermentation was carried out using bamboo basket method, heap method and sack method. Before fermentation weight of fresh beans was recorded. After fermentation the beans were dried and dry weight was recorded. Then fermentation recovery was estimated and expressed in percent.

Fermentation Recovery = (weight after fermentation (g))/(Fresh weight before fermentation (g)) × 100

Cut test

Cut test or fermentation index was used to determine the extent of fermentation of fermented cocoa beans. Based on the colour, beans were characterized into fully fermented, partially fermented, not fermented, slaty and mouldy. White colour at center or full dark brown colour indicated as fully fermented, partly pink colour or brown colour across and along margin indicated as partially fermented beans, fully purple colour indicated as not fermented, dark black colour indicated as slaty beans. The value is expressed in percent based on the number of beans recorded under each category [5].

Moisture

Moisture content of the fermented beans were estimated by the method of A.O.A.C [6]. To determine the moisture content, ten grams of the sample was taken in a petridish and dried at 6°C to 70°C in a hot air oven, cooled in a desiccator and weighed. The process of heating and cooling was repeated till constant weight. The moisture content of the sample was calculated from the loss in weight during drying.

Moisture content (%) = (Initial weight-Final weight)/(Initial weight) × 100

pH estimation

pH of selected hybrids after fermentation was recorded. Five gram samples of beans were homogenized for 30s in 100ml of hot distilled water and vacuum filtered through Whatman filter paper. A 25 ml aliquot was pipetted into a beaker and the pH was measured using a pH meter.

Lipase activity

Lipase activity was estimated by the method took 20 ml of substrate (Emulsion of 2ml cocoa butter, 25 ml water, 100 mg bile salt and 2gm gum Arabic) in a 500ml beaker. Add 5ml of phosphate buffer. Set the beaker on the top of a magnetic stirrer cum hot plate, maintain the temperature at 35°C. Note the pH of reaction mixture and adjust it to 7.0. Add enzyme extract (0.5 ml), record the pH and set the timer on and let it be pH on zero time. At frequent intervals (10min), the pH drops by about 0.2 unit add 0.1 N NaOH to bring the pH to initial value. Continue the titration for 30 min-60 min period. Note the volume of alkali consumed [5].

Activity meq/min/g sample = (Volume of alkali consumed × Strength of alkali)/(Wt of sample(g) × Time in min)

Total fat

The fat content of the fermented cocoa beans were estimated using the method [5]. Five gram of sample was taken in a thimble and

plugged with cotton. The material was extracted with petroleum ether for six hours without interruption by gentle heating in a soxhlet apparatus. Extraction flask was then cooled and ether was removed by heating and the weight was taken. The fat content was expressed in gram per 100 g of the sample.

Free fatty acid analysis

1 gm-10 gm of cocoa butter extracted from fermented cocoa beans was weighed into 250 ml conical flask to which added 50 ml of a mixture of equal volumes of alcohol and diethyl ether previously neutralised, after the addition of 1ml of phenolphthalein indicator. The solution was titrated with N/10 KOH with constant shaking until pink colour persists for 15 seconds. The titre value in ml (V) was noted [5].

Acid value = (Titre value × Normality of KOH × 56.1)/(Weight of sample(gm))

ANALYSIS OF DATA

The data were recorded and analysed as Completely Randomised Design (CRD). The physico-chemical qualities of fermented beans were compared using ANOVA.

RESULTS AND DISCUSSION

Bean recovery

Bean recovery of cocoa beans was worked out using different fermentation methods during different periods of fermentation interpreted using two factor analysis are presented in Table 1. Based on two way ANOVA, no statistically significant difference was observed between different types of fermentation namely basket, heap and sack method. However, with regards to days of fermentation, significant changes were observable for all the three periods (5th, 6th and 7th day) of fermentation. The highest bean recovery of 34.07% was noticed in heap method of fermentation, but with no significant difference.

Heap method is a large scale traditional standard method for fermentation of cocoa beans while basket and sack methods are small scale traditional standard method. In heap method, size of batch was 50 kg, but for sack and basket method it was 3 and 2 kg respectively. No significant difference in bean recovery was observed between fermentation methods and fermentation periods [7].

Table 1: Bean recovery of cocoa beans with different fermentation methods and fermentation periods

Fermentation methods	Periods of fermentation		
	5 th day	6 th day	7 th day
Basket	44.16	37.08	28.81
	0	-15.18	-23.86
Heap	44.24	39.38	34.07
	0	-10.86	-13.44
Sack	44.97	40.97	28.84
	0	-14.9	-20.93

Figures in parenthesis indicates the percentage deviation

LSD (5% level): Fermentation methods (NS); Fermentation period (7.127); Fermentation methods × Fermentation period (NS).

Cut test/fermentation index

Cut test/fermentation index was found out for the cocoa beans using different fermentation methods during different periods of fermentation and is presented in Table 2. The beans after fermentation were scored based on the degree of fermentation into fully fermented, partially fermented and not fermented. Fermentation index was observed highest in heap method at seventh day of fermentation with 84.99% of fully fermented beans, 10.66% of partially fermented beans and 4.33% of not fermented. The basket method exhibited 72.22% fully fermented beans and 59.77% partially fermented beans and 28.99 not fermented beans. The sack method was observed with least fully fermented beans at seventh day of fermentation (69.10%) and highest per cent in non-fermented beans among all the treatments.

Highest mean fermentation index through cut test score was 63.76% in the heap method. In the present study, fermentation index of different methods of fermentation at seventh day observed above this range. Optimum fermentation index aid in the improvement of quality of cocoa products and it results in the development of flavor and reduction in sourness, astringency and bitterness through biochemical reactions observed that fermentation index was highest in Hyb. 17 with 78% fully fermented beans in basket method [7-9].

Table 2: Fermentation index of cocoa beans during fermentation

Fermentation methods	Fermentation index of fermented beans			
	Fermentation periods	Fully fermented score (%)	Partially fermented score (%)	Not fermented score (%)
Basket	5 th day	28.99	35.99	34.99
	6 th day	59.77	25.33	14.86
	7 th day	72.22	19.88	7.88
Heap	5 th day	43.55	35.77	21.77
	6 th day	72.22	16.88	10.88
	7 th day	84.99	10.66	4.33
Sack	5 th day	29.88	34.88	35.21
	6 th day	54.66	31.8	13.22
	7 th day	69.1	24.44	7.32

Moisture

Moisture content in cocoa beans using different methods and periods of fermentation were interpreted using two factor analysis and are presented in Table 3. The lowest moisture content of 37.8 percent was recorded in heap method at seventh day of fermentation. No significant difference was noticed in moisture content between the different types and periods of fermentation. Moisture content was observed in the range between 44.40% in heap method to 47.40% in basket method at fifth day of fermentation but it reduces into 37.83 and 39.43 at fifth day of fermentation.

Fermentation of cocoa bean pulp by microbial action causes cell rupture and release of intracellular juices, thereby reducing the amount of moisture retained by beans. 43.7% moisture content was in fermented cocoa beans, which is similar to the findings of present study [10].

Table 3: Per cent moisture content of cocoa beans during fermentation

Fermentation methods	Moisture content (%) for different fermentation periods			
	Initial	5 th day	6 th day	7 th day
Basket	54.4	47.4	42.37	39.43
		-11.24	-8.28	-9.67
Heap	53.67	44.4	40.83	37.83
		-9.09	-11.58	-11.8
Sack	50.4	44.83	42.03	39.8
		-4.13	-7.41	-10.7

Figures in parenthesis indicates the percentage deviation

LSD (5% level): Fermentation methods (NS); Fermentation period (NS); Fermentation methods × Fermentation period (NS).

pH

The changes in pH of different treatments during fermentation was interpreted using two factor analysis Table 4 . Based on the percentage deviation, the pH did not have any significant difference with regard to fermentation methods namely basket, heap and sack method. However, with days of fermentation significant changes were observable in all fermentation methods from fifth to sixth day of fermentation at LSD of 5% level. No significant difference was observed with 6th and 7th day of fermentation. Indicated that acids produced by microorganisms during fermentation cause an increase in acidity and consequent decrease in pH. In this study also a steady decline in pH with days of fermentation were noticed in all methods of fermentation [11].

Table 4: Per cent pH of cocoa beans during fermentation

Fermentation methods	pH of different fermentation periods			
	Initial	5 th day	6 th day	7 th day
Basket	5.93	4.61	4.43	4.28
		-22.06	-3.63	-3.32
Heap	6.11	4.52	4.45	4.38
		-26.23	-1.09	-1.6
Sack	5.82	4.53	4.39	4.26
		-22.08	-3.2	-2.74

Figures in parenthesis indicates the percentage deviation

LSD (5% level): Fermentation methods (NS); Fermentation period (4.340); Fermentation methods × Fermentation period (NS).

Total fat

The fat content of cocoa beans using different methods and periods of fermentation is represented in Table 5. Generally,

the fat content decreased with the increase in the fermentation periods. No significant difference was observed with respect to the fermentation methods and period of fermentation. However the fat content was slightly more in cocoa beans fermented in heap method with 40.11%, 37.00% and 32.89% at fifth, sixth and seventh day of fermentation respectively. There was no significant difference between fermentation methods and fermentation periods. The fat content was decreased from 51.95 to 48.82 per cent with the increase in fermentation periods [12].

Table 5: Per cent of fat content of cocoa beans during fermentation

Fermentation methods	Per cent fat content for different fermentation periods			
	Initial	5 th day	6 th day	7 th day
Basket	40.44	36.33	33.66	31
		-9.36	-7.45	-8.5
Heap	42.66	40.11	37	32.89
		-8.7	-7.67	-8.08
Sack	41	39.44	35.55	32.55
		-8.63	-7.53	-7.73

Figures in parenthesis indicates the percentage deviation

LSD (5% level): Fermentation methods (NS); Fermentation period (NS); Fermentation methods × Fermentation period (NS).

Lipase activity

The lipase activity of cocoa beans using different methods and periods of fermentation is presented in (Table 6). Based on the percentage deviation, the lipase activity did not have a significant response for fermentation methods.

However, in the traditional heap method the lipase activity was observed with a decrease in per cent deviation at 25.46%, 25.30% and 22.19% in 5th, 6th and 7th days respectively. Lipase activity decrease with fermentation periods in all three fermentation methods. In basket method the activity varied from 0.0027 to 0.0011, in heap method it varied from 0.0022-0.0005 and for sack it decreased from 0.0026 to 0.0012 respectively.

One of the most important enzymes found in cocoa beans is lipase. This lipolytic enzyme plays an important role in fatty acid metabolism [8].

There was decrease in lipase activity during fermentation periods in all methods. Such a reduction in lipase activity due to decrease in pH of cotyledon contribute to the decrease in lipase activity. Changes in the temperature of the beans during fermentation seem to affect the lipase activity [13].

Table 6: Per cent of lipase activity of cocoa beans during fermentation

Fermentation methods	Lipase activity for different fermentation periods(Milli mol/min/mg protein)			
	Initial	5 th day	6 th day	7 th day
Basket	0.0027	0.0018	0.0014	0.0011
		-24.18	-20.25	-30.27
Heap	0.0022	0.0014	0.0012	0.0005
		-25.46	-25.3	-22.19
Sack	0.0026	0.0019	0.0015	0.0012
		-25.1	-20.12	-21.62

FP Fermentation periods. Figures in parenthesis indicates the percentage deviation

LSD (5% level): Fermentation methods (NS); Fermentation period (NS); Fermentation methods × Fermentation period (NS).

Free fatty acid

The free fatty acid content of cocoa beans using different methods and periods of fermentation is presented in Table 7. Based on the percentage deviation, the free fatty acid had a statistically significant response to fermentation methods, namely basket, heap and sack method and the difference in period of fermentation namely fifth, sixth and seventh were also significant.

The three different types of fermentation had significantly different decrease with the maximum decrease in free fatty acid observed with respect to traditional heap method (42.96%, 13.48% and 32.10% respectively in 5th, 6th and 7th days). However as regards to days of fermentation significant decreased changes were observable on all the three days of fermentation under observation with maximum observable decrease after five days of fermentation (40.07%, 42.96% and 35.93% in basket, heap and sack methods respectively) and the trend continued as there was a telescopic decreasing tendency noticeable even up to seven days of fermentation. It is desirable that further days of fermentation may have a decrease in free fatty acid.

Though the interaction effects of fermentation methods and fermentation periods were not significant, the heap method with seven days of fermentation combination was observed as with the lowest free fatty acid content (0.80) and a higher percentage deviation (32.10 %).

An increase in the percentage of FFA is one of the clear indications of deterioration in cocoa quality [14]. Free Fatty Acids (FFA) are carboxylic acids released from triglycerides through the effect of a lipase observed that the free fatty acid content after fermentation of cocoa beans was 0.87%. This result is similar to the free fatty acid content of present study [15].

The per cent free fatty acid (%FFA) generally decreased with fermentation period. Our findings confirm results of who revealed that duration of cocoa beans fermentation seemed to have a critical effect of increasing the chances for FFA formation. He recorded slight increase in FFA in cocoa beans with varying increase in duration of fermentation [16-18].

Table 7: Percentage free fatty acid of cocoa beans during fermentation with different fermentation methods

Fermentation methods	Per cent free fatty acid content for different fermentation periods			
	Initial	5 th day	6 th day	7 th day
Basket	2.51	1.49	1.3	1.05
		-40.07	-12.27	-18.35
Heap	2.39	1.36	1.17	0.8
		-42.96	-13.48	-32.1
Sack	2.52	1.61	1.42	1.18
		-35.93	-11.78	-19.91

Figures in parenthesis indicates the percentage deviation

LSD (5% level): Fermentation methods (4.902); Fermentation period (4.902); Fermentation methods × Fermentation period (NS).

CONCLUSION

Optimisation studies of various cocoa bean fermentation processes have revealed that good operational small scale practices lead to reduction in free fatty acid content (>1.75%). Free fatty acid content of cocoa butter are of interest to both producers and chocolate manufacturers since higher percentage leads to quality reduction in fermented cocoa beans as well as decrease in hardness of the cocoa butter. Heap method of fermentation attained the best results with maximum per cent of beans and lowest free fatty acid of <80%. This adheres with the international standards which promotes the export potential of cocoa beans.

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