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Influence of pulp-preconditioning and fermentation on fermentative quality and appearance of Ghanaian cocoa (*Theobroma cacao*) beans

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Abstract: Studies were conducted to evaluate effects of pod storage (as a means of pulp pre-conditioning) and fermentation time on the fermentative quality and appearance of Ghanaian cocoa beans. The fermentative quality (cut test and fermentation index [FI]) and colour (L, a, b) of the cocoa beans were studied using standard methods. Increasing pod storage and fermentation time significantly ($P<0.05$) influenced the fermentative quality and appearance of the beans. Fermentation caused significant increases in FI at all periods of pod storage whilst increasing pod storage resulted in darker, yellower and less red beans. Cut test scores revealed that storage of pods for 7 and 14 days increased the percentage of brown beans by 15 and 38% respectively by the sixth day of fermentation. Thus, Ghanaian cocoa pods could be stored for up to 14 days prior to fermentation with optimum fermentative quality and desired colour attained after 4 days of fermentation.

Key words: ***Theobroma cacao*, Forastero, pod storage, fermentation index, cut test, bean colour**

Introduction

The quality of cocoa beans depends on the complex chemical and biochemical changes which occur in the beans during fermentation and drying. The principal varieties of the cocoa tree *Theobroma cacao* (family *Sterculiacae*) are: *Criollo*, which is rarely grown because of disease susceptibility; *Nacional* with fine flavour and distinctive aroma and flavour characteristics, representing only 5% of global cocoa production, and grown in Ecuador; *Forastero* from the Amazonas region and grown largely in West Africa and South-east Asia, forming most of the "bulk" or "basic" cocoa market with over 90% of global production; and *Trinitario*, a hybrid of *Forastero* and *Criollo* (Afoakwa *et al*., 2007; Fowler, 2009).

World annual cocoa bean production is approximately 3.6 million metric tonnes and major producers are the Ivory Coast, Ghana, Indonesia, Brazil, Nigeria, Cameroon, Ecuador and Malaysia (Schwan and Wheals, 2004; ICCO, 2009; Afoakwa, 2010). These cultivars exhibit some similarities and differences in the appearance of pods, yields of beans, resistance to pests and disease, acidification (pH and acidity) concentrations, as well as flavour characteristics, intensity and release (Wood and Lass, 1985; Afoakwa *et al*., 2008; Kratzer *et al*., 2009; Rodriguez-Campos *et al*., 2011).

Fermentation is a key processing stage that causes the death of the bean and facilitates removal of the pulp and subsequent drying. During this stage, there is initiation of flavour precursor formation and colour development, and a significant reduction in bitterness (Biehl *et al*., 1990, Voigt *et al*., 1994). Cellular disruption and seed death initiate various enzymatic and non-enzymatic reactions between seed components. These reactions develop a range of flavour precursors (peptides, amino acids, reducing sugars and polyphenols), and also affect the colour of the beans (Schwan *et al*., 1995; Adeyeye *et al*., 2010). These precursors undergo further transformations during roasting, to form the final chocolate flavour compounds (Lopez & Dimick, 1991; Thompson *et al*., 2007; Ouattara *et al*., 2008; Rodriguez-Campos *et al*., 2011). During fermentation, the rate of diffusion of organic acids into the cotyledons, timing of initial entry, duration of the period of optimum pH and final pH are crucial for optimum flavour formation (Biehl *et al*., 1985). Beans of higher pH (5.5-5.8) are considered unfermented - with low fermentation index and cut test score - and those of lower pH (4.75-5.19), well fermented. Fermentation techniques can reduce acid notes and maximize chocolate flavours (Holm *et al*., 1993; Beckett, 2008; Afoakwa and Paterson, 2010).

Indicators of well-fermented and dried quality beans are a good brown colour, low astringency and bitterness, and an absence of off-flavours such as smoky notes and excessive acidity. Sensory

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assessment of cocoa beans dried using different strategies, i.e. sun drying, air-blowing, shade drying and oven drying suggested sun-dried beans were rated higher in chocolate development with fewer off-notes (Dias and Avila, 1993; Buyukpamukcu *et al*., 2001; Kyi *et al*., 2005; Granvogl *et al*., 2006). Off-notes from incomplete drying or rain soaking may result in high levels of water activity and mould contamination, producing high concentrations of strongly flavoured carbonyls, leading to alterations in bean flavour, producing hammy off flavours, which is also correlated with over-fermentation (Dimick and Hoskin, 1999; Misnawi *et al*., 2003; Afoakwa and Paterson, 2010; Páramo *et al*., 2010).

The technique of pod storage (as a means of pulp pre-conditioning) of cocoa beans has been reported to be beneficial to fermentation outcomes (Sanagi, 1997; Meyer *et al*., 1989; Nazaruddin *et al*., 2006). Previous reports have shown that, pod storage results in decreases in pulp volume per seed due to water evaporation and inversion of sucrose; diminishes total sugar content leading to reduced acid production during fermentation (Biehl *et al*., 1989). The flavour quality of Malaysian beans has been reported to improve by pod storage for up to 21 days prior to fermentation (Duncan *et al*., 1989; Aroyeun *et al*., 2006). As well, reductions in acidity and polyphenol content of Malaysian cocoa beans have been reported (Nazaruddin *et al*., 2006). Previous findings on the chemical and physical quality characteristics, changes in acidification, proteolysis, sugars, free fatty acids, polyphenolic and anthocyanin concentrations of Ghanaian cocoa as influenced by pulp pre-conditioning and fermentation have been published (Afoakwa *et al*., 2011a,b,c). However, the extent to which this technique of pulp pre-conditioning would influence the fermentative quality and appearance of Ghanaian cocoa beans still remains unknown.

The objective of this study was to investigate the influence of pod storage (as a means of pulp pre-conditioning) on the fermentative quality and appearance of Ghanaian cocoa (*Theobroma cacao*) beans.

Materials and Methods

Samples

Ripe cocoa pods from mixed hybrids (of two *Forastero* cultivars - Amelonado and Amazonica) were harvested from the experimental plots of Cocoa Research Institute of Ghana (CRIG), Tafo in the Eastern Region of Ghana. The cocoa pods were selected according to their ripeness and maturity levels. The beans were pulp preconditioned by storing about 5000 pods (on the cocoa plantation) at ambient temperature (25-28°C) and relative humidity (85-100%) for periods of 0, 7, 14 and 21 days respectively. The stored pods (~ 1200) were then split after these predetermined storage times and fermented using the traditional heap method.

The fermentation was done by heaping about 50 kg of the extracted cocoa beans on the fermenting platform covered with banana leaves. The heaped beans were again covered with banana leaves and fermented for six days with consecutive openings and turnings after every 48 h. Samples of the unfermented beans were picked into a sterile polythene bag and after every 2 days of fermentation till the end of the sixth days, for drying and subsequent analysis. After each sampling time, the samples were immediately transported to the laboratory for drying by spreading the cocoa beans approximately 5 cm deep on metal trays (40 cm × 60 cm), and placed in a temperature controlled, forced air oven for about 24 h at a temperature of 45-50°C until dried (to moisture content below 8%). The dried beans were bagged in airtight black plastic bags and stored at ambient temperature (25-28°C) in a dark room free from strong odours until used for the analyses.

Experimental design

A 4 x 4 full factorial design was conducted with experimental factors as pod storage (0, 7, 14 and 21 days) and fermentation time (0, 2, 4 and 6 days). The fermentative quality (cut test and fermentation index) and colour (L-, a-, b-values) of the cocoa beans were studied.

Measurements of cut test

The cut test was performed according to the International method described by Guehi *et al*. (2008). A total of 300 beans were cut lengthwise through the middle in order to expose the maximum cut surface of the cotyledons. Both halves were examined in full daylight and placed in one of the following categories: purple, partly brown/partly purple, brown, slaty, germinated and mouldy.

Determination of fermentation index (FI)

FI were determined according to the method described by Gourieva and Tserrevitinov (1979). In this procedure, 0.5 g ground cocoa nibs was extracted with 50 ml of 97:3 mixture of methanol:HCl. The homogenate was allowed to stand in refrigerator (8°C) for 16–19 h and then vacuum filtered. The filtrate was made up to volume in 50 ml volumetric flask. The filtrate was read separately in a Spectrophotometer (LKB Biochrom Novaspec II UV Spectrometer,

Birmingham, UK) at 460 nm and 530 nm absorbances respectively. The fermentation index of the sample was obtained by calculating the ratio of absorbance at 460 nm to the absorbance at 530 nm. Three replicate readings were obtained for each sample and the mean values reported.

Colour determination

The colour of each sample was measured with a Minolta CR-310 Tristimulus Colorimeter (Minolta Camera Co. Ltd, Tokyo, Japan). Measurement were based on the $L^*a^*b^*$ colour system. The colour space parameters L^*, a^* and b^* of the samples were calculated as: L^* (L_s-L_0) for lightness (100 = perfect/ brightness to 0=darkness/blackness); a^* (a_s-a_o) for the extent of green colour (in the range from negative = green to positive = redness); b^* (b_s-b_o) qualifies blue in the range of negative= blue to positive = yellow. A reference white porcelain tile with the following readings: L_0= 97.51, a_0= 0.29 and b_0= 1.88 was used.

Statistical analyses

The data were analysed using Statgraphics software version 13.0 (STSC Inc, Rockville, MD, USA) for analysis of variance (ANOVA). Least significant difference (LSD) was used to separate and compare the means and significance was accepted at 5% level (p<0.05). All treatments and measurements were conducted in triplicates and the mean values reported.

Results and Discussion

Fermentation index (FI) of pulp pre-conditioned and fermented cocoa beans

Table 1 shows the mean data of FI from different days of pod storage and fermentation. Results of colour fraction spectral measurements (Table 1) showed that cocoa beans from all the pod storage treatments prior to fermentation (unfermented beans) had maximum absorption value of 1.70 at 530 nm and this could be attributed to the presence of high amounts of anthocyanin pigments in the unfermented beans. Increasing fermentation time of the beans led to drastic decreasing absorbances from 1.70 to 0.44 at 530 nm within the 6 days of fermentation whiles only marginal decreases of 0.63 to 0.46 were observed in the absorbances at 460 nm. These resulted in increasing fermentation index of the beans from 0.372 to 1.050 between the 0 and 6th day fermentation. Generally, similar trends in colour fraction spectral measurements and FI were noted during fermentation of all the different pod storage treatments (Table 1). These changes could be due to the breakdown of anthocyanin pigments during fermentation as reported previously (Afoakwa *et al*., 2011c), with subsequent formation of more and more condensation products of anthocyanin, such as cyanidin-3-β-D-galactosid and cyanidin-3-α-L-arabinosid as fermentation progressed (Kim and Keeney, 1984).

Table 1. Effect of fermentation time and pod storage on colour fractions absorbance value and fermentation index of cocoa beans

Pod storage (days)	Fermentation time (h)	Colour fractions absorbance values		Fermentation Index (Fraction II / Fraction I)
		Fraction I (530 nm)	Fraction II (460 nm)	
0	0	1.70	0.63	0.372
	2	0.80	0.56	0.706
	4	0.65	0.50	0.774
	6	0.44	0.46	1.050
7	0	1.54	0.61	0.399
	2	0.57	0.42	0.751
	4	0.52	0.41	0.779
	6	0.39	0.41	1.052
14	0	1.65	0.61	0.368
	2	0.60	0.40	0.661
	4	0.40	0.41	1.025
	6	0.23	0.29	1.278
21	0	1.54	0.59	0.382
	2	0.58	0.36	0.619
	4	0.28	0.29	1.040
	6	0.29	0.28	0.962

The table also showed rapid changes in FI during the first 4 days of fermentation, which subsequently slowed down after the sixth day, probably due to the fact that the condensation product became less soluble with increased fermentation. Analysis of variance showed that pod storage and fermentation time as well as the interaction of these factors had significant effects (p<0.05) on the fermentation index of the cocoa beans (Table 2). Multiple range test revealed that the different pod storage treatments were significantly different (p<0.05) from each other. Overall, pod stored for 7 and 14 days were noted to have FI value of more than 1 after 6 days of fermentation while those stored for 14 achieved FI of 1.025 after 4 days of fermentation. Cocoa bean is considered under fermented when it yields an FI of less than 1.0 and any values above this is considered fully fermented (Gourieva and Tserrevitinov, 1979).

Colour of pulp pre-conditioned and fermented cocoa beans

Colour is one of the most important quality attribute of a food. This is because no matter how nutritious, flavoured or well textured a food product is, it is unlikely to be accepted unless it has the right colour (Serna-Saldivar *et al*., 1990). Results of the tristimulus colour measurements on cocoa powders

obtained from the pulp preconditioned fermented cocoa beans showed that the lightness of the cocoa samples ranged from 44.09 to 46.23 for the cocoa beans that were not stored prior to fermentation (Table 3). Generally, the samples became darker as fermentation increased and lightness also decreased progressively with increasing pod storage. This might be due to the destruction of anthocyanins by enzymic hydrolyses, which is accompanied by bleaching and subsequent browning of the beans as reported in our earlier work (Afoakwa *et al*., 2011a). Multiple range tests showed that the different pod storage treatments (0, 7, 14 and 21 days) were statistically different from each other with beans stored for 21 days being the darkest.

Table 2. ANOVA summary table showing F-ratios for variation in fermentation index and colour of pulp preconditioned and fermented cocoa beans

Variables	Fermentation Index	L*	a*	b*
Pod storage	1766.27*	116598.79*	48375.59*	862404.00*
Fermentation time	231111.63*	662340.54*	335066.84*	1237338.00*
Interaction	7487.42*	42428.22*	103.41*	155220.22*

* Significant at p<0.05

Redness a*, however, decreased progressively during fermentation, and higher decremental rates were observed during the fourth to sixth day of fermentation for all the pod storage treatments. This decrease could be attributed to the breakdown of anthocyanins which normally imparts the purple colour to under fermented beans. It was also observed (Table 2 and 3) that as pulp preconditioning increased the redness of the samples decreased significantly (p<0.05) probably due to the diffusion of the polyphenols along with cell liquids from their storage cells during pod storage (Wollgast and Anklam, 2000).

Table 3. Colour measurements of cocoa beans as affected by pod storage and fermentation time

Pod storage (days)	Fermentation time (days)	L*	a*	b*
0	0	46.23 ± 0.35	12.10 ±0.43	6.45 ±0.52
	2	45.35 ±0.11	11.95 ±0.08	9.03 ±0.03
	4	44.79 ±0.41	9.23 ±0.09	8.19 ±0.14
	6	44.09 ±0.48	8.69 ±0.114	9.00 ±0.11
7	0	46.80 ±0.54	12.09 ±0.17	7.90 ±0.09
	2	44.39 ±0.14	12.91 ±0.04	9.11 ±0.03
	4	44.26 ±0.04	10.12 ±0.02	9.82 ±0.05
	6	41.19 ±0.23	6.89 ± 0.04	13.26 ±0.04
14	0	46.53 ±0.53	11.54 ±0.03	5.86 ±0.09
	2	44.60 ±0.66	9.80 ±0.05	10.61 ±0.20
	4	43.60 ±0.01	8.90 ±0.02	11.69 ±0.02
	6	40.73 ±0.05	5.88 ±0.02	16.50 ±0.02
21	0	46.40 ±0.34	11.07 ±0.06	8.16 ±0.18
	2	44.84 ±0.25	9.98 ±0.03	13.23 ±0.38
	4	41.27 ±0.10	9.39 ±0.05	17.44 ±0.05
	6	38.92 ±0.42	5.80 ±0.04	16.02 ±0.06

The yellowness of the cocoa beans increased as fermentation days increased and as pod storage days increased. Increase in yellowness during fermentation could be due to the presence of oxidized polyphenols, as a result of enzymatic oxidation by polyphenol oxidase in the beans. For the four different pod storage treatments it was observed from Table 3 that at the end of fermentation, cocoa beans became darker, more yellowish but less reddish. However these changes became more prominent as the pod storage days increased. Thus, it can be inferred that pulp preconditioning influenced the fermentation of cocoa beans and resulted in the formation of darker beans. This agrees with the spectral measurement (Table 1) in which the spectral changes were higher in the pulp preconditioned fermented beans than the unfermented beans.

Cut test scores of pulp pre-conditioned and fermented cocoa beans

The cut test is used as an index of fermentation and relies on changes in colour of cocoa beans. It is the standard test used to assess the suitability of cocoa beans for chocolate processing. It is also the standard method of assessing quality as defined in grade standards and can be used to estimate two major off-flavours (mouldy and unfermented beans). It identifies other defects which can affect the keeping quality (Wood and Lass, 1985). The cut test was carried out on cocoa beans that had been fermented for 4 and 6 days based on the results obtained for the fermentation index, which illustrated that beans were fermented between the 4th and 6th day (Table 4).

In order to assess the degree of fermentation, the cut beans were divided into six different categories: purple, partly brown/partly purple, brown, slaty, germinated and mouldy (Table 4). Generally, reductions in the purple beans were noted between the 4th and 6th days of fermentation with values reducing from 21 to 10, 11 to 6 and 1 to 0 in the pods stored for 0, 7 and 14 days respectively. Beans from the pods stored for 21 days had no purple beans after both 4th and 6th days of fermentation (Table 4). On the contrary, the brown beans were noted to increase with increasing fermentation time from the 4th to the 6th day. Table 4 shows increasing brown beans from 41 to 58, 59 to 67, 77 to 80 and 88 to 76 respectively for the beans from pods stored for 0, 7, 14 and 21 days. The increasing changes in brown beans with fermentation is suspected to be resulting from changes in anthocyanin and oxidation products of the polyphenol oxidase activities might have contributed to the brown pigments formation in the cocoa beans during the fermentation period. The brown pigments

Table 4. Effect of fermentation time and pod storage on surface colour and cut test score of dried cocoa beans

Pod storage (days)	Fermentation time (days)	Purple (%)	Purple/Brown (%)	Brown (%)	Slaty (%)	Germinated (%)	Mouldy (%)
0	4	21	38	41	-	-	-
	6	10	32	58	-	-	-
7	4	11	30	59	-	-	-
	6	6	27	67	-	-	-
14	4	1	21	77	-	2	-
	6	-	20	80	-	-	-
21	4	-	1	88	-	11	3
	6	-	1	76	-	18	5

might also be produced from complexation of condensed tannin, a high molecular weight product of flavonoid polymerization, with protein, via hydrogen bonding (Shamsuddin and Dimmick, 1986).

The proportion of partly purple and partly brown beans did not exceed 50% for all the pod storage days and this gives an indication that the beans were adequately fermented and will not give rise to bitter and astringent flavours. Beans described as 'partly brown' and 'partly purple' are not defective and should be present at least to the extent of 20% (Wood and Lass, 1985). The 14 and 21 days pod storage had their beans falling within this range whilst the 0 and 7 days of pod storage were higher (27-38%), however, still within the acceptable range (30-40%). For good cocoa flavour development the degree of fermentation (% fully brown beans) should be above 60%. Beans stored for 7 days and beyond fell within this range and this was in agreement with spectral and tristimulus colour measurements (Table 1 and 3).

A number of defects were detected in the beans that were stored for 14 and 21 days and fermented. Germination occurred in beans stored for 14 and 21 days prior to fermentation, though it was lesser in the 14 days pod storage. The incidence of germination occurred because of the prolonged storage of the pods which resulted in the rotting of pods and consequently penetration of oxygen into the pods creating optimum conditions for growth of the beans. By 21 days of pod storage, the proportion of germinated beans had increased considerably and this increased further during fermentation. It was expected that the heat produced during fermentation coupled with the diffusion of some metabolites (ethanol and acetic acid) into the bean could result in the death of the bean hence arresting germination. However, enough acids were not generated in the beans as shown in the physicochemical analysis of the beans as reported previously (Afoakwa *et al*., 2011b). This suggests that beans stored for 21 days were not adequately fermentation. Germinated beans are considered a defect because the hole left by the emerging radicle provides an easy entrance for insects and moulds. They are also considered to lack good chocolate flavour (Wood and Lass, 1985). Slaty beans were absent in the fermented beans for all the pod storage days.

Mouldy beans (18%) were also detected in the beans stored for 21 days before fermenting. According to Wood and Lass (1985), internal moulds are the major causes of off-flavours during cocoa processing, and samples of beans with as little as 4% of internal moulds can produce off- flavours in their finished products. Beans stored for 21 days exceeded this limit and this could be ascribed to the invasion of mould species (*phytophthora palminovora* and *Botryodiplodia theobrommae*) during the prolonged pod storage and as a result the beans may produce off-flavours when roasted. Moulds inside the beans can also increase the free fatty acid (FFA) content of the cocoa butter (Wood and Lass, 1985) and this might have accounted for our previous findings of high FFA values obtained for the beans from the pods stored for 21 days (Afoakwa *et al*., 2011b).

Conclusion

Increasing pod storage and fermentation significantly influenced the fermentative quality and appearance of the beans. Fermentation caused significant increases in FI at all periods of pod storage whilst increasing pod storage resulted in darker, yellower and less red beans. Cut test scores revealed that storage of pods for 7 and 14 days increased the percentage of brown beans by 15 and 38% respectively by the sixth day of fermentation. Fermentation index of ~1.0 (well fermented level) was attained by all pulp preconditioned beans after 6 days of fermentation. Pods stored for 14 and 21 days, however, attained the fermentation index of 1.0 after 4 days of fermentation. As well, cocoa beans pulp preconditioned for 14 and 21 days were over 60% fully brown by the fourth day of fermentation suggesting that pod storage for 14 days could be used to reduce fermentation time of Ghanaian cocoa beans from 6 to 4 days. However, cut test scores revealed that the pods stored for 21 days had 11% and 18% of germinated beans respectively which were well

above the acceptable level of 5% in well fermented beans. It was therefore concluded that Ghanaian cocoa pods could be stored for up to 14 days prior to fermentation for optimum generation of fermentative quality and desired colour.

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