Changes in Nib Acidity, Proteolysis and Sugar Concentration as Influenced by Pod Storage and Roasting Conditions of Fermented Cocoa (*Theobroma cacao*) Beans

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Abstract: Changes in nib acidity, protein and sugar concentration during roasting of pulp pre-conditioned and fermented cocoa (*Theobroma cacao*) beans were investigated. A 4×4 full factorial design with the principal experimental factors as pod storage (0, 3, 7 and 10 d) and roasting time (0, 15, 30 and 45 min) were used. The roasted samples were evaluated for pH, titratable acidity, protein content and sugars concentrations using standard methods. Increasing pod storage caused consistent increases in pH with concomitant decreases in titratable acidity, whereas increasing roasting time caused only marginal and insignificant changes in pH but significantly decreased the titratable acidity. The protein content decreased significantly ($P < 0.05$) with increasing pod storage and roasting time. Reducing sugars increased marginally with increasing pod storage treatments while increasing roasting time significantly ($P < 0.05$) decreased the reducing sugars of the beans for all pod storage. The non-reducing sugar and total sugar content of the beans decreased significantly from 3.493 mg/g to 2.641 mg/g and from 9.284 mg/g to 8.891 mg/g, respectively, for pods stored from 0 to 10 days while roasting time caused slight decreases in non-reducing sugars with a considerable decrease in total sugars. Pod storage up to seven days decreased considerably the nib acidity (non-volatile acids), non-reducing sugars and total sugars while roasting up to 45 min at 120 °C caused dramatic decreases in the nib acidity and reducing sugars with only marginal decreases in non-reducing sugars and total sugars.

Key words: Cocoa, *Theobroma cacao*, acidity, pulp pre-conditioning, pod storage, roasting, sugars.

1. Introduction

Cocoa processing involves two unique stages, the primary stage (harvesting, fermentation, drying of fermented beans, sorting and packaging) and the secondary stage such as roasting, alkalization, pressing and pulverization [1]. The roasting process causes important chemical and physical changes in the beans, and thus transforms the flavor precursors formed

during the fermentation and drying into pronounced flavor volatiles [2-4]. Cocoa beans can be roasted in a conventional oven [5] or a Barth automatic gas roaster [6]. Conventional method of roasting cocoa is mostly used when raw cocoa beans are exposed to temperatures of 110 °C to 150 °C for 5-120 min depending on several conditions of the beans and equipment used [7, 8].

The choice of roasting conditions depends on the type of beans (variety) and quality, the cultivation conditions, period of harvesting, their origin,

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postharvest treatments such as pulp pre-conditioning [4], type of flavour desired [9], construction and dimensions of roasters, the manner of heating gas supply, the method of shifting of roasted material in the roasting chamber and the particle size of the roasted material [2, 4]. The characteristics of roasted beans such as total acidity, fat content, colouration and flavour volatile concentrations depend mainly on the roasting conditions such as temperature and time [7, 9] during processing.

Roasting of cocoa beans removes the undesirable compounds such as acetic acids which are produced during fermentation. The high roasting temperature reduces specifically the volatile acids with low boiling point such as ethanoic acid and acetic acid, making the cocoa beans less acidic [8, 10-12]. The less volatile acids, such as ethanedioic (oxalic), citric, tartaric, succinic and lactic acids remain largely unchanged by the roasting process [8]. Also, the roasting procedure initiates other chemical reactions such as Maillard reaction and Strecker degradation to produce colour, typical roasty and sweet odorants of the cocoa [12, 13]. The free amino acids such as leucine, alanine, phenylalanine and tyrosine [1] or short-chain peptides and reducing sugars produced during fermentation [12, 14] interact during Maillard reaction to form the colour and chocolate flavour.

Dried unfermented cocoa beans are strongly bitter and astringent, and produce poor or no chocolate flavour [1, 15, 16] after roasting and further processing such as milling and conching of the liquor since reducing sugars (glucose and fructose) [17] as well as amino acids are limited. Sucrose forms the major component of sugar thus about 90% of the total sugars present in the unfermented cocoa beans, followed by fructose (about 6%) [18]. The cotyledons of cocoa beans from fully ripe pods that are unfermented contain between 10% to 16% of protein and a low level of free amino acids on dry weight [18-20].

Cocoa fermentation helps to develop the right flavour precursors in the beans such as organic acids,

amino acids, peptides, sugars and fatty acids [11]. The technique of pulp pre-conditioning of cocoa beans after harvesting has become a major area of concern to researchers in the improvement of the quality of cocoa bean. Pulp pre-conditioning involves changing the properties of the pulp around the cocoa beans prior to fermentation [21]. This affects the sequence of microorganisms such as yeast and bacteria during the spontaneous fermentation process [22] hence, affecting the production of ethanol by yeasts and subsequent acids production by lactic acid and acetic acid bacteria causing an increase in the pH [9, 22].

Variation in the methods of pulp pre-conditioning before fermentation affect the pH, titratable acidity and total polyphenols [9] as well as proteins, amino acids and sugars. Cocoa pod storage as a means pulp pre-conditioning has been found to reduce nib acidification during subsequent fermentation as well as cause a reduction in the acid note in Ghanaian cocoa beans [23] but the extent to which this technique affects the outcome (nib acidity, proteins and sugars as flavor precursors) during roasting of Ghanaian cocoa beans still requires in-depth understanding. Thus, the objective of this study was to investigate variations in nib acidity, proteolysis and sugar concentration of fermented cocoa beans as affected by roasting and pod storage (as a means of pulp pre-conditioning).

2. Materials and Methods

2.1 Materials

2.1.1 Raw Materials and Sample Preparation

Fully ripe mixed hybrid variety of cocoa was obtained from the cocoa plantation of the Cocoa Research Institute of Ghana (CRIG) at New-Tafo in the Eastern Region of Ghana.

Freshly and fully ripe good looking cocoa pods were harvested, sorted out to remove the bruised ones and divided into four parts, each containing 300 pods. The pods were stored in a heap form for four different storage time (0, 3, 7 and 10 d) on the bear concrete

floor under shade and broken after the specified days of storage. The beans were scooped out and fermented for six days using the basket fermentation technique. The fermenting cocoa beans were opened and mixed after every 48 hours until the fermentation process was over. The fermented cocoa beans were sun-dried with stirring four times each day to allow uniform drying of the beans.

Cocoa samples were randomly picked into black air tight bags at intervals and moisture content analysed until a moisture content ranged between 5.5% and 6% was attained. The cocoa beans were immediately packaged in air tight black plastic bags prior to roasting. Roasting was done according to the method described by Owusu et al. [24] with slight modifications. The fermented and dried mixed hybrid cocoa beans packaged in air tight black plastic bags were sorted to remove all the smaller and flat beans. About 500 g of the beans was weighed and roasted using hot air oven in batches at a temperature of 120 °C for 0, 15, 30 and 45 min. For each of the roasting treatments under investigation, the oven temperature was set at 120 °C and left to equilibrate for at least 30 min. The fermented dried cocoa beans (500 g) were spread in a single layer in the perforated metallic sample tray and then placed on the oven shelf close to the thermometer.

After roasting, the cocoa beans were transferred to another tray and allowed to cool to room temperature and placed in air tight black plastic bags according to the duration of pod storage and roasting. The samples were stored at ambient temperature (25-28 °C) in a dark room free from strong odours until used. The procedure was repeated for the different pulp pre-conditioned treatments. The cocoa beans were shelled manually using knife and milled using kitchen blender for further analyses. All treatments were conducted in duplicates.

2.1.2 Experimental Design

A 4 × 4 full factorial design with the principal experimental factors as pod storage (0, 3, 7 and 10 d) and roasting time (0, 15, 30 and 45 min) at 120 °C were

used for all the studied parameters. The milled cocoa nibs were analyzed for pH, titratable acidity, protein, reducing sugars and non-reducing sugars.

2.2 Methods

2.2.1 PH

The nib pH was determined according to the International Office of Cocoa, Chocolate and Sugar Confectionery method, IOCCC [25]. 10 g of the milled nibs were homogenised in 90 mL boiled deionised water. The homogenate was filtered using Whatman No. 4 filter paper and cooled to 25 °C. The pH of the resulting filtrate was measured using a pH meter (Model MP 230 Mettler Toledo, Mettler Company Limited, Geneva, Switzerland) standardized with buffers at pH 4.1, 7.0 and 9.2.

2.2.2 Titratable Acidity

The titratable acidity was determined according to the International Office of Cocoa, Chocolate and Sugar Confectionery method, IOCCC [25]. About 25 mL of the aliquot collected for the above pH determination was titrated with 0.1 N NaOH drop wise to a pH of 8.2, determined using a pH meter (Model MP 230 Mettler Toledo, Mettler Company Limited, Geneva, Switzerland) which was calibrated with buffers at pH 4.1, 7.0 and 9.2. The titratable acidity was calculated using the titre values and reported as milliequivalent sodium hydroxide per gram of dry nibs. Triplicate readings were made.

2.2.3 Protein Content

The total nitrogen was determined by the micro-Kjeldahl method according to the Association of Official Analytical Chemists (AOAC) [26] method 970.22. The percentage of nitrogen determined was converted to percentage of crude protein by multiplying by a factor of 6.25. The analysis was conducted in triplicates and the mean values were reported.

2.2.4 Reducing Sugars

The reducing sugars were determined using the Luff-Schoorlf method [27]. Exactly 3 g of the defatted cocoa powder was weighed into a wide-neck 250 mL

volumetric flask, 150 mL hot water was added and shaken to extract the water soluble matter. The mixture was filtered and 5 mL of Carrez I and II solutions were added to the filtrate to clarify it. The filtrate was filtered, 25 mL of Cu reagent was pipetted into two of 250 mL quick fit Erlenmeyer flask. To one 25 mL of distilled water will be added and the other 20 mL of the clarified sample solution and top up by 5 mL of distilled water to the mark. Anti-bumping granules were added and boiled for 10 min then cooled for 5 min in cooling bath. About 3 g of KI was added, swirl to dissolve and 20 mL of 6 N HCl was added. The flask content was titrated with 0.1 N thiosulphate until the iodine colour nearly disappeared. 1 mL of starch indicator was added and titrated until the blue colour changed to white or faint yellow precipitate of cuprous iodide with no trace of blue colour. The blank was also titrated.

2.2.5 Non-reducing Sugar

Exactly 6 mL of the clarified sample solution was measured into a 250 mL quick fit Erlenmeyer flask and 2 mL of 1 N HCl was added and topped up with 10 mL distilled water then subjected to refluxing for 10 min, cool and 2 mL of 1 N NaOH and 3 mL dH₂O added. The Cu reagent was added and preceded as the reducing sugar.

2.2.6 Total Sugars

The total sugars were determined using the phenol sulphuric acid method [28]. Approximately 0.5 g of the defatted cocoa powder was weighed into a round bottom flask and 30 mL of 80% ethanol was added. The mixture was boiled on a heating mantle under reflux for 30 min and the supernatant was decanted slowly into another round bottom flask. The procedure was repeated three times and supernatants were added together. The supernatant was concentrated under reduced pressure using rotary evaporator. 7.2 mL of 5% ZnSO₄ solution and 10 mL of 0.3 N barium hydroxide octahydrate (Ba(OH)₂·8H₂O) solution were added to the extract and the mixture filtered. A mixture of Zeo-Karb 225 (H⁺), anion and cation exchange

resins and deacidite FF (OH) was added to the filtrate and filtered. 0.2 mL of the extract was pipetted into a test tube, and 1 mL phenol reagent and 5 mL H₂SO₄ reagent were added. The solution was allowed to stand for about an hour and absorbance read at 490 nm using a UV/Visible spectrophotometer (Beckman Coulter spectrophotometer, model Du 730) equipped with one centimeter cuvette. Glucose at different concentrations (0, 20, 40, 60, 80 and 100 µg/mL) was used to draw the standard curve. The total sugars in the prepared sample were calculated.

2.3 Statistical Analyses

The data were analyzed using Statgraphics software version 15.0 (STSC, Inc., Rockville, MD, USA). Analysis of variance (ANOVA) was carried out on the results obtained to determine statistical differences between the studied attributes. The significance was established at a confidence level of 95% ($P \leq 0.05$). The combined effect of pod storage and roasting duration was studied using the response surface methodology. Models were developed to relate pod storage and roasting duration on the studied parameters. The nib acidity was measured by response variables from pH and titratable acidity; the proteolysis was measured by protein content and the sugar concentration was measured by response variables from reducing sugars, non-reducing and total sugars. The coefficients of the variables in the models and their contribution to the model's variation were calculated and reported as the coefficient of determination, R^2 . The values of R^2 were used to judge the adequacy of the models. For a good fit of a model, an R^2 of at least 60% was used. The R^2 of a model refers to the proportion of variation in the response attributed to the model rather than random error.

3. Results and Discussion

3.1 Changes in pH of Cocoa Nibs

The pH of cocoa beans is an important physicochemical property as it gives an indication on

the degree of acidification of the nibs during fermentation and the quality of fermentation. It also gives an indication of the degree of biochemical reactions that occurred during the fermentation as well as the taste or sourness of the bean.

Cocoa beans from the unstored pods (PS0) after fermentation and drying had a pH of 4.91 and those from pods stored for 3, 7 and 10 d had pH values of 5.31, 5.79 and 5.86, respectively. The unstored pods had the lowest pH while 10-day pod storage had the highest pH. Fermented and dried cocoa beans with pH range 5.0-5.5 produced higher flavour potentials while that with pH range 4.0-4.5 give low flavour potential [29, 30]. Similar trends were observed by Nazaruddin et al. [9] and Afoakwa et al. [23]. However, variable trends were observed for increasing pod storage and roasting time (Fig. 1). Increasing pod storage caused consistent increases in the pH of the beans, while only slight and insignificant changes in pH were noted with increasing roasting time (Fig. 1).

The pH of the unstored pods increased from 4.91 to

5.28 after 45 min of roasting, similar pH increases were observed for beans from pod stored for 3, 7 and 10 d with increasing roasting time. These findings corroborate with earlier reports [31, 32]. The significant increases in pH with increasing pod storage might be due to loss of moisture from the beans and the pulp during storage of the pods [23] which causes a reduction in the pulp volume as well as a reduction in sugar content due to respiration by the cocoa fruit and increase in aeration thereby decreasing alcohol production by the yeasts from sugar metabolism at the anaerobic phase of the fermentation process [21, 33].

Regression analysis of the data showed significant ($P < 0.05$) influence of the linear and quadratic factors for pod storage on the pH of the nibs (Table 1) while the linear and quadratic factors for roasting time showed insignificant ($P > 0.05$) influence on the pH of the cocoa nibs. There was also insignificant interaction between pod storage and roasting time. The regression model developed could explain 71.1% of the variation in the pH of the cocoa nibs due to pod storage and

Fig. 1 Effect of pod storage and roasting time on the pH of the cocoa beans.

Table 1 Regression coefficients and their R^2 values in the models for pH, titratable acidity, proteins, reducing sugars, non-reducing sugars and total sugars in the cocoa beans.

Variables	pH	Titratable acidity	Proteins	Reducing sugars	Non-reducing sugars	Total sugars
Constant	5.65596*	0.071861*	17.9646*	4.3970*	2.8945*	6.929*
X_1	0.43481*	-0.052872*	-2.8880*	-0.1460*	-0.7207*	-0.7702*
X_2	0.09862	-0.016556*	-1.6558*	-2.2914*	-0.0388	-2.1719*
X_1^2	-0.21354*	0.041443*	0.16558	0.3091*	0.2730*	0.8063*
X_2^2	0.04852	-0.002742	-1.0049*	-0.9076*	0.0395	-0.7583*
$X_1 \times X_2$	-0.10216	0.018391*	-0.7056*	-0.2267*	-0.0053	-0.2364
R^2	71.1%	84.2%	87.2%	95.8%	74.0%	87.4%
R^2 (adj.)	68.6%	82.8%	86.1%	95.4%	68.9%	86.4%

* Significant at $P < 0.05$; X_1 : pod storage; X_2 : roasting time.

roasting time (Table 1). This means 28.9% of the variation in the model was due to random error. The response surface curve (Fig. 1) showed a linear relationship between pod storage and pH at all roasting time.

3.2 Changes in the Titratable Acidity

The titratable acidity is one of the main quality parameters determined during processing of cocoa both on farm and industrial processing since cocoa nibs with very high titratable acidity after fermentation is an indication of poor fermentation. The titratable acidity for the beans from the unstored pods was 0.210 meq/NaOH which decreased to 0.102 meq/NaOH, 0.070 meq/NaOH and 0.06 meq/NaOH for beans from pods stored for 3, 7 and 10 d, respectively. The decrease in titratable acidity of the beans with increasing pod storage is corroborated by Nazaruddin et al. [9] and Afoakwa et al. [23]. Increasing pod storage caused reductions in the titratable acidity in the beans from pod stored for 0 d to 7 d and increased slightly again till the end of the 10 days of pod storage at all roasting time (Fig. 2). Similarly, increasing roasting time caused slight reduction in the acidity of the beans from day zero to day seven of pod storage (Fig. 2), further increases in pod storage (between 7 d and 10 d) had no effect on the acidity of the beans with increasing roasting time (Fig. 2) hence cocoa pod storage up to seven days could be used to reduce nib acidity significantly compared to roasting. The observed decrease in titratable acidity with increase in

roasting time is corroborated by earlier reports [31, 32].

The decreases in titratable acidity of the beans with increasing pod storage might be due to the loss of moisture from the beans and the pulp in the pod [23]. This causes reduction in the pulp volume as well as sugar content due to respiration by the cocoa fruit, thereby decreasing alcohol production by the yeast from sugar metabolism at the anaerobic phase of the fermentation process [21, 33]. This might have affected the rate of acid production with beans from the stored pods. The decrease in titratable acidity of the cocoa beans as observed with increase in roasting time was due to evaporation of lower molecular weight and volatiles organic acids such as acetic acid along with moisture during the roasting process [34, 35]. The response surface curve (Fig. 2) shows a curvilinear relationship between pod storage and roasting time on the titratable acidity of the cocoa beans. The multiple regression model could explain 84.2% of the variation that existed in the titratable acidity of the beans due to pod storage and roasting time as indicated by the R^2 value (Table 1), hence, 15.8% of the variation in the model was due to random error.

3.3 Changes in the Protein Content

The amino containing compounds that take part in Maillard reactions during drying and roasting are obtained from peptides, hydrophobic free amino acids and proteins. The flavour precursors especially peptides and amino acids are formed from protein degradation during fermentation [19].

Fig. 2 Effect of pod storage and roasting time on the titratable acidity of the cocoa beans.

The protein content of the dried fermented beans decreased with increasing pod storage (Fig. 3). It decreased from 21.69% for the unstored pods to 19.30%, 18.06% and 16.39% for pods stored for 3, 7 and 10 d, respectively. Afoakwa et al. [23] also observed a similar decrease in protein content of the cocoa beans with pod storage and attributed it to the action of protease enzymes in the pods during storage, initiating the process of proteolysis. The decrease in protein content during pulp pre-conditioning (pod storage) might result in an increase in peptides as well as amino acids which is necessary for formation of flavour compounds during roasting.

Results showed that the increasing of roasting time decreased the protein content in the cocoa beans (Fig. 3). The protein content in the unstored cocoa beans decreased significantly from 21.69% prior to roasting to 18.96% after 45 min roasting (Fig. 3). Similar trends were observed for 3, 7 and 10 d pod stored beans (Fig. 3). These findings corroborate earlier reports [36-38]. The significant decrease in protein content with increasing roasting time is probably due to the

involvement of the protein in Maillard reactions as well as oxidative deamination during roasting.

The regression analysis of the data showed a statistically significant ($P < 0.05$) influence of the linear factors for pod storage and roasting time on the protein content of the nibs (Table 1). The quadratic factor for pod storage had insignificant ($P > 0.05$) influence while that of roasting time was significant ($P < 0.05$) (Table 1). The interaction effect of pod storage and roasting time significantly ($P < 0.05$) influence the protein content of the beans (Table 1). The model developed had an R^2 of 87.2% implying that the model could explain about 87% of the variations in the proteins of the nibs, with the remaining 13% was due to other factors not investigated in this work (Table 1). The response surface curve (Fig. 3) showed a curvilinear relationship for roasting time and protein content at all pod storage treatments.

3.4 Changes in Reducing Sugars

The level of reducing sugars in cocoa beans after drying is very crucial to the amount of flavour

Fig. 3 Effect of pod storage and roasting time on the protein content of the cocoa beans.

compounds formed during roasting [8, 39]. Increasing pod storage increased the reducing sugars of the unroasted cocoa beans from 5.880 mg/g for the unroasted pods to 5.938 mg/g for pods stored for three days. It however decreased slightly to 5.638 mg/g by day seven and increased again to 6.250 mg/g by day 10 of pod storage (Fig. 4). The increase in the reducing sugars with increasing pod storage could be due to the activities of cotyledon invertase in the beans which hydrolyzed sucrose to fructose and glucose (the main reducing sugars).

Changes in reducing sugars in the beans during roasting for all pod storage treatments are shown in Fig. 4. Increasing roasting time decreased significantly the reducing sugars of the beans for all pod storage. The reducing sugars in the unroasted cocoa beans decreased from 5.880 mg/g prior to roasting to 2.095 mg/g after 45 min roasting (Fig. 4). Similar trends were observed for beans from pods stored for 3, 7 and 10 d after roasting. The decrease in reducing sugars during

roasting was in agreement with earlier findings by several researchers [12, 40, 41]. The reduction in the reducing sugars in the cocoa beans during roasting was probably due to their involvement in Maillard reactions by reacting with the free amino acids or proteins in the cocoa beans to form flavour volatiles and also develop the brown colour of roasted cocoa beans [12, 40, 41].

The regression coefficient (Table 1) indicated that the linear and quadratic terms of pod storage and roasting time significantly ($P < 0.05$) influence the reducing sugars of the cocoa nibs as well as the interaction between pod storage and roasting time (Table 1). The regression model had an R^2 of 95.8% implying that about 96% of the variations in the reducing sugars of the nibs could be explained by the model while the remaining 4% was due to other factors not investigated in this work (Table 1).

3.5 Changes in Non-reducing Sugars

Non-reducing sugar, mainly sucrose serves as the

Fig. 4 Effect of pod storage and roasting time on the reducing sugars of the cocoa beans.

reactant for both cotyledon and pulp invertases during fermentation and drying to produce reducing sugars such as glucose and fructose. These reducing sugars act as flavour and colour precursors during roasting [1].

The fermented and dried cocoa beans from the unstored pods had non-reducing sugar content of 3.493 mg/g which decreased significantly ($P < 0.05$) during pod storage. It decreased from 3.493 mg/g to 3.346, 2.195 and 2.641 mg/g for pods stored for 3, 7 and 10 d, respectively (Fig. 5). Results also showed that roasting time caused slight decrease in the non-reducing sugars (Fig. 5). It decreased from 3.493 mg/g at the start of roasting to 3.432 mg/g at the end of roasting (45 min) for the unstored pods. Similar trend of decrease was observed for pods stored for 3, 7 and 10 d. The significant decrease in the non-reducing sugars of the beans with increasing pod storage might be due to the action of cotyledon invertase during the fermentation and drying process. Bonvehi and Coll [36] observed a similar trend of marginal decrease in non-reducing

sugar with roasting. The marginal decrease in non-reducing sugar with roasting was due to their polymerisation with proteins and other polysaccharides to form insoluble complexes [42].

The regression model developed to predict the effect of pod storage and roasting time on the non-reducing sugar of the cocoa nibs showed a statistically significant ($P < 0.05$) influence of the linear and quadratic factors for pod storage on the non-reducing sugar content of the cocoa nibs (Table 1). Both the linear and quadratic factors for the roasting time insignificantly ($P > 0.05$) influence the non-reducing sugars of the cocoa nibs. The interaction factor for pod storage and roasting time insignificantly ($P > 0.05$) influence the non-reducing sugar (Table 1). The regression model could explain 74.0% of the variation that existed in the non-reducing sugar of the cocoa beans due to pod storage and roasting time as indicated by the R^2 value (Table 1). This means 26% of the variation in the model was due to other factors not investigated in this work.

Fig. 5 Effect of pod storage and roasting time on the non-reducing sugar content of the cocoa beans.

3.6 Changes in the Total Sugars

Response surface plot (Fig. 6) showed changes in the total sugar content of the cocoa beans during pod storage and roasting. Total sugar content in the fermented and dried cocoa beans decreased from 9.389 mg/g for the unstored pods to 9.284 mg/g for pods stored for three days and 7.583 mg/g for pods stored for seven days but increased slightly to 8.891 mg/g for 10 days pod storage. The decrease up to day seven might be due to the involvement of some of the reducing sugars in Maillard reactions. Changes in total sugars in the beans during roasting for all pod storage treatments are shown in Fig. 6. Increasing roasting time decreased significantly ($P < 0.05$) the total sugars of the beans for all pod storage. The total sugars in the unstored cocoa beans decreased from 9.389 mg/g prior to roasting to 5.962 mg/g after 45 min roasting (Fig. 6). Similar trends of decrease were observed for beans from pods stored for 3, 7 and 10 d after roasting. The decrease in total sugars during roasting was in agreement with earlier findings by Akomanyi [32]. The significant decrease in the total sugars in the cocoa beans during

roasting was due to the involvement of the reducing sugars in Maillard reactions to form flavour compounds [12, 40, 41] and the reduction in some amount of non-reducing sugar by polymerizing with proteins and other polysaccharides to form insoluble complexes during the roasting process [42].

The regression model developed for the effect of pod storage and roasting time on the total sugars of the cocoa nibs showed a significant ($P < 0.05$) influence of the linear and quadratic factors of pod storage and roasting time on the total sugars of the cocoa nibs (Table 1). The interaction between pod storage and roasting time had insignificant ($P > 0.05$) influence on the total sugars present in the cocoa nibs (Table 1). The regression model had an R^2 of 87.4% implying that about 87% of the variations in the total sugars of the nibs could be explained by the model while the remaining 13% was due to other factors not investigated in this work (Table 1).

4. Conclusions

Increasing pod storage and duration of roasting

Fig. 6 Effect of pod storage and roasting time on total sugars of the cocoa bean.

increased the pH of the fermented and dried cocoa beans with consequential decrease in titratable acidity. Increasing pod storage caused consistent increases in pH while only marginal and insignificant changes in pH were noted with increasing roasting time. Similarly, increasing roasting time caused slight reduction in the acidity of the beans from day zero to day seven of pod storage, and further increases in pod storage (between 7 d and 10 d) had no effect on the acidity of the beans. Cocoa pod storage up to seven days leads to reduction in nib acidity to attain optimum sourness in the fermented beans. Total sugars, non-reducing sugars and protein content of the beans decreased with increasing pod storage and roasting time while reducing sugars increased with pod storage, probably due to Maillard reaction and Strecker degradation. To attain optimum acidity and flavour precursors generation in pulp preconditioned cocoa nibs, cocoa pod could be stored for up to seven days, fermented,

dried to the required moisture level and roasted at 120 °C for 45 min.

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