

Changes in Biochemical and Physico-chemical Qualities during Drying of Pulp Preconditioned and Fermented Cocoa (*Theobroma cacao*) Beans

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Abstract

This study investigated the effects of post-harvest pod storage as a means of pulp preconditioning on the souring production, flavour precursors development and free fatty acids during drying of fermented Ghanaian cocoa beans. A 4 x 4 full factorial experiment was conducted with pod storage (0, 7, 14, 21 days) and drying times (0, 2, 4 and 6 days) as the principal factors. The souring/acidification indices (pH and titratable acidity), total sugar, total nitrogen and free fatty acids (FFA) were studied using standard analytical methods. The results showed that titratable acidity, total sugars and total nitrogen in cocoa beans decreased during drying and with increasing pod storage durations probably due to their participation in Maillard non-enzymatic reactions to form flavour volatiles and colour pigments as Amadori intermediates. By contrast, FFAs and pH increased during drying and with increasing pod storage durations. Pod storage for up to 7 days followed by 6 days of fermentation and drying respectively produced beans with acceptable FFA values below 1.75% whilst enhancing the flavour precursors development and reductions in nib acidity. However, these observed changes were more dependent on pod storage than on drying.

Keywords: Cocoa; *Theobroma cacao*; Pod storage; Pulp preconditioning; Fermentation; Flavour volatiles

Introduction

Theobroma cacao belongs to the family *Sterculiaceae*, with three main varieties - *Criollo*, *Forastero* and *Trinitario* dominating the world cocoa trade. Cocoa is grown principally in West Africa, Central and South America and South-East Asia, and the eight largest producing countries include Côte d'Ivoire, Ghana, Indonesia, Nigeria, Brazil, Cameroon, Ecuador and Malaysia. Typically, the *Forastero* variety is grown across the West African countries - Cote d'Ivoire, Ghana, Nigeria and Cameroon and accounts for ~ 70% of the beans sold on the global cocoa market [1-3].

Chocolate flavour originates from flavour precursors generated in cocoa beans during fermentation and drying and are

transformed into flavour notes during roasting. The fermentation process triggers an array of chemical reactions within the bean which generate the biochemical precursors of chocolate flavour, aroma and colour [1,4-8]. The process produces alcohol and acids, and generates heat, typically raising the temperature of the fermenting beans to about 45°C to 50°C within the first 72 hours. The acids lower the pH and cause acidification or souring of the bean which is a desirable quality in chocolate. The acid and heat generated kills the cocoa bean causing the cell walls within the bean to break down, allowing enzymes to come into contact with their substrates resulting in the beginning of the biochemical changes in the bean [9-11].

During fermentation, bitter and astringent flavours which are due to polyhydroxyphenols such as catechins, flavan-3-ols, anthocyanins, and proanthocyanadins are modified in a multi-step process, oxidised and condensed enzymatically into milder tasting substances. Anthocyanins are converted into anthocyanidin by glucosidase enzymes. Anthocyanidin are oxidized by polyphenol oxidase into quinone which combines with amino acids and proteins to form melanin. Polyphenols diffuse out of the bean during the fermentation and are also oxidised by polyphenol oxidases to produce mostly insoluble tannins. The enzymic reactions continue in several ways and yield flavour precursors and some flavour notes [10,12-14]. The great majority of chocolate flavour compounds are formed due to biochemical and enzymatic reactions that occur within the cotyledon [8,10,15-18].

Pulp preconditioning entails changing the properties of the pulp in cocoa beans prior to fermentation and since the pulp is the substrate metabolised during fermentation, changes in the pulp affect the production of acids by lactic acid bacteria, yeasts and acetic acid bacteria [15,19]. The technique reduces the formation of acids throughout the fermentation without enhancing the degradation of acids at the end of fermentation.

Methods of pulp preconditioning include, bean spreading, bean pressing, depulping and post harvest pod storage [19].

Pod storage as a method of pulp preconditioning involves keeping the harvested pod (fruit) for a period of time before breaking the pod. In West Africa, the pods are usually left on the ground to be gathered together for breaking by a team of workers after a few days of harvesting. Ghanaian farmers unknowingly adopted this technique of pod storage simply by their practice of using family labour to collect the harvested pods into piles before the pods are opened to remove the beans for fermentation. During pod storage, the beans within the pod lose moisture which allows more air to penetrate the pods into beans before the pods are broken to start the fermentation. This facilitates the fermentation process with accompanied faster temperature rises and thus resulting in improved quality of the cocoa beans [15,19,20]. Previous research findings revealed that fermentation of beans from stored pods are more rapid and result in higher brown bean counts [21]. As well, pod storage has been reported to reduce pulp volume and acidity, effecting subsequent reductions in the polyphenolic and anthocyanin components, resulting in enhanced bean flavour quality [3,16].

Drying is initiated when the fermentation process is complete and this reduces the moisture content of the beans from ca. 60% to ca. 7.0% an appropriate moisture content that provides security for the prolonged storage of the beans [5,22]. Above 8% moisture there is the danger of moulds developing within the beans, whilst below 5% the beans are very brittle [1]. Drying also allows some of the chemical changes which occur during fermentation to continue and improve flavour development. This helps to reduce bitterness and astringency and also the development of the chocolate brown colour of well fermented cocoa beans [13,23].

Even though some studies have been conducted on pulp pre-conditioning and its effects on the quality of cocoa beans, previous work have been focussed on elucidating the effects on acidification, biochemical constituents (mainly flavour precursors) and polyphenolic constituents during fermentation [3,11,14,18-20]. Knowledge on the further changes in acidification, sugar and protein nitrogen (flavour precursors) concentrations and free fatty acids during drying largely remains unknown. Thus, the objective of this work was to investigate changes in biochemical qualities (flavour precursors development and free fatty acids) and physico-chemical qualities (souring production by acidification) during drying of pulp pre-conditioned and fermented cocoa beans.

Materials and Methods

Materials

Fleshly harvested pods of mixed hybrids (Amelonado and Amazonica) were obtained from the cocoa plantation of the Cocoa Research Institute of Ghana (CRIG), Akim Tafo in the Eastern Region of Ghana and used for the study.

Sample preparation: Freshly harvested cocoa pods (fruits) were first sorted out (only healthy pods were picked) and kept in a heap for four different storage duration (0, 7, 14 and 21 days).

Each heap for the four storage periods was made up of 600 pods. The pods were stored under shade and were broken after the specified days of storage. The beans were fermented for six days using the heap fermentation technique. The fermenting cocoa beans were mixed after every 48 hours until the fermentation process was over. The fermented cocoa beans were dried in the open sun on raised platforms using the traditional process [2]. Drying started 8 am and ended at 5 pm each day for six days. The beans were stirred four times each day and were covered with palm mats in the evening till the next morning. Samples for analyses were taken immediately after fermentation (prior to drying) and after every 48 hours of drying intervals. The samples taken were packaged in air tight plastic bags and taken immediately to the laboratory for analysis. The sample treatments were conducted in duplicates.

Experimental Design: The studies were conducted using a 4 x 4 full factorial design with experimental factors as pod storage (0, 7, 14 and 21 days) and drying times (0, 2, 4 and 6 days). Changes in moisture content, pH, non-volatile (titratable) acidity, total sugars, protein nitrogen and free fatty acids were studied.

Methods

Moisture determination: The moisture content of the beans (nib) was determined using the International Organisation for Standards (ISO) method as modified by Hamid and Lopez (2000). Approximately 10 g of ground bean sample was placed into a pre-weighed dish (W_1) with a lid and re-weighed to the nearest mg (W_2). Dishes and contents were put in an oven at $103 \pm 2^\circ\text{C}$ for 16 hours. The results were calculated and the mean values reported.

Determination of pH and non-volatile (titratable) acidity: Non-volatile acidity of the cocoa beans were determined according to AOAC method 970.21, [24] (AOAC, 2005) and expressed as % acetic acid by titrating juice with 0.1N NaOH. Five grams samples of the beans were homogenized for 30 s in 100 ml of hot distilled water and vacuum filtered through Whatman filter paper No. 4. A 25 ml aliquot was pipetted into a beaker and the pH measured using a pH meter (model MP230 Mettler Toledo MP 230, Geneva, Switzerland). A further 25 ml aliquot was titrated to an end point pH of 8.1 with 0.01N NaOH and the values reported as moles of sodium hydroxide per 100 g dry nibs. The results were calculated and the mean values reported.

Determination of total sugars: Total sugars were determined using the phenol sulphuric acid method 9 [25]. Dry weights of the samples were determined by drying the fresh samples overnight in an oven at 105°C . The sample was boiled in 80% ethanol under reflux for 30-40 min and the supernatant removed. The ethanol was evaporated under reduced pressure in a rotary evaporator. Some water was added to make sure the sugar present was in solution. After removal of the alcohol, alcohol-soluble, water-insoluble substances were precipitated. The precipitate was filtered into a clean flask through Whatman No. 41 filter paper followed by washing of the paper with distilled water. The extract was clarified by adding 5 ml of the ZnSO_4 solution to the extract followed by 4.94 ml of the $\text{Ba}(\text{OH})_2$ solution. The solution was allowed to stand for about 5 min. and

filtered through Whatman No 41; the filter paper was washed with distilled water to remove any sugar still trapped in the precipitate. A mixture of Zeokarb 225 (H⁺), a cation exchange resin, and Deacidite FF (OH) was added to the filtrate, shaken and then filtered. To 1 ml of the extract, 1 ml phenol reagent and 5 ml of H₂SO₄ was added and allowed to stand for an hour to develop colour. The absorbance of the sample was read at 480 nm and the results calculated. The analysis was conducted in triplicates and the mean values reported.

Determination of protein: Protein content of the nibs was determined by the Kjeldahl method using the [24] AOAC (2005) method 970.22. The percent protein was calculated by multiplying the percent nitrogen by the conversion factor 6.25. The analysis was conducted in triplicates and the mean values are reported.

Determination of free fatty acids (FFA): Fat from the samples was extracted with petroleum ether (40-60°C) in a Soxhlet apparatus using [24] AOAC (2005) method 963.15. Free fatty acid of the oils extracted was determined using [26] IOCCC method 42-1993, (IOCCC, 1996). Five (5) gram of the oil was weighed into a dry 250 ml stopper conical flask and 25 ml of 95% Ethanol/Ether (1:1) and phenolphthalein indicator. The solution was titrated with 0.1N NaOH, shaking constantly until pink colour persisted for 30 seconds and the percentage free fatty acid determined. The analysis was conducted in triplicates and the mean values reported.

Statistical analysis

The data were analysed using Statgraphics software version 3.0 (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 ($p < 0.05$). Further analyses were conducted to evaluate the combined effect of pulp preconditioning and drying time on the studied parameters using response surface methodology. The data obtained were evaluated using stepwise multiple regression procedures. Models were developed to relate pulp preconditioning and drying time on the moisture content, souring production (non-volatile acid and pH), protein nitrogen, total sugars and free fatty acids in cocoa beans. The coefficients of the variables in the models and their contribution to the model's variation were reported. The R² values were used to judge the adequacy of the models. The R² of a model refers to the proportion of variation in the response attributed to the model rather than random error. For a good fit of a model, an R² of at least 60% was used. All sample treatments were conducted in duplicates, the analyses in triplicates and the mean values reported.

Results and Discussion

Effect of drying time and pod storage time on pH of cocoa nibs

The pH of cocoa beans is a very important physico-chemical property as it indicates the level of souring production or acidification of the bean after fermentation. High pH indicates low acidification and vice versa. The level of pH also determines the

fermentation status, biochemical reactions and tastes of the bean. For instance, beans with low pH tastes acidic and beans with high pH above 7 are likely to have been over-fermented [1,2,9,10,27].

The data obtained for the changes in pH during drying of pulp pre conditioning of cocoa beans was fitted into a multiple regression model as follows;

$$Z = 5.29095 + 0.15462X_1 + 0.06441X_2 - 0.00290X_1^2 - 0.00234X_2^2 + 0.00126X_1 * X_2 \text{ with } R^2 = 96.9\%. \text{ Where } Z = \text{pH}, X_1 = \text{pod storage time}, X_2 = \text{drying time}$$

The initial pH of the fresh non pod stored beans after fermentation was 5.14. After six days of drying the pH increased slightly to 5.69. The initial pH of the 7, 14 and 21 days pod stored beans after fermentation was 6.50, 6.83 and 7.13 respectively. The result as represented in Figure 1 indicates that, pH increased as drying time increased. These increases in pH were however very slight and non-significant (Table 1). The increases in pH during drying might be due to the fact that some of the volatile organic acids mainly acetic acids might have been lost along with the evaporating moisture from the beans [1].

The significant ($p < 0.001$) increases in pH of pod stored beans after fermentation is suspected to be due to the reduction in pulp volume during pod storage suppresses the anaerobic phase of fermentation and under these conditions the development of lactic acid bacteria is suppressed and the respiration of sugars by yeasts is considerably increased over alcoholic fermentation and therefore nib pH value does not fall below 5.0 [16,19] During fermentation (with non pod stored beans) the sticky pulp causes anaerobic conditions and ethanol fermentation to predominate as long as sugars are available during the first hours of fermentation [1,9].

The response surface plots developed (Figure 1) showed a curvilinear nature of the effect of pod storage time on pH, meaning that as pod storage durations increased pH increased drastically. The significant increases in pH during pod storage and drying is a very important phenomenon as it produces the desired sourness in the fermented beans and also has great influence on Maillard non-enzymatic browning, and flavour development during subsequent processing [13].

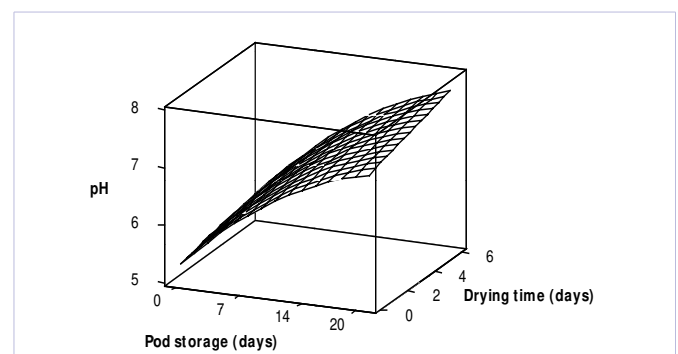


Figure 1: Response surface plot of effect of drying time and pod storage on pH of cocoa nibs.

Table 1: F-values from ANOVA for the changes in biochemical and physico-chemical constituents of pod stored beans during drying.

Variables	F-values					
	Moisture	pH	Titratable acidity	Total sugars	Protein	FFA
Constant	22.989*	37.500*	32.741*	21.757*	45.948*	7.166*
X ₁	-3.587*	7.177*	-14.884*	-2.154*	-6.665*	1.399
X ₂	-8.655*	0.854	-2.896*	-4.387*	-13.135*	-0.035
X ₁ ²	-0.655	-3.154*	7.554*	-0.312	0.466	0.039
X ₂ ²	2.912*	-0.208	0.814	1.036	5.767*	0.954
X ₁ *X ₂	4.826*	0.492	1.306	1.136	6.698*	1.888
R ²	0.974	0.969	0.989	0.950	0.986	0.907

* Significant at p≤0.05

Effect of drying time and pod storage time on titratable acidity of cocoa nibs

Volatile acidity formed during cocoa beans fermentation reaches approximately 2% of the dry basis. This acidity is the result of some components such as acetic, propionic, butyric, isobutyric and isovaleric acids but 90% of these components are acetic acid, which has an important role in souring production or acidification as well as the catalysis of enzymatic reactions for production of components of desirable sensorial characteristics in the fermented beans [13,23].

The data obtained for the changes in titratable acidity during drying of the pulp preconditioned cocoa beans was fitted into a multiple regression model as follows:

$$Z = 1.38080 - 0.09584X_1 - 0.06526X_2 + 0.00207X_1^2 + 0.00273X_2^2 + 0.00100X_1 * X_2, \text{ with } R^2 = 98.9\%. \text{ Where } Z = \text{titratable acidity, } X_1 = \text{pod storage time, } X_2 = \text{drying time}$$

From the model there was strong significant (p≤0.001) influence of both the linear and quadratic factor of pod storage time on the model. There was significant (p=0.016) influence of the linear factor of drying time on the model. The quadratic factor of drying time as well as the interactions factor had no significant influence on the model (Table 1). The model could explain 98.9% of the variations in titratable acidity.

Response surface plot developed from the model (Figure 2) showed a slight curvilinear nature of the effect of pod storage time on titratable acidity, as pod storage duration increased, titratable acidity decreased drastically. The response surface plot also showed that drying time increases caused gradual decline in titratable acidity. The significant decrease in total acidity during pod storage is a very important phenomenon as it has great influence on the flavour development during processing [13].

The initial titratable acidity of the fresh non pod-stored beans after fermentation was 1.43 meq/NaOH. After drying for six days the titratable acidity was 1.07 meq/NaOH. The initial titratable acidity of the pod stored beans after fermentation was 0.73, 0.49 and 0.27 meq/NaOH for the 7, 14 and 21 days pod stored beans respectively. The results (Figure 2) indicated that titratable acidity decreased as drying time increased. It also shows that increasing pod storage durations decreased titratable acidity of the cocoa nib significantly after fermentation (Table 1).

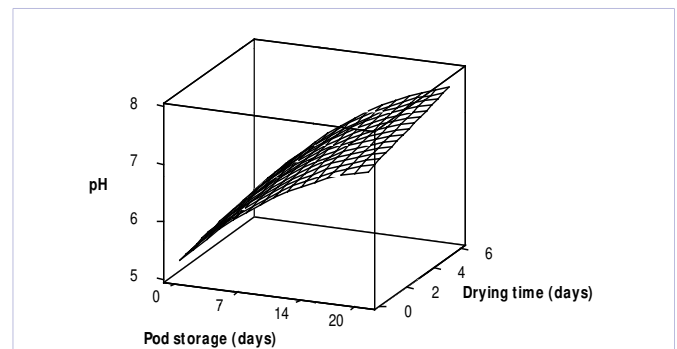


Figure 2: Response surface plot of titratable acidity of cocoa nib as a function of drying time and pod storage time.

The gradual decrease in total acidity during drying was due to loss of some of the volatile organic acids through the evaporating moisture from the beans [1]. These gradual changes are not so significant during the initial stages of drying as less moisture is lost in the bean but as the drying proceeds up to about 4-6 days there is significant decrease in total acidity due to the fact that by then more of the moisture and for that matter more of the volatile organic acids have been evaporated.

The decrease in acidity during the pod storage process was in line with similar findings by Faborode. They indicated that that there was great reduction in pulp volume during pod storage prior to fermentation which affects the production of acids by lactic acid bacteria, yeasts and acetic acid bacteria. Further research by [16] showed that under these conditions the development of lactic acid bacteria is suppressed and the respiration of sugars by yeasts is considerably increased over alcoholic fermentation and therefore nib acidification is greatly reduced.

Effect of drying time and pod storage time on moisture of cocoa beans

The recommended percentage moisture after drying has been established at 7.5% for secure storage of cocoa for a couple of months in the tropics. Above 8% moisture there is the danger of moulds developing within the beans, whilst below 5% the beans are very brittle [5,22,23,28,29].

The initial moisture content of the fresh non-stored beans after fermentation was 55.31%. After six days of drying the moisture content decreased to 8.94%. The initial moisture of the pod stored beans were 50.58%, 35.02% and 26.58% for the 7,

14 and 21 days pod-stored beans respectively. The final moisture contents of the pod-stored beans after six days of drying were all lower than that of the non pod-stored beans as shown in Figure 3. The results indicated that the initial moisture in cocoa beans after fermentation decreases significantly (Table 1) with increasing pod storage.

The data obtained for the changes in moisture during drying and pulp pre-conditioning (pod storage) was fitted into a multiple regression model as follows;

$Z = 58.4508 - 1.3923X_1 - 11.7595X_2 - 0.0101X_1^2 + 0.5900X_2^2 + 0.2235X_1 * X_2$, with an $R^2 = 97.4\%$. Where; Z = moisture, X_1 = pod storage time, X_2 = drying time

From the model there was significant influence of the linear factors of both drying time and pod storage time on moisture content of the beans (Table 1). The influence of drying time on moisture was more significant ($p < 0.05$) than that of pod storage time. Both drying and pod storage times had no significant quadratic effect on the model. The model could explain about 97.4 % of the variations in moisture. Thus, only 2.6 % of the variation was due to other factors not included in the model.

The rate of moisture loss as represented in Figure 3 shows clearly that the rate of moisture loss in the beans from the unstored pods was much steeper than those from the stored pods. This means that the rate of moisture loss during drying was greater for pods stored for 0 days. Also the actual amount of water lost in total after six days of drying was greater for pods stored for 0 days and decreased with increased days of pod storage. The crucial distinguishing factor is the initial moisture content available. The pod stored beans had lower initial moisture content and this where the effect of pod storage is relevant. The idea in cocoa drying is to reduce the moisture content quickly so as to reduce or eliminate the incidence of mould growth.

The model was used to generate response surface plot (Figure 3). The plot indicated a drastic decline in moisture content as the drying time increases. However, increasing the pod storage time resulted in a gradual decline in moisture content. There was significant ($p < 0.001$) interaction effect of both the drying time and pod storage time on moisture changes, which means the effect of either of the factors on moisture loss depended on the levels of each factor.

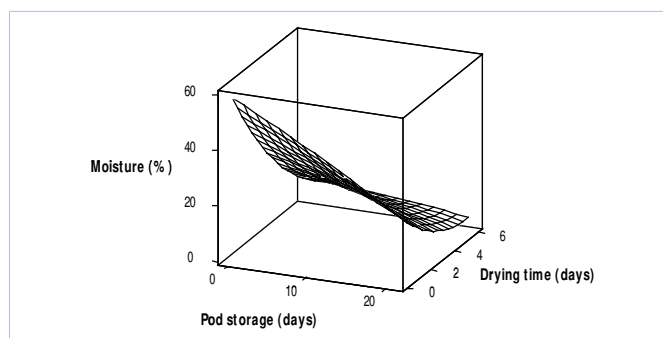


Figure 3: Response surface plot showing effect of pod storage and drying time on of moisture of cocoa beans.

Moisture loss during drying is a very important phenomenon in the primary processing of cocoa as it has great influence on the rate of Maillard browning reaction during subsequent roasting[30,31]. After six days of drying moisture content of the non pod stored beans was 8.94% which is above the recommended 7.5% indicating that six days of sun drying was not enough to reduce the moisture content of non pod stored beans to 7.5%. The final moisture content of the 7, 14 and 21 days pod stored beans after six days of drying was 7.84%, 6.34% and 5.06% respectively indicating that after 7 days of pod storage beans moisture lost increases and that pod stored beans should not be dried beyond six days of sun drying.

Effect of drying time and pod storage on total sugars content of cocoa nibs

The level of total sugars in cocoa after drying is very important as it contributes to the amount of aroma substances formed during subsequent roasting. Reducing sugars, especially glucose are transformed during Maillard or caramelization reactions to Amadori compounds which undergo further transformations in subsequent reactions into colour and flavour development [13].

The data obtained for the changes in total sugars during drying of pulp preconditioned cocoa beans was fitted into a multiple regression model as follows;

$Z = 97.0406 - 1.4671X_1 - 10.4573X_2 - 0.0091X_1^2 + 0.3684 X_2^2 + 0.0923X_1 * X_2$ with $R^2 = 95\%$. Where Z= total sugars, X_1 = pod storage time, X_2 = drying time

From the model, there was strong significant influence of the linear factor of drying time on the model. There was also slight significant ($p = 0.05$) influence of the linear factor of pod storage on the model. There was no significant influence of quadratic factors of both drying time and pod storage time on the model and the model could explain about 95.0% of the variations in total sugar content (Table 1). The response surface plots developed (Figure 4) showed a sharp linear decrease in total sugar as drying time increased. However, increasing the pod storage time showed a gradual decline in total sugar content.

The initial total sugar content of the fresh non pod stored beans after fermentation was 89.58 mg/g. After six days of drying the total sugars content decreased to 44.28 mg/g. The initial total sugars content of the pod stored beans after fermentation was 84.24, 79.95 and 65.10 mg/g for the 7, 14 and 21 days respectively. The total sugar contents of the pod stored beans after six days of drying were all lower than that of the non pod stored beans as indicated in Figure 4.

The results indicated that the total sugars in cocoa beans decreases as it undergoes fermentation after pod storage. The participation of sugars in Maillard non enzymatic reactions to form flavour volatiles and colour pigments (Amadori intermediates) accounts for the reduction in total sugar content of cocoa nibs during drying. This is in line with the findings by [5] which indicated that decreases in the amount of total reducing sugars could reach 71% of the initial amount after 24 hours of oven drying at 40 °C to 80 °C.

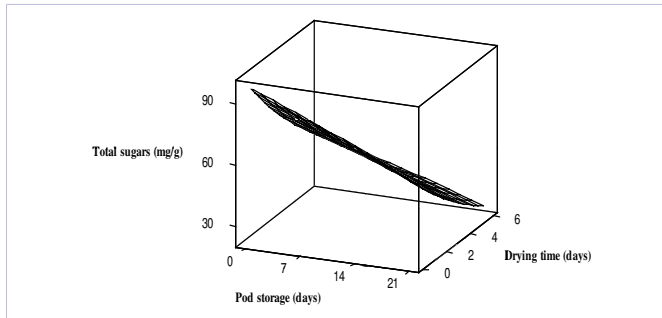


Figure 4: Response surface plot on effect of pod storage and drying time on total sugar of cocoa beans.

The reduction in total sugars after the fermentation of pod stored cocoa beans could be attributed to two possible causes, the first is the loss of sugars through respiration concomitantly with reduction of the pulp volume due to water evaporation and the second is the consumption of sugars through non enzymatic browning during fermentation of the beans [14,16,19]. Fermentation of pod stored beans was faster than in non pod stored beans. Therefore, six days of fermentation is more likely to cause over fermentation in pod stored beans than in non pod stored beans. Over fermentation is known to cause depletion of flavour volatile precursors such as sugars, amino acids and proteins [19,32-34].

Effect of drying time and pod storage time on protein content of cocoa nibs

The level of protein in cocoa after drying is very important as it contributes to the amount of aroma substance formed during subsequent roasting. Protein nitrogen, when broken down gets converted to peptide nitrogen and this is transformed during Maillard reactions to Amadori compounds [5,13,28] with resulting effects on flavour volatiles in cocoa beans.

The data obtained for the changes in percentage nitrogen during drying of pulp preconditioned cocoa beans was fitted into multiple regression models as follows;

$$Z = 7.06249 - 0.15641X_1 - 1.07891X_2 + 0.00047X_1^2 + 0.07064X_2^2 + 0.01875X_1 * X_2, \text{ with } R^2 = 98.6\%. \text{ Where } Z = \text{percentage nitrogen, } X_1 = \text{pod storage time, } X_2 = \text{drying time}$$

The model indicated strong significant ($p < 0.001$) influence of the linear factor of both drying time and pod storage on the model. There was also significant ($p < 0.001$) influence of the quadratic factor of drying time on the model. There was no significant influence of quadratic factors of pod storage time on the model and the model could explain about 98.6% of the variations in nitrogen (Table 1). The response surface plots developed from the model (Figure 5) showed a drastic decrease in nitrogen as drying time increased.

Effect of drying time and pod storage time on percentage free fatty acids

Free fatty acids (FFA) are carboxylic acids released from triglycerides through the effect of a lipase [34]. Higher FFA content leads to quality reduction in fermented cocoa beans as

well as decrease in hardness of cocoa butter [35] and must be considered as a raw cocoa commercial value reducing factor both for producers and chocolate manufacturers. For reasons of quality therefore, the directive 73/241/EEC (EEC, 1973) limits the maximum FFA content to 1.75% oleic acid equivalent in cocoa butter [34].

The data obtained for the changes in percentage free fatty acids during drying of pulp preconditioned cocoa beans was fitted into multiple regression models as follows;

$$Z = 0.890600 + 0.026541X_1 + 0.002331X_2 - 0.000032X_1^2 + 0.009453X_2^2 + 0.004275X_1 * X_2, \text{ with } R^2 = 90.7\%. \text{ Where } Z = \text{percentage free fatty acid, } X_1 = \text{pod storage time, } X_2 = \text{drying time}$$

The model showed that at 95% confidence level, there was no significant influence of both the drying time and pod storage on free fatty acid in cocoa nibs (Table 1). The response surface plots developed from the model (Figure 6) showed that the effect of either pod storage or drying time on rise in free fatty acids depends on the levels of either of the factors.

The initial FFA of the fresh non-stored beans after fermentation was 0.87%. After six days of drying the %FFA content increased to 1.18%. The initial %FFA of the 7, 14 and 21 days pod stored beans after fermentation was 1.04%, 1.21% and 1.45% respectively. The results obtained (Figure 6) indicated significant increases in the %FFA content as pod storage duration increases. This result is contrary to findings by [33] who concluded that pod storage had no significant effect on %FFA. However, their work was

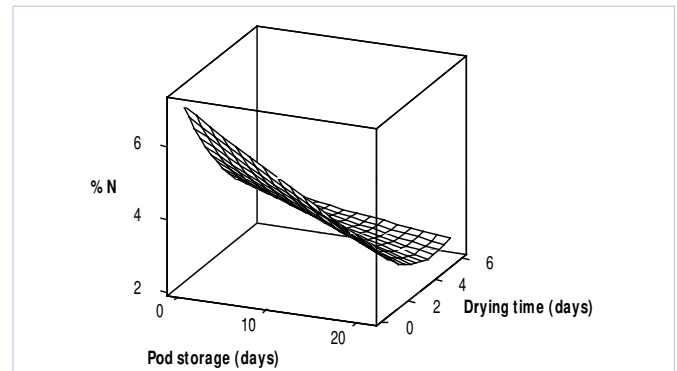


Figure 5: Response surface plot of protein content of cocoa nib as influenced by drying time and pod storage.

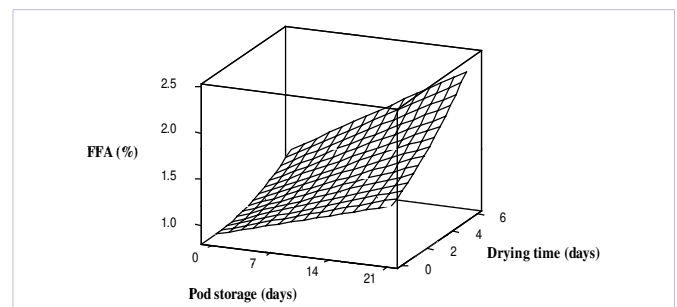


Figure 6: Response surface plot of effect of drying time and pod storage on FFA of cocoa nibs.

restricted to 9 days of pod storage and did not include six days of fermentation. The formation of FFA in raw cocoa beans has been attributed to the activity of the lipase enzymes from moulds and beans. Other factors such as over fermentation, storage period, water activity and bean germination have been found to favour lipase activity in raw cocoa beans [33-36].

Cocoa butter naturally contains about 1.08% FFA Six days of slow sun drying provided enough time for significant lipase enzyme activity to considerably increase the FFA. Six days of fermentation after pod storage is likely to cause over fermentation [16] which allows enough time for lipase producing moulds such as *R. oryzae* and *A. corymbifera* to multiply and produce lipase to cause changes in the beans [33].

The EEC, 1973 limits the concentration of FFA in cocoa nibs to 1.75 % [33]. FFA values of 1.80% and 2.55% were recorded in the nibs from the 14 and 21 days pod stored beans respectively after six days of drying time. Mould growth and percent germination were found to be very high in the 14 and 21 days pod stored beans [11]. The increase in free fatty acids as pod storage time and drying time increases is rather due to mould growth, over fermentation and germination which resulted from pod storage periods beyond seven days [33,36].

Conclusion

Both pod storage and drying influenced to varying levels the nib acidification, flavour precursors (protein nitrogen and total sugars) and FFA levels in fermented cocoa beans. Increasing pod storage consistently decreased the moisture content, non-volatile acidity, protein nitrogen and total sugars with concomitant increases in pH and FFAs during drying of the fermented beans. Drying had minimal effect on protein nitrogen and total sugars but drastically decreased moisture content of beans while pH and FFA of the fermented beans increased consistently at all levels of pod storage. The beans from the pods stored for more than 10 days had FFA levels exceeding 1.75% after drying. This suggests that to obtain well fermented cocoa beans with acceptable FFA levels, cocoa pods should not be stored for more than 10 days after harvesting. Thus, both pod storage and drying influenced the souring or nib acidification process (pH and non-volatile acidity), flavour precursors development (sugars degeneration and protein degradation) and FFA levels in fermented cocoa beans. However, the rates of change in souring, sugars degeneration, protein degradation and FFA concentrations were more dependent on pod storage than on drying.

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