

**CHANGES IN SOME BIOCHEMICAL QUALITIES DURING DRYING
OF PULP PRE-CONDITIONED AND FERMENTED COCOA
(*THEOBROMA CACAO*) BEANS**

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ABSTRACT

Fermentation and drying are critical to the development of flavour precursors that generate into distinctive chocolate flavour notes during industrial manufacture. These processes also lead to reduction in acidity and free fatty acids of nibs, which dictates the levels of bitterness and colour development in chocolates. This study investigated changes in nib acidity, flavour precursors (sugars concentration and proteins) and free fatty acids during drying of pulp pre-conditioned and fermented cocoa beans using a 4 x 3 full factorial experimental design with pod storage (0, 3, 7 and 10 days) and drying time (0, 3 and 6 days) as the principal factors. Non-volatile (titratable) acidity, pH, sugars (reducing, non-reducing and total sugars), changes in protein content and free fatty acids of the beans were studied using standard analytical methods. Increasing pod storage consistently increased pH of the fermented nibs at the end of drying with consequential decrease in titratable acidity. The pH increased from 4.92 for the freshly harvested pods to 6.00 for pods stored for 10 days at the end of the drying process. Similarly, pH of the fermented beans increased with increasing drying time for all pod storage treatment except for pods stored for 10 days. The pH of fermented beans whose pods were stored for 3 and 7 days were 5.26 and 5.56 respectively after drying for 7 days. Protein, reducing sugars, non-reducing sugars and total sugars decreased significantly ($p < 0.05$) with increasing duration of drying at all pod storage periods. Pod storage and drying significantly ($p < 0.05$) increased the free fatty acids content of the fermented nibs. The FFAs of the dried beans increased from 0.47% for the unstored (freshly harvested) pods to 0.55% for pods stored for 3 and 7 days and 0.58% for pods stored for 10 days. However, FFAs content of all the dried fermented beans were below the acceptable limits of 1.75% oleic acid equivalent in cocoa butter at all pod storage periods. Storage of cocoa pod between 3–7 days with 7 days of drying (after 6 days fermentation) led to considerable reductions in nib acidity, reducing sugars, non-reducing, total sugars and proteins and acceptable FFA levels.

Key words: *Cacao*, pod storage, drying, acidity

INTRODUCTION

Cocoa beans are dried after fermentation in order to reduce the moisture content from about 60% to between 6–8% [1]. Above 8% moisture, there is the danger of moulds developing within the beans while below 5% moisture, the beans are very brittle [2]. Drying fermented cocoa beans also allows some of the chemical changes which occur during fermentation to continue and improve flavour development and this continues either until the moisture content drops to below 7% or until the enzymes are inactivated [3]. Drying practices influence market quality, the development of flavour, final bean acidity, moldiness and the presence of off-flavour such as smoky notes in the beans [4].

During the drying process, there is physical loss of acidity through outward migration of volatile acids [5]. Laiu [6] provided evidence that the reduction of acidity during drying is mainly an oxidation process brought about by enzymes, while Bonaparte [4] observed that the factors which inhibit enzyme activity, such as high temperature and reduced moisture, contribute to acid retention. Hashim and Chaveron [7] and Cros and Jeanjean [8] have suggested that during the drying of fermented cocoa beans, reducing sugars participate in non-enzymatic browning reactions (Maillard reactions) to form volatile fractions of pyrazines. Oberparleiter and Ziegleder [9] have confirmed earlier findings by Hashim and Chaveron [7] and Cros and Jeanjean [8] by identifying Amadori compounds, the first intermediates of Maillard reaction in dried, unroasted cocoa beans. These Amadori compounds are the first intermediates of the reaction of free amino acid and glucose. Ziegleder [10] reported that although the formation of these Amadori compounds is not detectable by colour or odour and may even be reversible at this stage, these initial reactions are important because Amadori intermediates will during subsequent roasting decompose into numerous volatile components.

Nib acidity, flavour precursors and free fatty acids (FFA) affects the quality as well as the economic value of cocoa beans. Nib acidity plays a crucial role in the formation of flavour precursors (mainly amino acids, peptides and reducing sugars) as it affects enzymes responsible for the formation of these flavour precursors. These precursors react with each other during the roasting process to produce the typical cocoa and chocolate flavour. However, high nib acidity is also deleterious to the quality of the beans as this result in the production of highly acidic beans. The quality of raw cocoa beans also depends widely on their FFAs content as it gives the measure of rancidity of cocoa beans and high FFAs content is reported to be a serious quality defect which reduces the technical and economic value of the cocoa beans [11].

Pod storage as a means of pulp pre-conditioning cocoa beans is basically storing the harvested cocoa pods for a period of time before opening the pods and fermenting the beans. It has been reported to have high beneficial effect on the chemical composition of cocoa beans and subsequent development of chocolate flavour precursors [11]. Earlier work by Afoakwa *et al.* [12] reported that pod storage reduced nib acidification, increased the formation of flavour precursors such as reducing sugars and amino acids and produced cocoa beans with free fatty acid content within acceptable limits during fermentation. However, the extent to which pod storage would influence nib acidity,

free fatty acids content and the formation of flavour precursors (mainly sugars and proteins) during drying of fermented cocoa beans remains unknown. The objective of this study was thus to investigate changes in nib acidity, flavour precursors (sugars and proteins concentrations) and free fatty acids content during drying of pulp preconditioned and fermented cocoa (*Theobroma cacao*) beans.

MATERIALS AND METHODS

Material

Ripe cocoa pods (mixed hybrids) were obtained from the Cocoa Research Institute of Ghana (CRIG), Tafo-Akim, Eastern Region. Cocoa pods of uniform ripeness were harvested by traditional methods (under ambient temperature during the day; 28–30°C) and transported to the fermentary where they were stored. The beans were pulp preconditioned by storing the harvested pods for a period of time before splitting. About 1,200 pods were stored (on the cocoa plantation) at ambient temperature (25–28°C) and relative humidity of 85–100% for periods of 0, 3, 7 and 10 days, respectively. The respective pods were then split after these predetermined storage times and fermented using the traditional basket fermentation method.

About 30 kg of extracted cocoa beans were placed in woven baskets lined with banana leaves. The surface were also covered with banana leaves and fermented for six days with consecutive opening and turning every 48 h.

Drying of fermented cocoa beans

The fermented cocoa beans were dried in the open sun on raised platforms using the traditional process as described by Afoakwa [13]. Drying started at 8am and ended at 5 pm each day for 7 days. The beans were stirred four times each day and were covered with palm mats in the evening till the next morning. Samples were taken at 0 (undried samples or immediately after fermentation), 3 days, and 7 days of drying from 7.00 a.m. to 5.00 p.m. under 30–32°C and RH 70–80% daily. The samples were then packaged in air tight plastic bags and taken to the laboratory for analyses. All the treatments were conducted in duplicates.

Experimental design

A 4 x 3 full factorial experimental design was used for the study. The principal factors investigated were pod storage (0, 3, 7, 10 days) and drying time (0, 3, 7 days). Changes in the pH, non-volatile (titratable) acidity, reducing sugars, non-reducing sugars, total sugars, protein content and the free fatty acids content of the beans were studied.

METHODS

Determination of pH and non-volatile (titratable) acidity

Non-volatile acidity of the cocoa beans was determined according to the Association of Official Analytical Chemists' (AOAC) [14] method 970.21 and expressed as the percentage of acetic acid by titrating juice with 0.1N NaOH. Five gram samples of 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, Mettler Company Limited, 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 analysis was conducted in triplicates and the mean values are reported.

Determination of reducing sugars

Reducing sugars of the cocoa beans was determined using the phenol-sulphuric acid method as described by Brummer and Cui [15] with slight modifications. Fat from the samples was extracted with petroleum ether (40–60°C) using the Soxhlet extraction method (AOAC [14] method 963.15). About 0.5 g of defatted cocoa powder was boiled in 30 ml 80% ethanol under reflux for 30 minutes. The supernatant decanted into another round bottom flask and the process repeated twice. The collected supernatant was concentrated (not to dryness) under reduced pressure using a rotary evaporator. After the removal of ethanol, the extract was then clarified using 7.2 ml of 5% ZnSO₄ and 10 ml of 0.3 N barium hydroxide octahydrate [Ba(OH)₂ · 8H₂O] to precipitate proteins, colour, and other organic substances out of the solution and allowed to stand for about 5 minutes and then filtered.

A mixture of Zeokarb 225 (H⁺), a cation and anion exchange resin and deactivated Fe(OH)₂ was added to the filtrate to rid it of ions, shaken and filtered. 1ml phenol and 5 ml H₂SO₄ reagents were added to 1ml of the extract and allowed to stand for an hour and absorbance read at 480 nm. A standard solution of 20, 40, 60, 80, and 100 ppm glucose was prepared and the absorbance read at 480 nm and a standard curve drawn. From the standard graph, the amount of reducing sugars present in the samples was calculated and results expressed as mg/g of cocoa beans. The analysis was conducted in triplicates and the mean values reported.

Determination of non-reducing sugars

Non-reducing sugars were determined using the phenol sulphuric acid method as described by Brummer and Cui [15] with slight modifications. To the remaining residues (from the ethanol extraction), 20 ml of 1.5 N H₂SO₄ was added and the mixture digested for 1 hr, allowed to cool, filtered and neutralized with barium carbonate. The mixture was then centrifuged at 10,000 rpm for 30 minutes and supernatant decanted. 7.2 ml of 5% ZnSO₄ and 10 ml of 0.3N barium hydroxide octahydrate [Ba(OH)₂ · 8H₂O] to precipitate proteins, colour, and other organic substances out of the solution and proceeded as described for the reducing sugars. The analysis was conducted in triplicates and the mean values reported.

Determination of total sugars

Total sugars were determined by adding the values of reducing and non-reducing sugars.

Determination of protein content

Protein content of the defatted cocoa powder was determined by the Kjeldahl method using the AOAC [21] 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 (FFAs)

Fat from the samples was extracted with petroleum ether (40–60°C) using the Soxhlet extraction method (AOAC [14] method 963.15). FFA of the oils extracted was determined using the International Office of Cocoa, Chocolate and Confectionery (IOCCC) [16] method 42-1993. Five grams of the oil was weighed into a dry 250 ml stoppered conical flask and 25 ml of 95% ethanol/ether (1:1) and phenolphthalein indicator were added. The solution was titrated with 0.1N NaOH by shaking constantly until pink colour persisted for 30 s and the percentage FFA (as % oleic acid) was determined. The analysis was conducted in triplicates and the mean values are reported. The %FFA (% oleic acid) was calculated using the following equation:

$$\%FFA = \frac{\text{volume of NaOH used} \times \text{Normality of NaOH (0.1N)} \times \text{Equivalent factor (28.2)}}{\text{Weight of sample}}$$

Statistical analyses

Statgraphics software version 3.0 (STSC, Inc., Rockville, MD, USA) was used to analyze the data for analysis of variance (ANOVA). Least significant difference (LSD) was used to separate and compare the means, and significance was accepted at 5% level ($p < 0.05$). The combined effects of pod storage (pulp preconditioning) and drying time on the parameters of interest were studied using the response surface methodology. Models were developed to relate pod storage and drying time on the studied parameters. The coefficients of the variables in the models and their contribution to the model's variation were reported. The R^2 values were used to judge the adequacy of the models. The R^2 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 R^2 of at least 60% was used (was accepted in this work). All analyses were conducted in triplicates and the mean values reported.

RESULTS

Changes in pH profile of cocoa beans

Response surface plot (Figure 1) showed an increase in pH of the fermented beans with increasing drying time for all pod storage treatment except for pods stored for 10 days. On the drying mats, the pH increased steadily from 4.80 at the start of drying to 4.92 at the end of the drying process (7 days) for the unstored pods.

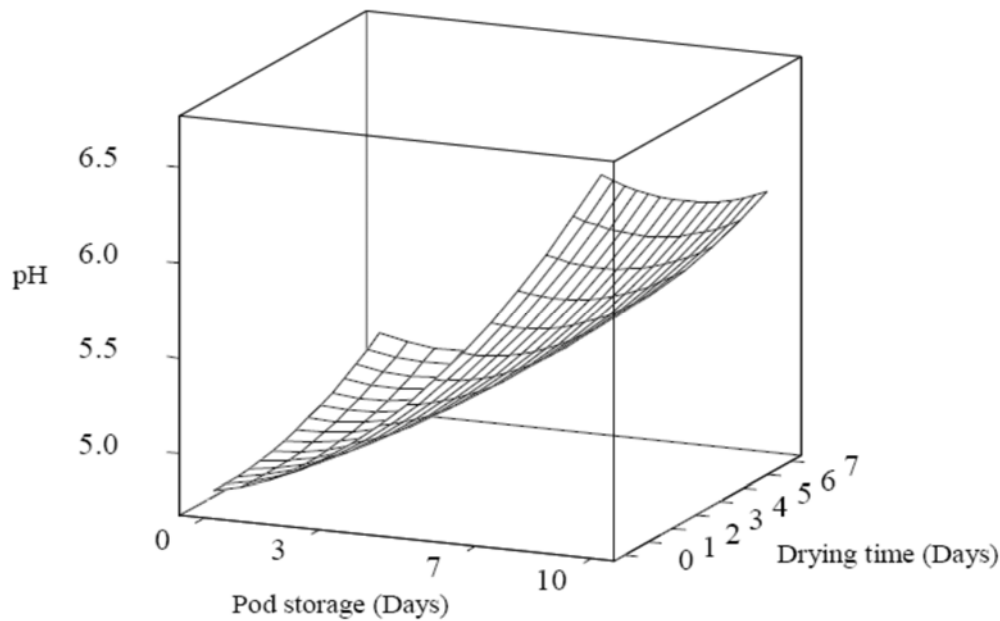


Figure 1: Response surface plot displaying pH of cocoa beans as affected by pod storage and drying time

Similar trend of increase was observed for pods stored for 3 days (5.10–5.26) and 7 days (5.36–5.56). In contrast, pH for pods stored for 10 days decreased from 7.01 at the start of drying to 6.00 after 7 days of drying. Pod storage generally increased the pH of the fermented cocoa beans at the end of the drying process. The pH increased from 4.92 for the unstored pods to 6.00 for pods stored for 10 days at the end of the drying process.

Regression analysis of the data showed significant ($p < 0.05$) influence of both the linear and quadratic factors of pod storage (PS) on the pH of the nibs. Both the linear factor and the quadratic factor of the drying time (DT) had no significant ($p > 0.05$) influence on the pH of the nibs. There was however, significant ($p < 0.05$) influence of the interaction between pod storage and drying time on the pH of the nibs. The model developed could explain about 77% of the variations in the pH of the cotyledons, suggesting that 23% of the variations were due to other factors not investigated in this work (Table 1).

Changes in non-volatile (titratable) acidity of cocoa beans

There were decreases in acidity of the fermented beans with increasing drying time (Figure 2) at all pod storage treatment except for pods stored for 10 days. Titratable acidity decreased from 0.277 at the start of drying to 0.197 meq NaOH/100g at the end of the drying process for the unstored pods. Similar trend of decrease was observed for pods stored for 3 days (0.188–0.146 meq NaOH/100g) and 7 days (0.158–0.075 meq NaOH/100g). In contrast, TA for pods stored for 10 days increased from 0.030 at the start of drying to 0.037 meq NaOH/100g after 7 days of drying.

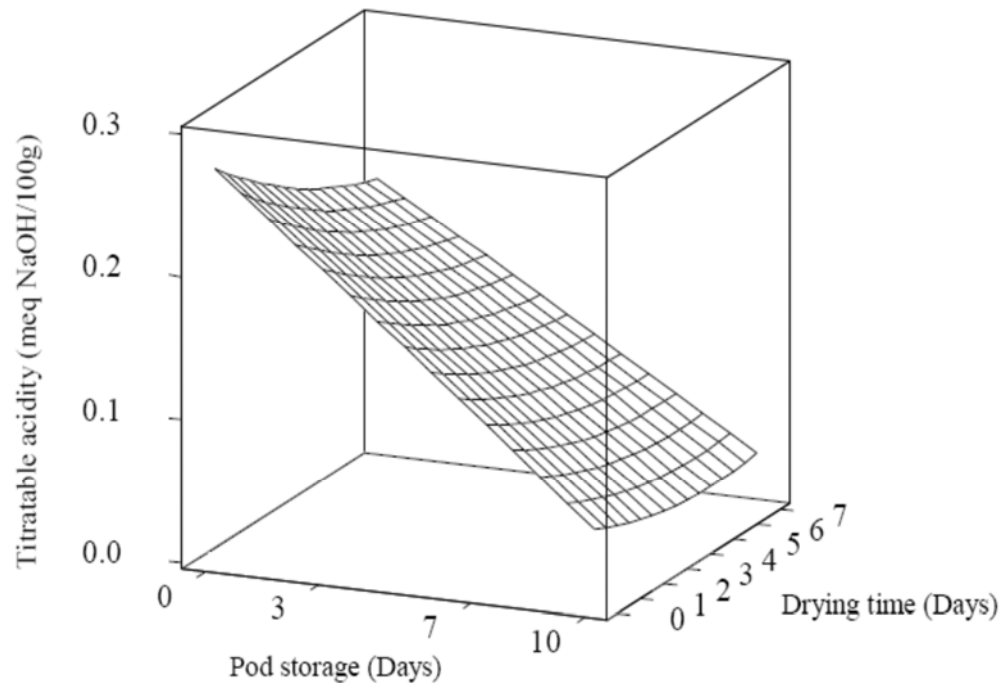


Figure 2: Response surface plot showing titratable acidity of cocoa beans as affected by pod storage and drying time

Regression analysis of the data showed significant ($p < 0.05$) influence of the linear factor of drying time (DT) and pod storage (PS) on the titratable acidity (TA) of the nibs. The quadratic factors of PS and DT had no significant ($p > 0.05$) influence on the TA of the nibs. There was significant ($p < 0.05$) influence of the interaction between PS and DT on the TA of the nibs. The model developed had an R^2 of 96% implying that the model could explain about 96% of the variations in the TA of the cotyledons. The model also suggests that 4% of the variations in the TA of the cotyledons were due to other factors not investigated in this work (Table 1).

Changes in reducing sugars

Response surface plot (Figure 3) showed that reducing sugars of the fermented cocoa beans decreased significantly ($p < 0.05$) with increasing drying time for all pod storage periods. It decreased from 10.69 mg/g at the onset of drying to 3.42 mg/g at the end of drying for the unstored pods.

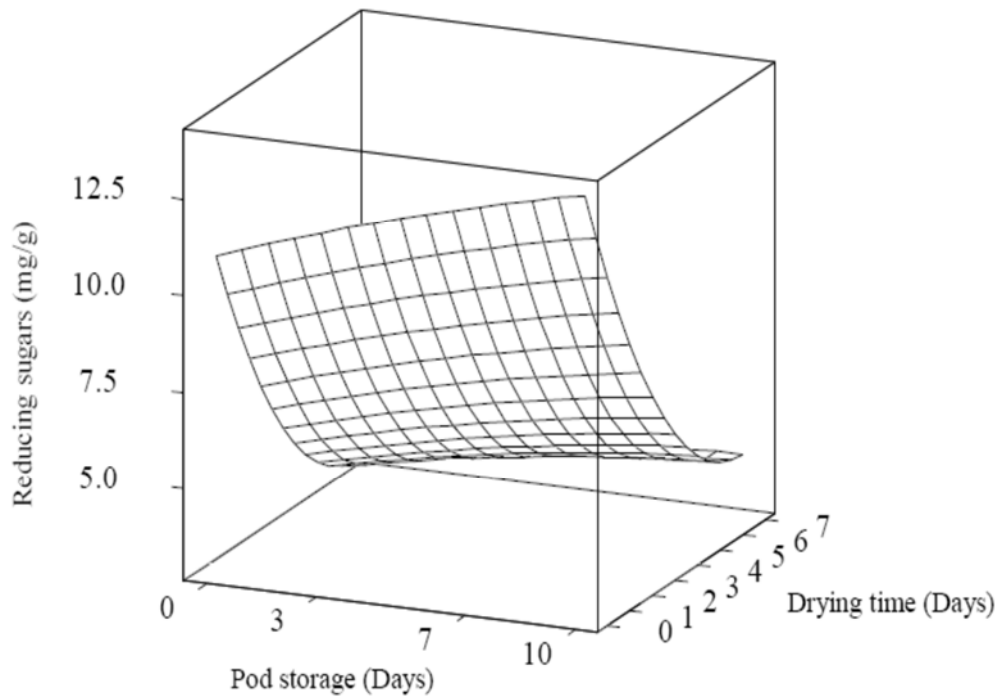


Figure 3: Response surface plot showing changes in reducing sugars during drying of fermented pulp pre-conditioned cocoa beans

Similar decreasing trend was observed for the stored pods where reducing sugars decreased from 11.94–3.58 mg/g, 13.34–4.00 mg/g, and 13.56–4.47 mg/g for pods stored for 3, 7, and 10 days, respectively. Puziah *et al.* [19] observed a decrease in the concentrations of total reducing sugars from about 3.5 mg/g to 1.3 mg/g after 24 h of drying fermented cocoa beans representing about 38% reduction.

Regression analysis of the data showed significant ($p < 0.05$) influence of the linear factor of pod storage (PS) and drying time (DT) and quadratic factor of DT on the reducing sugars of the nibs. The model developed could explain about 93% of the variations in the reducing sugars of the cotyledons, suggesting that 7% of the variations were due to other factors not investigated in this work (Table 1).

Changes in non-reducing sugars

There were decreases in the concentrations of non-reducing sugars during drying of fermented cocoa beans for all pod storage periods (Figure 4). It decreased from 6.11 mg/g at the onset of drying to 2.09 mg/g at the end of drying for the unstored pods. A similar trend of decrease was also observed for the stored pods where non-reducing sugars decreased from 5.07–2.03 mg/g, 4.43–1.99 mg/g, and 4.47–2.24 mg/g for pods stored for 3, 7 and 10 days respectively. Puziah *et al.* [19] observed similar trend of decrease in sucrose (the main non-reducing sugar in cocoa beans) from 2.2 mg/g at the onset of drying to 1.0 mg/g after 24 h of drying. The decrease in concentrations of the

non-reducing sugars was drastic within the first 3 days of drying, and then slowed down towards the end of drying (7 days).

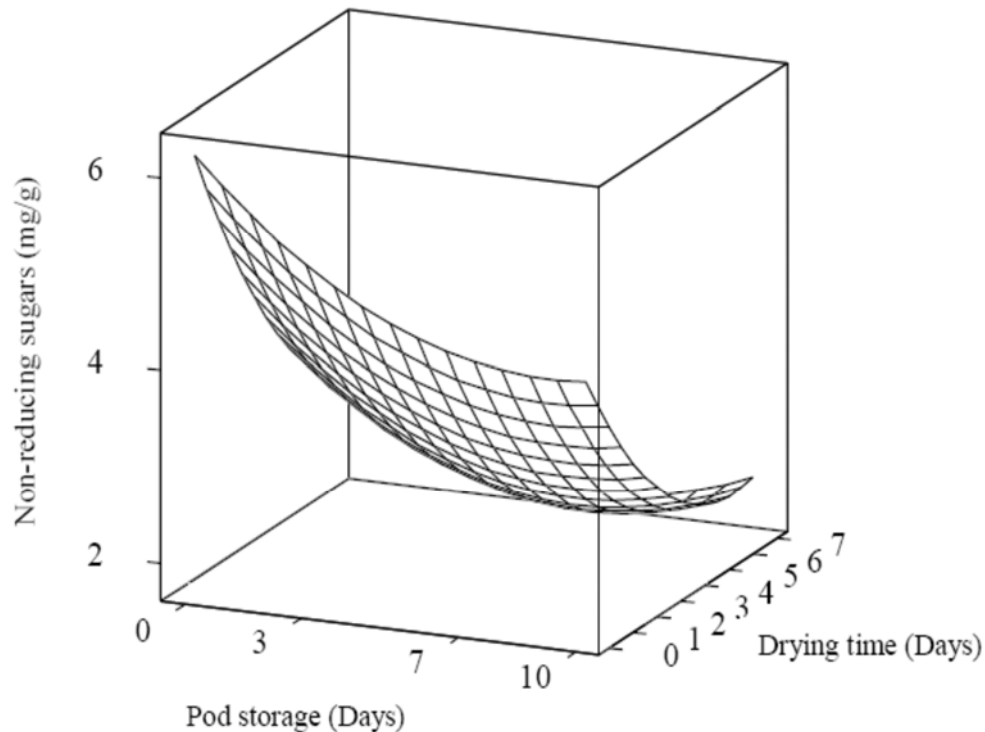


Figure 4: Response surface plot showing changes in non-reducing sugars during drying of fermented pulp pre-conditioned cocoa beans

Regression analysis of the data showed significant ($p < 0.05$) influence of the linear factor of pod storage (PS) and drying time (DT) and quadratic factor of drying time (DT) on the non-reducing sugars of the nibs. There was no significant ($p > 0.05$) influence of the interaction between PS and DT on the non-reducing sugars of the nibs. The model developed could explain about 73% of the variations in the reducing sugars of the cotyledons, suggesting that 27% of the variations were due to other factors not investigated in this work (Table 1).

Changes in total sugars

Changes in the concentrations of total sugars during drying for all pod storage treatments are shown in Figure 5. Total sugars of the beans decreased significantly ($p < 0.05$) during drying for all pod storage periods. It decreased from 16.81 mg/g at the start of drying to 6.05 mg/g at the end of drying for the unstored pods. It also decreased from 17.01–5.61 mg/g for pods stored for 3 days, 17.77–6.00 mg/g for pods stored for 7 days and 18.03–6.71 mg/g for pods stored for 10 days.

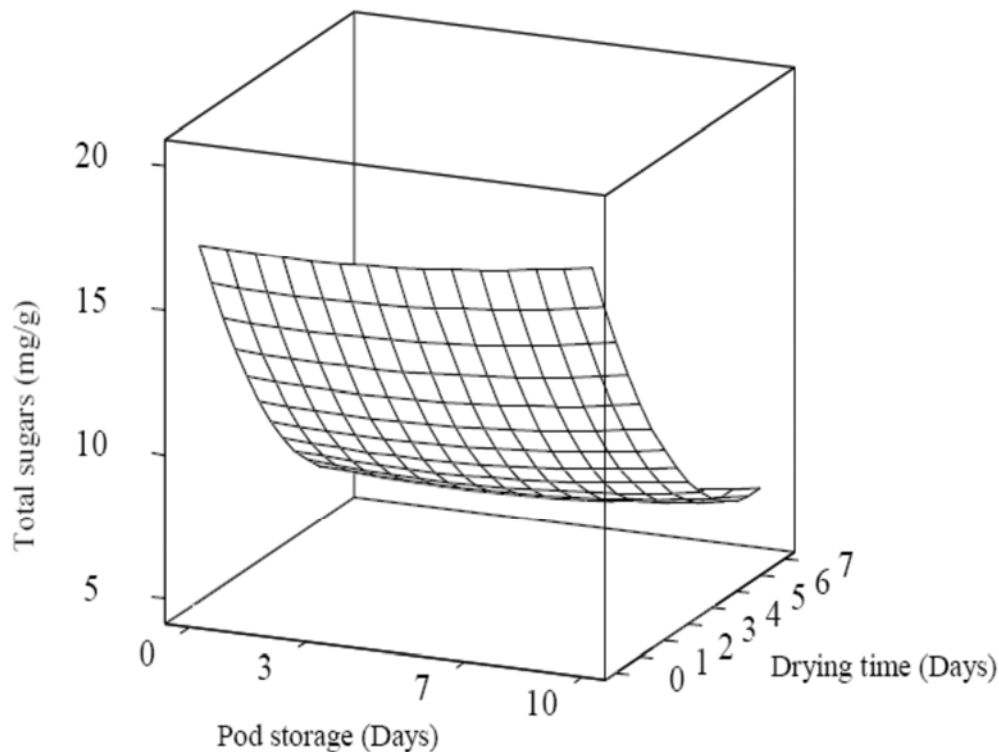


Figure 5: Response surface plot showing changes in total sugars during drying of fermented pulp pre-conditioned cocoa beans

Regression analysis of the data showed significant ($p < 0.05$) influence of the linear factor and quadratic factor of drying time (DT) on the total sugars of the nibs. The linear and quadratic factors of pod storage (PS) as well as the interaction between PS and DT had no significant ($p > 0.05$) influence on the total sugars of the nibs. The model developed could explain about 90% of the variations in the reducing sugars of the cotyledons, suggesting that 10% of the variations were due to other factors not investigated in this work (Table 1).

Changes in protein content during drying

Response surface plot (Figure 6) showed changes in the protein concentrations of the fermented cocoa beans during drying for all the pod storage treatments. Protein concentrations decreased significantly at $p < 0.05$ as drying progressed from day 0 to day 7. It decreased from 24.7% at the onset of drying to 20.3% at the end of drying (7 days) for the unstored (freshly harvested) pods. It also decreased from 23.2% to 18.3%, 22.7% to 15.4% and 21.6% to 14.5% at the end of the drying process for pods stored for 3, 7 and 10 days respectively.

Again, protein content reduced significantly ($p < 0.05$) with increasing pod storage at all drying times (Figure 6). The protein content of the dried cocoa beans reduced from 20.3% (unstored pods) to 14.5% (pods stored for 10 days).

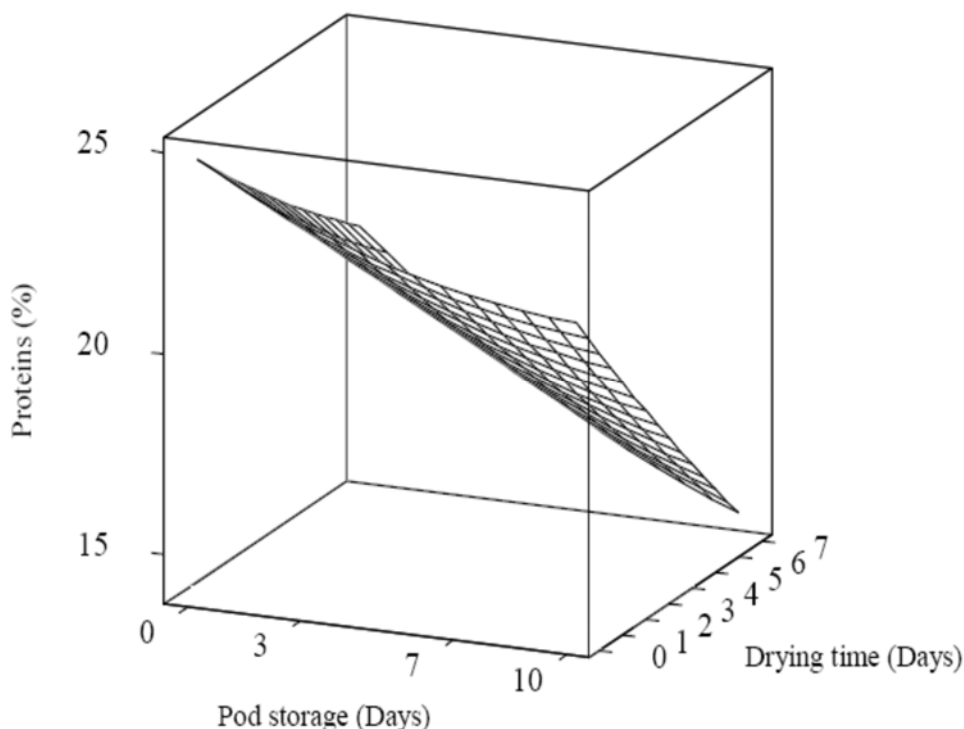


Figure 6: Response surface plot showing effect of pod storage and drying time on the protein content of cocoa beans

Regression analysis of the data showed significant ($p < 0.05$) influence of the linear factor of pod storage (PS) and drying time (DT) as well as the interaction between PS and DT on the protein content of the cotyledons. The quadratic factor of PS and DT had no significant ($p > 0.05$) influence the protein content of the cotyledons. The model developed could explain about 88% of the variations in the protein content of the cotyledons, suggesting that 12% of the variations were due to other factors not investigated in this work (Table 1).

Changes in free fatty acids (FFAs)

There were general increases in FFAs levels with increasing drying time (Figure 7). The FFAs increased from 0.42% at the start of drying to 0.47% at end of drying for the unstored pods, 0.51–0.55% for pods stored for 3 days, 0.52–0.55% for pods stored for 7 days and 0.52–0.58% for pods stored for 10 days. The FFAs also increased with increasing pod storage. The FFAs of the dried beans increased from 0.47% for the unstored pods to 0.55% for pods stored for both 3 and 7 days and 0.58% for pods stored for 10 days at the end of drying. These findings confirmed earlier findings by Akomayi [20] who reported an increase in FFAs in Ghanaian cocoa beans with increasing pod storage (PS) and drying times (DT).

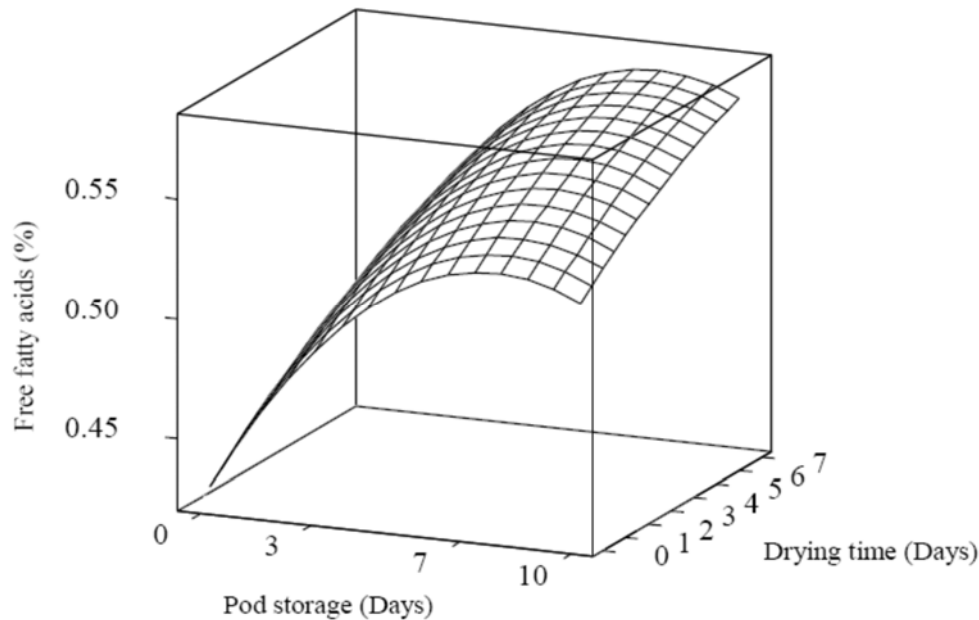


Figure 7: Response surface plot showing effect of pod storage and drying time on the free fatty acids of cocoa beans

Regression analysis of the data showed that there were significant ($p < 0.05$) influence of the linear factor of pod storage (PS) and drying time (DT) and quadratic factor of PS on the FFAs of the cotyledons (Table 1). There was no significant ($p > 0.05$) influence of the interaction between PS and DT on the FFAs of the cotyledons. The model developed could explain about 85% of the variations in the FFAs of the cotyledons, suggesting that 15% of the variations were due to other factors not investigated in this work (Table 1).

DISCUSSION

Changes in pH profile of cocoa beans

During the drying of fermented cocoa beans, there is loss of volatile acids and water from the beans when this process occurs slowly, resulting in an increase in pH of the cotyledons [21]. In contrast, pH for pods stored for 10 days decreased from 7.01 at the start of drying to 6.00 after 7 days of drying. Jinap *et al.* [5] observed that rapid drying of the beans result in case hardening which prevents outward migration of acetic acid from the beans. Pod storage for 10 days resulted in reduced moisture content of the beans which might have led to rapid drying of the beans during the initial stage of drying. This might have resulted in case hardening which prevented outward migration of acetic acid from the beans leading to acid retention and hence, reduction in pH. Laiu [6] also provided evidence that the reduction of acidity during drying is mainly an oxidation process brought about by enzymes. Other studies have reported increases in pH during the drying of fermented cocoa beans. Hii *et al.* [17] found the pH of dried cocoa beans to increase from 4.91 to 5.39 for different loadings. Takrama *et al.* [18] also observed an increase in pH from 4.2 to about 5.3 at the end of drying (14 days).

Work by Bonaparte [4] reported that factors which inhibit enzyme activity, such as high temperature and reduced moisture, contribute to acid retention. Pod storage for 10 days resulted in reduced moisture content of the beans and this might have contributed to acid retention resulting in reduction in pH.

The pH of fermented beans whose pods were stored for 3 and 7 days were 5.26 and 5.56 respectively after drying for 7 days. These findings suggests that pod storage for 3 and 7 days with 6 days fermentation and 7 days drying could be effectively used to improve on the flavour potentials of Ghanaian cocoa beans during roasting. Higher chocolate flavour potentials are reported to be produced during cocoa roasting when the pH of the dried fermented cocoa beans is between 5.0–5.5.

Changes in non-volatile (titratable) acidity of cocoa beans

Volatile acidity formed during cocoa beans fermentation reaches approximately 2% of the dry basis [22]. Acetic acid is reported to form about 90% of these components, which has an important role in the catalysis of enzymatic reactions for producing components of desirable sensorial characteristics [23]. Cocoa drying is a continuation of the oxidative stage of fermentation and therefore plays an important role in reducing acidity of the cocoa beans [24]. The drying process must not be too rapid otherwise the beans tend to retain an excessive amount of acetic acid, and this is deleterious to flavour [19].

TA for pods stored for 10 days increased from 0.030 at the start of drying to 0.037 meq NaOH/100g after 7 days of drying. Rapid drying of the beans result in case hardening which prevents outward migration of acetic acid from the beans. Pod storage for 10 days resulted in reduced moisture content of the beans. Hence, there was rapid drying of the beans during the initial stage of the drying process. This might have resulted in case hardening which prevented outward migration of acetic acid from the beans leading to a buildup of acidity in the beans.

Changes in reducing sugars

The reduction in reducing sugars during the drying process could partly be due to their participation in the non-enzymatic browning reactions to form volatile compounds. Hashim and Chaveron [7] and Cros and Jeanjean [8] both suggested that during the drying of fermented cocoa beans, reducing sugars participate in non-enzymatic browning reactions (Maillard reactions) to form volatile fractions of pyrazines. Oberparleiter and Ziegleder [9] confirmed these findings by Hashim and Chaveron [7] and Cros and Jeanjean [8] by identifying Amadori compounds, the first intermediates of Maillard reaction in dried, unroasted cocoa beans. These Amadori compounds are the first intermediates of the reaction of free amino acid and glucose.

Changes in non-reducing sugars

The drastic reduction in the concentrations of non-reducing sugars during the first 3 days of drying indicates that the fermentation process still proceeds during the early stage of drying, where the hydrolysis of sucrose by invertase enzyme continues until the enzyme becomes slowly inactive due to the increase in temperature and a drop in moisture content [19]. Lehrian and Patterson [25] reported that during high temperature

drying, case hardening occurs, whereby the testa adheres to the nib and reduces the availability of oxygen in the beans due to a drop in moisture content, limiting the biological oxidation, such as enzymatic reactions; in this case, the invertase would become inactive.

Changes in total sugars

The drastic reduction in the concentrations of total sugars during drying might be due to two factors; the continuous breakdown of non-reducing sugars (sucrose) in the cotyledons by invertase [19] and also the participation of reducing sugars in the non enzymatic browning reactions that is, the Maillard reactions to form volatile fractions such as pyrazines [7, 8].

Changes in proteins during drying

The decrease in protein content during drying might be caused by the continuous breakdown of cocoa bean proteins to oligopeptides and free amino acids, confirming results previously reported by other researchers [20]. The reduction in protein content might also be due to the complexation of proteins with polyphenols as the latter is polymerized. The reduction in protein content during pod storage (pulp preconditioning) is reported to be due to the action of protease enzymes in the pods during storage and thus initiating the process of proteolysis [26].

Changes in free fatty acids (FFAs)

The European parliament and European council directive 73/241/EEC [27] limits the maximum FFAs content to 1.75% oleic acid equivalent in cocoa butter. To be able to meet the acceptable level, Dand [28] reported that the FFAs levels should be less than 1% in fresh cocoa beans and less than 1.75% in dried cocoa beans.

The gradual increase in the FFAs of cocoa beans during both pod storage and drying could be attributed to the activity of lipase enzyme present in the natural cocoa beans and acts to breakdown the triglycerides into separate groups of the fatty acids and glycerol thereby freeing the fatty acids [28]. Even though the FFAs levels in the cocoa beans increased with both drying time and pod storage, the levels were all however, below the maximum acceptable limits of 1.75% oleic acid equivalent in cocoa butter. Results from this study suggest that cocoa pods could be stored up to 10 days and beans fermented for 6 days and dried up to 7 days without adversely affecting the FFAs levels in the dried beans.

CONCLUSION

The pH of the fermented beans increased with increasing drying time for all pod storage treatments with consequent decrease in titratable acidity except for pods stored for 10 days which recorded a decrease in pH with concomitant increase in titratable acidity. The increase in pH with consequential decrease in titratable acidity during drying might be due to the loss of volatile acids. The pH of fermented beans whose pods were stored for 3 and 7 days were 5.26 and 5.56, respectively after drying for 7 days. Pod storage for 3 and 7 days with 6 days fermentation and 7 days drying could be effectively used to improve on the flavour potentials of Ghanaian cocoa beans during roasting.

Increasing duration of drying consistently decreased the reducing sugars, non-reducing sugars, total sugars and protein content of the beans. Ten (10) days storage of cocoa pods and 7 days drying (after 6 days fermentation) produced beans with 0.58% FFAs which was below the acceptable limits of 1.75% oleic acid equivalent. Cocoa pods could be stored up to 10 days and beans fermented for 6 days and dried up to 7 days without adversely affecting the FFAs levels in the dried beans. Attainment of pH of between 5.0 and 5.5 after drying of the pulp preconditioned cocoa beans would lead to improved chocolate flavour during roasting of cocoa beans. Further work could investigate the flavour profiles and character from the beans from the unstored and pod stored pods.

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Table 1: Regression coefficients and their R² values in the models for pH, titratable acidity, sugars, protein and FFA of cocoa beans

Variables	pH	Non-volatile acidity	Reducing sugars	Non-reducing sugars	Total sugars	Protein	Free fatty acids
Constant	5.19058*	0.129135*	5.8309*	2.5843*	8.4152*	19.5348*	0.545562*
X ₁	0.70506*	-0.095627*	0.9183*	-0.5943*	0.3239	-2.2195*	0.047163*
X ₂	-0.06187	-0.024750*	-4.2564*	-1.4001*	-5.6564*	-2.9664*	0.022500*
X ₁ ²	0.37698*	-0.001290	-0.1872	0.5820	0.3948	0.6058	-0.042659*
X ₂ ²	0.09452	0.009926	2.4040*	0.7000*	3.1040*	0.1947	-0.005195
X ₁ . X ₂	-0.22856*	0.015043*	-0.4622	0.3352	-0.1271	-0.7752*	0.000775
R ²	76.6%	95.9%	93.2%	73.0%	90.1%	89.1%	84.6%
R ² (adjusted)	73.9%	95.4%	92.4%	69.8%	88.9%	87.8%	82.7%

Notes: *Significant at p<0.05; X₁= Pod storage; X₂= Drying time

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