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Changes in chemical quality of cocoa butter during roasting of pulp pre-conditioned and fermented cocoa (*Theobroma cacao*) beans

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Abstract

Investigations were conducted to ascertain changes in chemical quality of cocoa butter during roasting of pulp pre-conditioned and fermented cocoa beans. A 4×4 full factorial design was used with pod storage (0, 3, 7, 10 days) and roasting time (0, 15, 30 and 45 minutes) as the principal factors. Samples were evaluated for free fatty acids (FFA), saponification value, iodine value, peroxide value and extinction value using standard analytical methods. Pod storage as a means of pulp pre-conditioning caused increases in the FFA, peroxide value, iodine value and extinction value of the cocoa butter. Similarly, increasing roasting time led to consistent increases in the peroxide value and extinction value of the cocoa butter but had only marginal and insignificant effect on the FFA, iodine value and saponification value. The varied increases in the chemical quality characteristics of the cocoa butter as a result of 10 days pod storage and roasting time did not have any remarkable negative effect on the chemical quality of the resultant cocoa butter. However, the observed changes were more dependent of pod storage than or roasting time.

Introduction

Cocoa butter is the most abundant component of the cocoa nib, constituting about 53–65% of the nibs on a dry weight basis compared to the other components (sugars, polyphenols, alkaloids, starch, proteins, theobromine, caffeine, non-volatile acids such as oxalic acids, malic acid and minerals) (Biehl and Ziegleder, 2003; Borges *et al*., 2006; Afoakwa, 2010; Afoakwa *et al*., 2011a). It is the most valuable chemical component of the cocoa beans, due to its unique physical and chemical characteristics which gives it great demand in the food industry. These special characteristics, non-comparable with any other edible vegetable fat, are very useful in the manufacture of a wide variety of products in the chocolate, cosmetic and pharmaceutical industries (Liendo *et al*., 1997; Sukha, 2003; Howell *et al*., 2005; Beckett, 2009).

Chemically, cocoa butter is made up of about 95% triacylglycerols, 2% diacylglycerols, less than 1% monoacylglycerol, 1% polar lipids and 1% free fatty acids (Biehl and Ziegleder, 2003). Triglycerides consist of about 37% oleic (O), 32% stearic (S), 27% palmitic (P) and 2–5% linoleic (L) acids (Saldaña *et al*., 2002; Biehl and Ziegleder, 2003; Kaphueakngam *et al*., 2009; Quast *et al*., 2011). Trace amount of lauric acid (C12) and myristic acid (C14) also exist (Kaphueakngam *et al*., 2009). The chemical quality of cocoa butter largely influence the physical quality characteristics of chocolate such as hardness at room temperature, brightness, fast and melting behaviour (Saldaña *et al*., 2002). Dried unfermented cocoa beans are strongly bitter and astringent and produce poor or no chocolate flavour (Ardhana and Fleet, 2003; Schwan and Wheals, 2004) after roasting. The flavour characters of the beans depends mainly on the variety or genotype and partly on post-harvest treatments such as pod storage and fermentation (Faborode *et al*., 1995; Afoakwa, 2010).

During fermentation, various biochemical processes important for taste and flavour development are initiated in the cocoa beans. The processes result in the formation of flavour precursors, such as free amino acids, short-chain peptides and reducing sugars (Counet *et al*., 2002; Frauendorfer and Schieberle, 2008) from which the typical cocoa aroma is generated during subsequent roasting process.

Roasting is critical to the flavour quality of cocoa beans and it is dependent on the time and temperature as well as post-harvest processes of fermentation and drying. The roasting conditions determine the quality of the final products (Krysiak and Motyl-Patelska, 2006). During roasting, several physical and chemical changes occur in the cocoa beans such as evaporation of volatile acids from the beans causing a reduction in

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the cocoa beans acidity hence reducing the sourness of and the bitterness of the cocoa beans and produce desirable chocolate flavours and colour by maillard reaction (Afoakwa, 2010).

In recent times, the technique of pulp pre-conditioning by postharvest pod storage has been of major interest to researchers due to its reported improvement on the quality of fermented cocoa beans in terms of reduction in the nib acidity and enhanced flavour note Several researchers have reported on the beneficial influence of pulp pre-conditioning by pod storage on the quality of cocoa beans (Biehl *et al.*, 1989; Sanagl *et al.*, 1997; Schwan and Wheals, 2004 Afoakwa *et al.*, 2011a). Recent workdone by Afoakwa *et al.* (2011b) suggested that the chemical components of cocoa butter such as the free fatty acids are affected by post-harvest treatments such as pulp pre-conditioning by pod storage and fermentation. Pod storage of cocoa beans up to 21 days increased the % free fatty acids of the cocoa butter significantly above the acceptable international standard of 0.75 % (Afoakwa *et al.*, 2011b).

Further research in reducing the duration of pod staorge prior to fermentation revealed that storing cocoa pods up to 10 days reduces the FFA content of the derived cocoa butter to acceptable levels whilst still enhancing the nib acidification and flavor precursors in the beans (Afoakwa *et al.*, 2013). However, the extent to which the reduced pod storage duration and varied roasting conditions influences the other chemical quality characteristics of cocoa butter such as iodine value, peroxide value, saponification value and extinction value (conjugated dienes) still remains unknown. This study was therefore aimed at investigating changes in chemical quality of cocoa butter during roasting of pulp pre-conditioned and fermented cocoa beans.

Materials and Methods

Raw materials

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

Sample preparation

Freshly and fully ripe good looking cocoa pods were harvested, sorted out to remove the bruise ones and divided into four parts, each containing three hundred (300) pods. The pods were stored in a heap form for four different storage times (0, 3, 7 and 10 days) 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 to 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*. (2011) with slight modifications. The fermented and dried cocoa beans sampled 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 minutes. For each of the roasting treatments under investigation, the oven temperature was set at 120oC and left to equilibrate for at least 30 minutes. 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 and labeled appropriately. 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 deshelled manually using knife and milled using kitchen blender for further analyses. All treatments were conducted in duplicates.

Experimental design

A 4×4 full factorial design with the principal experimental factors as pod storage (0, 3, 7 and 10 days) and roasting time (0, 15, 30 and 45 minutes) at 120^{o}C were used to study the changes in the free fatty acids (% FFA), saponification value, peroxide value, iodine value and extinction values (conjugated dienes) of the cocoa butter.

Determination of free fatty acids (% FFA)

The free fatty acids (% FFA) were determined by the International Office for Coffee, Cocoa and Sugar Confectionery Official Method 42-1993 (IOCCC, 1996) as modified by Guehi *et al.* (2008). Exactly 5 g of the extracted liquid cocoa fat sample was weighed into a conical flask and 50 ml of 95% ethanol/diethylether (1:1, v/v) added. Two (2) drops of phenolphthalein was added and titrated with 0.1 N

NaOH with constant shaking until the appearance of faint pink colouration that persisted for 15 seconds. The end titre values were recorded. Free fatty acids (% oleic acid) were calculated and the mean values reported.

Determination of saponification value, peroxide value and iodine value

The saponification value, peroxide value and iodine values were determined by the official methods 920.160B; 965.33 and 993.20 for AOAC (2005), respectively.

Determination of extinction values (conjugated dienes)

The extinction values were determined using Krysiak (2011) method with slight modifications. About 0.1 g of the extracted cocoa butter was weighed and dissolved in 10 ml of n-hexane placed in a test tube. Absorbance of the sample was measured after 2 minutes at a wavelength of 233 nm using UV/Visible spectrophotometer (Beckman Coulter spectrophotometer, model Du 730) equipped with one centimetre curvette. Analyses were conducted in triplicate and the mean calculated.

Statistical analyses

The data were analyzed using Statsgraphics software version 15.0 (STSC, Inc., Rockville, MD, USA) for Analysis of variance (ANOVA) at $p < 0.05$. Least significant difference (LSD) was used to separate and compare the means, 5% level ($p < 0.05$) was accepted as significance. Line graphs were used to show the effect of pod storage and roasting time on the cocoa butter quality characteristics.

Results and Discussion

Changes in free fatty acid (FFA)

Free fatty acid content of cocoa butter are of interest to both producers and chocolate manufacturers since higher percentage leads to quality reduction in fermented cocoa beans as well as decrease in hardness of the cocoa butter. Cocoa butter from fermented and dried cocoa beans from the unstored pods had free fatty acid content of 0.480% oleic acids and those from pods stored for 3, 7 and 10 days had free fatty acid content of 0.483% oleic acids, 0.480% oleic acids and 0.537% oleic acids respectively. Increasing pod storage increased the % FFA in the butter significantly ($P < 0.05$) for cocoa beans from 10 days pod storage whiles no observable increase in free fatty acids for butters from the unstored pods, 3 and 7 days stored pods (Figure 1). Similar trend was

Table 1. ANOVA summary table showing F-ratios for variations in cocoa butter quality characteristics of pod stored and fermented cocoa beans during roasting

Variables	FFA (% oleic acid)	S.V (mgKOH/g)	Iodine value (mg/100 g)	Peroxide value (mmol/kg)	Extinction value
Pod storage (PS)	44.07*	9.54*	2.80*	6.94*	8.20*
Roasting time (RT)	2.00	0.76	0.31*	22.73*	15.83*
Interaction (PS x RT)	1.62	0.64	2.02*	1.24	7.17*

*Significant at $p < 0.05$; SV – Saponification value; FFA – free fatty acids; Extinction value of 1% butter sample at wavelength 233nm [$E1\%_{233}$]

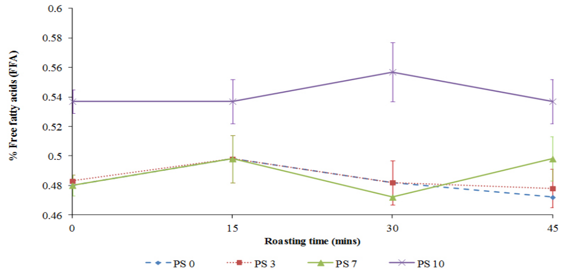

Figure 1. Effect of pod storage (PS) and roasting time (RT) on the free fatty acid content (% oleic acids) of the cocoa butter

observed by Afoakwa *et al.* (2011b). The significant influence of pod storage on the % FFA might probably be due to microbial activity as well as endogenous lipase activities leading to germination of some of the cocoa beans during the pod storage period (Dand, 1996; Fowler, 1999; Afoakwa *et al*., 2011b).

During roasting, the % FFA of the butter from cocoa beans from the unstored pods increased from 0.480% oleic acids prior to roasting to 0.498% oleic acids after 15 minutes roasting and decreased to 0.472% oleic acids after 45 minutes (Figure 1). Similar trend was observed for butter from cocoa beans from 3 and 7 days pods storage (Figure 1). Butter from beans pod stored for 10 days showed different trend with roasting (Figure 1). Roasting time caused insignificant ($p > 0.05$) influence on the free fatty acids of the butter (Table 1). The interaction between pod storage and roasting time also had insignificant ($p > 0.05$) influence on the free fatty acids in the cocoa butter. The trend was in agreement with earlier findings by Krysiak (2011) during cocoa roasting. The cocoa butter from the fermented, dried and unroasted beans had percentage free fatty acids content below the maximum industrial limit of 1.75% and that of roasted beans 3.1% oleic acid (Chaiseri and Dimick, 1989; Shukla, 2003; Krysiak, 2011) or 1.75% oleic acids.

Changes in saponification value

Saponification value provides a measure of the quality and purity of oils and fats. Saponification value measures the mean molecular weight of the fatty acids present in the oil or fat (Krysiak, 2011). The saponification values (SV) for the cocoa butter for the unroasted cocoa beans decreased marginally

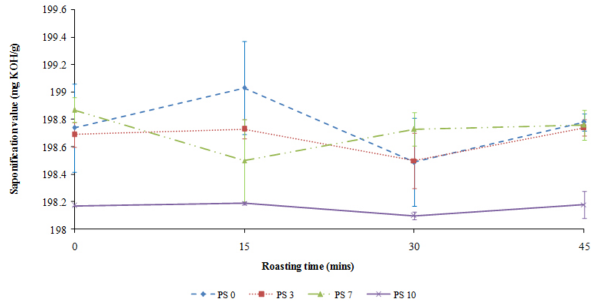

Figure 2. Effect of pod storage (PS) and roasting time (RT) on the saponification value (SV) of the cocoa butter

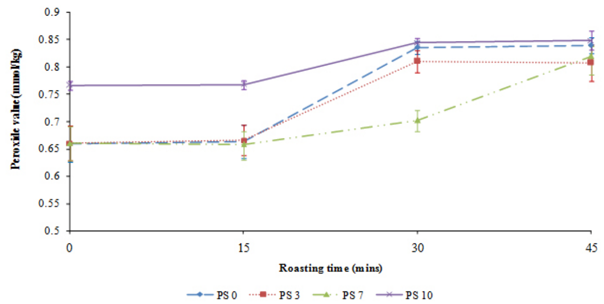

Figure 4. Effect of pod storage (PS) and roasting time (RT) on the peroxide value (PV) of the cocoa butter

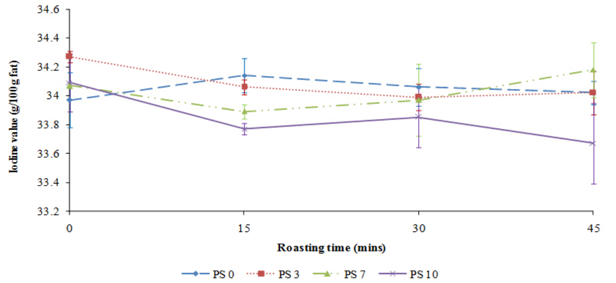

Figure 3. Effect of pod storage (PS) and roasting time (RT) on the iodine value (IV) of the cocoa butter

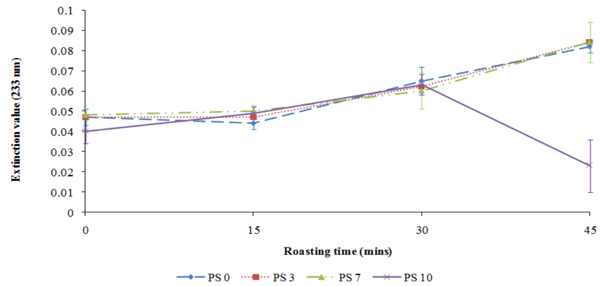

Figure 5. Effect of pod storage (PS) and roasting time (RT) on the Extinction value of 1% cocoa butter at wavelength 233nm [$E1\%_{233}$]

from 198.74 for the unstored pods to 198.17 mg KOH/g cocoa butter for pods stored for 10 days (Figure 2). The saponification values recorded in the current study were higher than literature value of 188 to 198 mg KOH/g cocoa fat (Codex Standard 86, 1981; Krysiak, 2011) and that reported by Chaiseri and Dimick (1989) of 195.07 to 195.92 mg KOH/g for cocoa beans grown in five countries in South America.

The roasting process also caused insignificant ($p > 0.05$) changes in the saponification values of the cocoa butter for all pod storage treatments (Figure 2). The interaction effect of pod storage and roasting time on the saponification values of the butter was also statistically insignificant at $p < 0.05$ (Table 1). The saponification values of the butter from the roasted pulp pre-conditioned cocoa beans were within cited literature values of 188-198 mg KOH/g cocoa butter (Codex Standard 86, 1981; Chaiseri and Dimick, 1989; Shukla, 2003; Krysiak, 2011).

Changes in iodine value

The iodine value (IV) estimates the degree of unsaturation and hardness of the cocoa butter. A high iodine value indicates a high level of unsaturation of the triglycerides in the butter, contributing to the soft texture of the cocoa butter (Liendo *et al.*, 1997). Increasing roasting time and pod storage caused variable trends in the iodine values of the cocoa butter (Figure 3). The butter from the unstored pods had iodine value of 33.97 g I/100 g cocoa fat and those from pods stored for 3, 7 and 10 days had iodine values of 34.27 g I/100 g cocoa fat, 34.07 g I/100 g cocoa fat and 33.79 g I/100 g cocoa fat, respectively (Figure 3). Pod storage caused a significant change in the iodine value of the cocoa butter at $p < 0.05$ (Table 1).

Increasing roasting time caused a consistent decrease in the iodine value of the butter from 3 days pod stored beans whiles 7 days pod stored beans decreased from 34.07 g I/100 g fat to 33.89 g I/100 g fat after 15 minutes roasting and increased consistently for 30 and 45 minutes roasting. That of 10 days pod stored beans decreased from 34.09 g I/100 g to 33.77 g I/100 g fat and increased to 33.85 g I/100 g cocoa fat after 30 minutes roasting but decreased again to 33.67 g I/100 g cocoa fat after 45 minutes roasting (Figure 3). The roasting time caused significant ($p < 0.05$) change in the iodine value of the butter (Table 1). The interaction between pod storage and roasting time also caused significant change in the iodine values of the butter at $p < 0.05$ (Table 1).

The iodine values for all pod storage treatments at all durations of roasting were within the acceptable limits by the Codex Standard of 33-42 (Codex Standard 86, 1981). Work done by Biehl and Ziegleder (2003) also reported iodine values (36.5 g I/100 g) for cocoa butter extracted from Ghanaian cocoa beans to be within the Codex Standard. The iodine values observed in this research indicated that the cocoa butter were slightly hard.

Changes in peroxide value

Peroxide values measures the degree of rancidity in oils and fat. The peroxide value for the cocoa butter from the fermented and dried cocoa beans from the unstored pods was 0.659 mmol/kg fat which increased significantly ($p < 0.05$) during pod storage (Table 1). It increased from 0.659 mmol/kg fat for the unstored pods to 0.660 mmol/kg fat, 0.660 mmol/kg fats and 0.766 mmol/kg for butters from cocoa beans from 3, 7 and 10 days storage respectively (Figure 4). The increase in the peroxide values with pod storage might be due to increased aeration of the pulp and diffusion of oxygen into the beans during fermentation and drying which caused oxygen molecules to react with some of the double bonds (oleic and linoleic fatty acids) in the triacylglycerol molecules to form hydroperoxides.

Increasing roasting time led to consistent increase in peroxide values of the cocoa butter from all pod storage treated cocoa beans (Figure 4). The rate of increase in peroxide values of the butter was rapid after 30 minutes roasting for 0, 3, and 10 days pod stored cocoa beans, after which no increase resulted except butter from 7 days pod stored cocoa beans (Figure 4). The peroxide value for the butter from cocoa beans from unstored pods increased from 0.659 prior to roasting to 0.839 mmol/kg after 45 minutes roasting. Similar increases were observed for beans from 3, 7 and 10 days of pod storage. These findings are consistent with that of Oomah *et al.* (1998) and Krysiak (2011).

The significant increase ($p < 0.05$) in the peroxide values of the butter with increase in roasting time might be due to thermal oxidation of the cocoa butter during roasting. The interaction between pod storage and roasting time caused insignificant ($p > 0.05$) influence on the peroxide values of the butter (Table 1). The cocoa butter from the cocoa beans from the different treatments had peroxide values below the maximum value cited in literature of 3.5 mmol O_2/kg fat that causes unsatisfactory sensory properties in fat (Krysiak, 2011) or 5 mmol O_2/kg fat (10 meq/kg fat) (Shahidi, 2005).

Changes in the extinction value (conjugated dienes)

Extinction value is the absorbance of fat or oil sample in hexane at a particular wavelength. It measures the degree of rancidity in fats and oils and has been shown to related to peroxide values. Increasing pod storage increased significantly ($p < 0.05$) the extinction values (conjugated dienes) of the butter of the unroasted cocoa beans from 0.047 for the unstored pods to 0.047 and 0.048 for butters from pods stored for 3 and 7 days, respectively (Figure 5). It however, decreased slightly to 0.040 by day 10 (Figure 5). The increase in the extinction with pod storage might be due to increased aeration of the pulp and diffusion of oxygen into the beans during fermentation and drying which caused oxygen molecules to react with some of the double bonds (oleic and linoleic fatty acids) in the triacylglycerol molecules.

Also, increasing roasting time increase significantly ($p < 0.05$) the extinction values of the cocoa butter (Table 1). The extinction value (conjugated dienes) for the butter extracted from the unstored pods increased significantly from 0.047 prior to roasting to 0.082 after 45 minutes roasting (Figure 5). Similar trend was observed for cocoa butter from 3 and 7 days pod stored beans. However, cocoa butter from 10 days pod stored beans increased from 0.040 prior to roasting to 0.063 after 30 minutes roasting and decreased to 0.023 after 45 minutes roasting (Figure 5).

The interaction between pod storage and roasting time had a significant influence ($p < 0.05$) on the extinction values of the butter (Table 1). The extinction values (conjugated dienes) for the cocoa butter from the different treatments were below the European Union value of 0.25 for oils and fats (EEC, 2003) and that stated in Dezaan cocoa manual (2009) of extinction maximum of 0.5 for a pure prime pressed cocoa butter.

Conclusion

Increasing pod storage increased the % FFAs, peroxide value, saponification value and iodine value and increased the extinction values (conjugated dienes) for cocoa butters from 0, 3 and 7 days pod stored beans but decrease that of 10 days. Increasing roasting time insignificantly influenced the % FFAs and saponification values but caused significant effect on the iodine values, peroxide values and extinction value (conjugated dienes) of the cocoa butter. Cocoa beans can be pod stored for 3 to 7 days to cause no significant change in % FFAs, peroxide value and saponification value and roasted for 45 minutes to cause marginal change in the %FFAs and saponification values with peroxide values below the maximum value of 3.5 mmol O_2/kg fat that causes unsatisfactory sensory properties in fat or 5 mmol O_2 kg^{-1} fat (10 meq/kg^{-1} fat) and conjugated dienes below the European Union value of 0.25 for oils and fats or extinction maximum of 0.5 for a pure prime pressed cocoa butter.

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