



## Fat content and free fatty acid level of cocoa beans (*Theobroma cocoa*) relative to fermentation and storage periods

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### ABSTRACT

A study was carried out to evaluate the effect of inadequate fermentation of cocoa beans (*Theobroma cocoa* L.) on fat content and FFA levels in storage. Different samples were subjected to various days of fermentation, starting with one day up to five days. After each fermentation period, the cocoa beans were sun-dried. The samples were stored for 90 days but analyzed at 30 days interval starting from zero day of storage. Generally, the fat content significantly decreased ( $P<0.05$ ) with the increase in storage and fermentation periods. However, interaction of storage and fermentation periods also showed statistically significant effects ( $P<0.05$ ) on the percent fat in the dry nib of cocoa beans. This indicates that the effect of length of storage on percent fat content depends on fermentation period. After 90 days of storage, 5 days fermentation significantly resulted in the least fat content (43.18%) from the baseline data. Generally, percentage free fatty acids (% FFA) increased with length of storage ( $P<0.05$ ). Fermentation period was not relevant in production in percent FFA. However, its interaction with storage period was significant ( $P<0.05$ ), indicating that the % FFA depended on the number of days fermentation was done.

**Key words:** Cocoa beans, Fat content, Fermentation, Free fatty acids, Storage

The processing of cocoa beans (*Theobroma cacao* L.) consists of two major steps, namely fermentation and drying (Hii et al., 2009). Fermentation begins immediately after the beans embedded in the mucilaginous pulp are removed from the pods. Fermentation methods vary considerably from country to country (Baker et al., 1994). However, the two widely used methods are the heap and box fermentation (Nielson et al., 2007). Fermentation takes 6–7 days during which time the cocoa beans are mixed twice to ensure even fermentation. In order to moderate the initially bitter flavour of cocoa beans and to develop the typical flavour, the cocoa beans must be subjected to a fermentation process during which highly bitter tannins present in them are oxidized, resulting in the formation of aromatic substances and the development of the typical brown to deep red-brown colour (Thompson et al., 2001). The reduction in bitterness and astringency is the result of diffusion of alkaloids (30% fall) and

polyphenols (20% fall) out of the cocoa beans (Camu et al., 2008). Research has shown that during fermentation, the polyphenol content of the cocoa beans decreases. If fermentation is terminated at three, four, five and six days, the polyphenol values will be 10.7%, 8.2%, 7.6% and 6.01% (w/w) respectively (Aikpokpodion and Dongo, 2010).

It has been observed that some farmers being under pressure from the buyers of cocoa beans do not allow their beans to undergo adequate fermentation. Some of the cocoa farmers just allow fermentation for a few days, while a few do not allow fermentation at all. However, there is no scientific data that show what happen to the fat content and FFA levels of cocoa beans in storage when the cocoa beans are not adequately fermented. This study was, therefore, conducted to determine the effect of inadequate fermentation of cocoa beans in storage.

### MATERIALS AND METHODS

#### *Fermentation and drying of cocoa beans*

Matured and fully ripe cocoa pods were harvested

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from cocoa plantation at the Cocoa Research Institute of Ghana (CRIG), Tafo. The harvested pods were opened using a wooden stick. The beans were separated from the placenta, and any black or diseased beans, germinated beans, placenta fragments or pieces of shell were removed. The wet cocoa beans were grouped into five samples. Plantain leaves were neatly laid on the ground, covering an area big enough to avoid the beans touching the ground and 50 kg of the wet cocoa beans were piled up in a heap on the leaves. The beans were covered tightly with banana leaves and other plant materials were added to prevent the top leaves from blowing off. This was done for each of the samples. Various samples were subjected to days of fermentation, starting with one day up to five days with consecutive openings and turning after every 2 days. This was done to get the purple beans for the experiment, but the recommended fermentation period is six days which will produce brown cocoa beans. After each fermentation period, the cocoa beans were sun dried by spreading them on a bamboo mats on a raised platform. The mats were rolled up at night to cover the cocoa beans from dew and rain. The beans were hand stirred and mixed constantly to ensure uniform drying. Each sample of the fermented cocoa beans was dried to 7.5% m.c. The samples were packed into jute bags and stored.

#### *Storage of dry cocoa beans*

Five samples (2 kg each) representing 1, 2, 3, 4, and 5 days' fermentation were stored in a miniature prototype 30 cm × 30 cm jute sacks for 90 days (three months) at  $30 \pm 2^\circ\text{C}$  and r. h. of  $70 \pm 2\%$  based on the prevailing condition at the cocoa warehouses in Ghana (Jonfia-Essien, 2004). Completely randomized design was used and each treatment was replicated four times. The jute sack was used to conform to the approved standard of storing cocoa beans in Ghana.

#### *Cut test*

Cut test was used to determine the purpleness of the dry cocoa beans. It is one of the cocoa grading schemes based on visual assessment of cocoa quality, which relies on changes in colour of the beans. A sample of 300 cocoa beans was taken from each jute sack and cut length-wise through the middle to expose the internal surface of the two cotyledons. The cut cocoa beans were examined visually in good daylight and the percentage of total purple (deep, pale, partly brown/ partly purple) cocoa beans were determined and recorded. This was done before storage and monthly during storage for each of the dry cocoa beans that have been fermented for the different days. Each treatment

was replicated four times.

#### *Free fatty acid analysis*

Recommended method of Federation of Cocoa Commerce (FCC) was used for the FFA analysis, and double extraction was carried out. Round bottom flasks of 250 mL were dried in the oven at  $105^\circ\text{C}$ , cooled in the desiccator, weighed and 180 mL of hexane was measured into the round bottom flasks. Each test sample of 10 g was measured into a thimble and was set up for extraction for 2 h using the Soxhlet apparatus. The set up was allowed to cool and the solvent drained into the round bottom flask. Each sample was ground with sand and set up for 2 h again. The solvent was concentrated into fat by evaporating the hexane using the rotary evaporator. The fat content was dried in the oven for 2 h and cooled in the desiccator. Weight of the extract and the flask were taken and recorded. The weighed fat extract was dissolved in 50 mL ethanol/Diethyl ether solution, 1/1 [v/v]. Three drops of phenolphthalein indicator were added to the fat in 50 mL ethanol/Diethyl ether. The mixture was titrated against 0.1 M sodium hydroxide in ethanol solution and the end-point taken and recorded for the FFA calculation.

## RESULTS AND DISCUSSION

Generally, the fat content significantly decreased ( $P < 0.05$ ) with the increase in the storage and fermentation periods. With the exception of the day 2 fermentation period, the reduction of the fat content over the storage period occurred in all the cocoa beans fermented in day 1, 3, 4, and 5. However, interaction of storage and fermentation periods also produced statistically significant effects on the percent fat in the dry nib of cocoa beans. This means the effect of length of storage on percent fat content depends on fermentation period. Consistently, percentage fat content was relatively high in cocoa beans fermented for 3 days, although the highest fat content (58.40%) was observed in cocoa beans fermented for 5 days and stored for 60 days. After 90 days of storage, 5 days' fermentation significantly reduced the least fat content (43.18%) from the baseline data (Table 1).

The percentage free fatty acid (%FFA) generally increased with length of storage (Table 2). Here, fermentation period was not relevant in the production of % FFA. However, its interaction with storage period was significant ( $P < 0.05$ ), implying that the % FFA depended on the number of days the fermentation was done. Though not a quality parameter, 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

Table 1 Percentage fat content of dry nib of varying fermentation period in storage

Storage period	Fermentation period					Mean storage period
	FP1	FP2	FP3	FP4	FP5	
ST30	51.95	49.36	51.51	48.16	48.82	49.96
ST60	52.71	49.48	54.68	49.36	58.40	52.92
ST90	50.78	50.65	50.06	47.99	43.18	48.53
Mean fermentation period	51.81	49.83	52.08	48.51	50.13	

LSD (5% level): Storage period (1.93); Fermentation period (2.49); Storage period × Fermentation period (4.31)

Table 2 Percentage free fatty acid of cocoa beans of varying fermentation period in storage

Storage Period	Fermentation period					Mean storage period
	FP1	FP2	FP3	FP4	FP5	
ST30	0.574	0.540	0.592	0.725	0.685	0.623
ST60	0.913	0.831	0.936	0.793	0.780	0.850
ST90	0.813	0.621	0.661	0.639	0.736	0.694
Mean fermentation period	0.767	0.664	0.730	0.719	0.733	

LSD (5% level): Storage period (0.0838); Fermentation period (0.1082); Storage period × Fermentation period (0.1874)

cocoa butter extracted from the dry cocoa beans. Consistently, % FFA was relatively high in beans stored for 60 days, although the highest amount (1.39 %) was observed in beans fermented for 3 days but not stored at all. Our findings confirm results of Simplice et al. (2003), 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. Also the initial and final FFA contents in cocoa beans fermented over 3 days were found to be higher than those in cocoa beans fermented below 3 days, collaborating with Guehi et al. (2008).

Different fermentation periods resulted in varying levels of purple cocoa beans. The purple content of cocoa beans decreases with the increasing period of fermentation. The polyphenol content in cocoa beans is among the factors responsible for the purple colour, thus if the polyphenol content in cocoa beans are high, the percentage of purple beans will be high. Aikopkpodion and Dongo (2010) reported a decrease in the polyphenol content with the increase in fermentation period, thus also decrease in the percentage of purple colour beans. He suggested that fermentation leads to gradual reduction of polyphenol in cocoa beans. Polyphenols are mostly responsible for the astringent sensation as well as bitter taste and colour (Misnawi et al., 2002). At the beginning of the storage period, there was high percentage of purple beans in cocoa beans fermented for 3, 4 and 5 days.

Guehi et al. (2010) reported a higher percentage of purple beans in cocoa fermented for 4 days than in cocoa fermented for 5 days using different method of fermentation. Under-fermented beans usually produce high percentage of purple beans, and cocoa with high percentage of purple beans gives a bitter and astringent chocolate. Although there was variation in the percentage of purple beans, the grade of the beans did not vary.

### CONCLUSION

The interaction of storage and fermentation periods had a significant effect on the percent fat in the dry nib of cocoa beans. Thus the effect of length of storage on percent fat content depends on fermentation period. The percentage free fatty acid (% FFA) generally increased with length of storage but the fermentation period was not relevant in the production of % FFA. However, its interaction with storage period was significant implying that the % FFA depended on the number of days fermentation was done. Under-fermentation of cocoa beans leads to the production of purple beans and cocoa beans fermented for one day produce slaty beans which are normally considered as unfermented cocoa beans.

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