Journal of Food Science and Engineering 3 (2013) 235-245

Effects of Pulp Preconditioning on Total Polyphenols, *O*-diphenols and Anthocyanin Concentrations during Fermentation and Drying of Cocoa (*Theobroma cacao*) Beans

Emmanuel Ohene Afoakwa[1], John Edem Kongor[1], Jemmy Felix Takrama[2], Agnes Simpson Budu[1] and Henry Mensah-Brown[3]

1. Department of Nutrition and Food Science, University of Ghana, P.O. Box LG 134, Legon-Accra, Ghana

2. Cocoa Research Institute of Ghana, P.O. Box 8, New Tafo-Akim, Eastern Region, Ghana

3. Department of Food Process Engineering, University of Ghana, Legon-Accra, Ghana

Received: January 18, 2013 / Published: May 20, 2013.

Abstract: Changes in total polyphenols, *O*-diphenols and anthocyanin concentrations during fermentation and drying of pulp pre-conditioned cocoa (*Theobroma cacao*) beans were investigated using standard analytical methods. Increasing pod storage, fermentation and drying led to variable reductions in total polyphenols, *O*-diphenols and anthocyanins content of the beans. The rates of reduction were however more pronounced during fermentation than pod storage and drying. Storage of cocoa pods between 3-7 days with 6 and 7 days of fermentation and drying respectively retained 85%-90% of the total polyphenol and *O*-diphenols of the cocoa beans. Similarly, anthocyanin content of beans from the 10 days of pod storage decreased by 70% in the sixth day of fermentation. Pod storage decreased the anthocyanin content at all periods of fermentation. These suggest that the post-harvest treatments of pod storage, fermentation and drying all results in variable reductions in polyphenolic content (total polyphenols and *O*-diphenols) and anthocyanins content of cocoa beans.

Key words: *Theobroma cacao*, pod storage, pulp pre-conditioning, fermentation, drying, total polyphenols, *O*-diphenols, anthocyanins.

1. Introduction

Polyphenols are naturally occurring compounds in cocoa beans stored in the pigment cells of the cotyledons [1] and depending on the amount of anthocyanins, those pigment cells, also called polyphenol-storage cells, are white to deep purple [2]. Polyphenols are responsible for the astringency of cocoa beans and contribute to bitter and green flavours [3-5]. Their most important attribute is their propensity to form complexes with protein, polysaccharide and alkaloid [6-7]. Hydrogen and hydrophobic bonding is involved in the protein-polyphenol interaction [8]. It is this capacity to precipitate proteins, in particular, the salivary proteins in the oral cavity, which give the polyphenols their astringent character [9].

Three groups of polyphenols can be distinguished in cocoa beans and these are: catechins or flavan-3-ols (ca. 37%), anthocyanins (ca. 4%) and proanthocyanidins (ca. 58%) [2]. The main catechin in the cocoa bean is (–)-epicatechin with up to 35% of polyphenol content with other catechins found in smaller amounts being (+)-catechin as well as traces of (+)-gallocatechin and (–)-epigallocatechin [1, 2]. The anthocyanin fraction consists mainly of cyanidin-3-α-L-arabinosid and

Corresponding author: Emmanuel Ohene Afoakwa, Ph.D., associate professor, research field: post-harvest technology of cocoa. E-mail: eoafoakwa@gmail.com.

cyanidin-3-β-D-galactosid [2] while proanthocyanidins are mostly flavan-3,4-diols, that are 4→8 or 4→6 bound to condensed dimers, trimers or oligomers with epicatechin as the main extension subunit [10]. Other polyphenols found in cocoa beans are the flavonol glycosides such as quercetin-3-O-α-D-arabinosid and quercetin-3-O-β-D-glucopuranosid [2]. Again, up to 17 phenolic acids and esters have also been reported and the total amount of seven of them comprises not more than 23 ppm of the seed dry weight (phloroglucinol, protocatechuic acid, vanillic acid, *O*-hydroxyphenylacetic acid, p-coumaric acid, caffeic acid and ferulic acid) [11].

The total amount of soluble polyphenols in the dried fat-free mass of fresh cocoa beans is 15% to 20% (equals approx. 6% in air dried cocoa beans, containing 54% fat and 6% water) [2]. Unfermented Forastero cocoa beans is reported to contain 120-180 g kg^{-1} of polyphenolic compounds with (–)-epicatechin being quantitatively the main compound (approximately 35%) [2, 12]. Nazaruddin et al. [13] reported total polyphenols content of 45 mg g^{-1} to 52 mg g^{-1} in cocoa liquor, 34 mg g^{-1} to 60 mg g^{-1} in cocoa beans and 20 mg g^{-1} to 62 mg g^{-1} in cocoa powder.

Polyphenols, originally compacted into vacuoles of specific cells, diffuse through the cotyledon during cocoa fermentation [14] and are subjected to biochemical modification through polymerization and complexation with protein, hence decreasing solubility and astringency [3]. The resulting compounds from polyphenol oxidation associate reversibly with proteins by hydrogen bonds or, irreversibly, by condensation with reactive groups of amino acids, peptides, proteins and polysaccharides [14]. These reactions are reported to be important to the development of cocoa flavour [15]. Work done by Porter et al. [16] concluded that total phenols in cocoa get reduced during fermentation to 30% of the initial concentrations and the (–)-epicatechin, the principal substrate of cocoa polyphenol oxidase (PPO), is reduced by 90%, with a proportional increase in catechin content. These reactions are both enzymatic and non-enzymatic and are catalyzed by the enzyme polyphenol oxidase, even though this enzyme is strongly inactivated during the first days of fermentation, remaining only 50% and 6% of enzyme activity after 1 and 2 days, respectively [17]. Also, anthocyanins are hydrolyzed to anthocyanidins.

After fermentation, the beans are then dried to reduce the moisture content from about 60% to between 6%-8% [18] to prevent mould infestation during storage and also allow some of the chemical changes which occurred during fermentation to continue and improve flavour development [19]. During drying, the amount of polyphenols is substantially reduced mainly by enzymatic browning catalyzed by polyphenol oxidase followed by non-enzymatic browning from quinone polymerization as well as diffusion outside of the beans [14, 19]. So, astringency of cocoa is reduced and the colour changes from purple to brown.

Polyphenol oxidase is the major oxidase in cocoa beans responsible for catalyzing the oxidation of polyphenols during the fermentation and drying processes. This enzyme is reported to become active during the aerobic phase of the fermentation as a result of oxygen permeating the cotyledon [20]. Among the factors that facilitate their activity include reduction in the amount of seed pulp, seed death, subsequent breakdown of subcellular membranes, and aeration of the bean by turning of the fermenting bean mass. Pod storage is reported to reduce pulp volume per seed due to water evaporation and inversion of sucrose causing an increase in micro-aeration within the pulp and the fermenting mass [12, 21]. This technique is expected to influence the activity of polyphenol oxidase during fermentation and drying, with resultant modifications in the polyphenolic and anthocyanin concentrations. This work was therefore aimed at investigating the effect of pod storage as a means of pulp preconditioning on total polyphenols, *O*-diphenols and anthocyanin concentrations during fermentation and drying of cocoa (*Theobroma cacao*) beans.

2. Materials and Methods

2.1 Materials

Ripe cocoa pods (mixed hybrids) were obtained from the Cocoa Research Institute of Ghana (CRIG), Tafo-Akim, Eastern Region. About 1,200 cocoa pods of uniform ripeness were harvested by traditional methods (under ambient temperature during the day; 28-30 °C) and transported to a fermentary (on the cocoa plantation) where they were stored (pulp preconditioned) at ambient temperature (28-30 °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. Samples were taken at 0, 3 and 6 days into a sterile polythene bag and oven-dried for about 48 h at a temperature of 45-50 °C until moisture content was between 7%-8%. The dried beans were then bagged in airtight black plastic bags and stored at ambient temperature (25-28 °C) in a dark room free from strong odours and used for analyses. Random sampling was done at the same time of the day and depth in the mass (40 cm to 80 cm from upper surface).

2.1.1 Drying of Fermented Cocoa Beans

The fermented cocoa beans were dried in the open sun on raised platforms using the traditional process [1]. Drying started at 8 am 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. The samples were then packaged in air tight plastic bags and taken to the laboratory for analyses. All the treatments were conducted in duplicates.

2.1.2 Experimental Design

A 4 × 3 full factorial experimental design with the principal factors being pod storage (0, 3, 7 and 10 days) and fermentation time (0, 3 and 6 days) was used to study the changes occurring during the fermentation process. The study also used a 4 × 3 full factorial design with pod storage (0, 3, 7 and 10 days) and drying time (0, 3 and 7 days) being the principal factors investigated to study the changes occurring during the drying process. Total polyphenols, *O*-diphenols and anthocyanins concentrations of the beans were studied during fermentation as well as the drying process.

2.2 Methods

2.2.1 Extraction of Phenolic Compounds

Extraction of the phenolic compounds was done following the procedures as described by Othman et al. [22] with slight modifications.

The dried cocoa nibs were finely ground using a kitchen blender and about 10 g of the resultant cocoa powder weighed into a thimble and the fat fraction removed by Soxhlet extraction (8 h) using petroleum ether (40-60 °C) according to the AOAC [23] method 963.15. The phenolic fraction was then extracted from the defatted cocoa powders. About 0.2 g of the resultant cocoa powder was homogenized in 30 mL mixture of 80% methanol:1% HCl for 2 h in falcon tubes using an orbital shaker at 420 rpm. The filtrate was decanted into fresh falcon tubes. The extract was used for the determination of total polyphenols, *O*-diphenols and anthocyanins concentrations.

2.2.2 Total Polyphenols

This was measured using the Folin-Ciocalteu's assay as described by Othman et al. [22] with slight modifications. 1 mL of the filtered sample was diluted with 49.0 mL of 80% methanol and 0.5 mL of this solution further diluted with 0.5 mL 80% methanol into test tubes making 1 mL of solution. Folin-Ciocalteu's phenol reagent was diluted to 10%, and then 5.0 mL of the 10% Folin-Ciocalteu's phenol reagent added to the 1.0 mL solution. This was followed by the addition of

4.0 mL saturated aqueous Na_2CO_3 solution and the mixture incubated at room temperature for 60 min and for another 60 min at -17 °C. The samples were taken from the freezer after the 60 min incubation and left to stand to attain a temperature of 30 °C. The absorbance at 760 nm was recorded. Results were expressed as catechin equivalents using a standard curve of catechin (0-100 μg mL^{-1}). The analysis was conducted in triplicates and mean values were reported.

2.2.3 *O*-diphenols

O-diphenols content was determined with Arrow's reagent (10 g $NaNO_2$ and 10 g Na_2MoO_4 in 100 mL distilled water). To 1mL of the methanol extract, 1mL of 0.5 N HCl, 1mL Arrow's reagent, 10 mL distilled water, and 2 mL of 1 N NaOH was added. The absorbance of the solution was read at 520 nm after 30 s. The analysis was conducted in triplicates and the mean values were reported. A working standard cathecol solution of 20, 40, 60, 80 and 100 ppm was prepared, the absorbance was read at 520 nm and a standard curve was drawn. From the standard graph, the amount of *O*-diphenols present in the samples was calculated.

2.2.4 Anthocyanins

Anthocyanins content was determined using the method described by Misnawi et al. [24]. The extract obtained for total polyphenol analysis was filtered using Whatman No. 4® filter paper and the supernatants were read spectrophotometrically for total absorbance (TOD) at 535 nm. The content of total anthocyanins was calculated as:

Total anthocyanins (mg kg^{-1}) = $TOD/(AvE_{535})^{1\%}{}_{1cm}/10 \times 1{,}000/1$

where, *TOD* is the total optical density (absorbance) and $(AvE_{535})^{1\%}{}_{1cm}$ is the average extinction coefficient for total anthocyanins when a 1cm cuvette and 1% (10 mg mL^{-1}) standard are used; the value is 982.

2.3 Statistical Analyses

Statgraphics software version 3.0 (STSC, Inc., Rockville, MD, USA) was used to analyzed 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 ($P < 0.05$). Again, the combined effects of pulp preconditioning and fermentation time and drying time on the studied parameters were studied using the response surface methodology. Models were developed to relate pulp preconditioning and fermentation time and also pulp preconditioning 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. All analyses were conducted in triplicates.

3. Results and Discussion

3.1 Changes in Total Polyphenols of Cocoa Beans during Fermentation

Total polyphenols decreased with increasing fermentation for all pod storage treatments as shown in the response surface plot (Fig. 1). It decreased from 171.54 mg g^{-1} at the start of fermentation to 153.58 mg g^{-1} at the end of the fermentation process for the unstored pods. It also decreased from 169.09 mg g^{-1} to 148.77 mg g^{-1} for pods stored for 3 days, 149.24 mg g^{-1} to 119.43 mg g^{-1} for pods stored for 7 days and 123.24 mg g^{-1} to 83.48 mg g^{-1} for pods stored for 10 days. Results from this study suggested that fermentation of cocoa beans leads to loss of total polyphenols in fermented cocoa beans. Aikpokpodion and Dongo [25] observed that polyphenols content of cocoa beans decreased from 16.11% (wt/wt) (161.1 mg g^{-1}) on day 0% to 6.01% (wt/wt) (60.1 mg g^{-1}) after six days of fermentation. Nazaruddin et al. [26] also reported that epicatechin and catechin content, respectively, were reduced to approximately 10%-70% during fermentation. Studies have also shown that loss of polyphenols during fermentation is not only due to the oxidation process

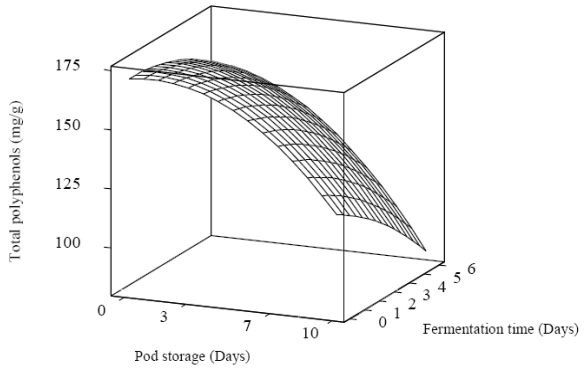

Fig. 1 Response surface plot showing changes in total polyphenols during fermentation of pulp pre-conditioned cocoa beans.

but also caused by diffusion of polyphenols into fermentation sweatings [2, 17] which had been confirmed by microscopic studies carried out by Brito et al. [27].

Again, results (Fig. 1) showed that increasing pod storage consistently reduced the levels of total polyphenols in the beans at all fermentation times. Total polyphenols in the unfermented cocoa beans decreased during pod storage. It decreased from 171.54 mg g^{-1} at the start of pod storage to 123.24 mg g^{-1} by 10 days of pod storage. At the end of fermentation, total polyphenols decreased by 12%, 14%, 25% and 48% for the unstored pods, pods stored for 3, 7 and 10 days, respectively. Similar observations were made by other researchers [26]. Pod storage is reported to reduce pulp volume per seed due to water evaporation and inversion of sucrose causing an increase in micro-aeration within the pulp and the fermenting mass [17]. This served to enhance the activity of polyphenol oxidase resulting in the oxidation of polyphenols with increasing pod storage.

Nazararuddin et al. [26] also reported that the reduction in pulp volume as a result of pod storage might facilitate the oxidation and polymerization of (–)-epicatechin and its oxidation products. Said et al. [28] also observed experimentally that there was significant reduction in the content of polyphenolic compounds, especially (–)-epicatechin in the pod storage period and degradation of the (–)-epicatechin and (+)-catechin was significant after 5-days fermentation. This suggests that pod storage could be effectively employed to reduce the polyphenol content of cocoa beans and hence reduce the astringency and bitter taste of cocoa beans.

Regression analysis of the data revealed significant ($P < 0.05$) influence of the linear and quadratic factors of fermentation time (FT) and pod storage (PS) as well as the interaction between PS and FT on the total polyphenols of the cotyledons. The model developed could explain about 96% of the variations in the total polyphenols of the cotyledons, suggesting that 4% of the variations were due to other factors not investigated in this work (Table 1).

3.2 Changes in O-diphenols during Cocoa Fermentation

Polyphenol oxidase, which is a copper-containing enzyme, catalyzes the aerobic regioselective oxidation of monophenols to *O*-diphenols followed by dehydrogenation to *O*-quinones [29]. During polyphenol oxidation, monophenols are first oxidized

Table 1 Regression coefficients and their R^2 values in the models for polyphenols, *O*-diphenols and anthocyanins of cocoa beans during fermentation.

Variables	Total polyphenols	*O*-diphenols	Anthocyanins
Constant	152.936*	22.252*	5.6314*
X_1	-29.450*	-2.565*	-1.6742*
X_2	-13.480*	-2.491*	-1.8928*
X_1^2	-15.487*	-2.652*	0.1686
X_2^2	-4.157*	-1.521*	0.1136
$X_1 \times X_2$	-5.515*	-1.377*	0.4271*
R^2	96.2%	93.0%	93.4%
R^2 (adjusted)	95.7%	92.2%	92.7%

*Significant at $P < 0.05$; X_1 = pod storage; X_2 = fermentation time.

to *O*-diphenols followed by dehydrogenation to *O*-quinones which can react in a number of different ways with the vast variety of compounds that are formed during the preceding stages and may also polymerize to form diphenols and diphenol-quinones [30].

Response surface plot (Fig. 2) showed that *O*-diphenols content in the beans decreased significantly ($P < 0.05$) with increasing fermentation time for all pod storage periods. With the exception of the unstored pods, the *O*-diphenols content of the beans recorded a continuous decrease with increasing fermentation time. It decreased from 23.65 mg g^{-1} at the start of fermentation to 18.79 mg g^{-1} at the end of fermentation (6 days) for the pods stored for 3 days. It also decreased from 22.05 mg g^{-1} to 16.72 mg g^{-1} for pods stored for 7 days and from 19.56 mg g^{-1} to 11.59 mg g^{-1} for pods stored for 10 days at the end of fermentation. The continuous reduction in the levels of *O*-diphenols in the cotyledons with fermentation might be due to the dehydrogenation of *O*-diphenols to *O*-quinones catalyzed by polyphenol oxidase [30]. However, with the unstored pods, the *O*-diphenols content increased from 21.47 mg g^{-1} at the start of fermentation to 22.38 mg g^{-1} at day 3 and then decreased to 19.70 mg g^{-1} at the end of the fermentation. This might be due to high monophenol content in the unstored pods prior to fermentation compared to the unfermented stored pods. Hence, the monophenols were first oxidized to *O*-diphenols to increase the content of the *O*-diphenols and then subsequently dehydrogenation to *O*-quinones.

Response surface plot (Fig. 2) also showed that *O*-diphenols content in the beans decreased significantly ($P < 0.05$) with increasing pod storage for all fermentation times. The *O*-diphenols content of the unfermented cocoa beans decreased from 21.47 mg g^{-1} for the unstored pods to 19.56 mg g^{-1} after 10 days of pod storage. The observed reduction in *O*-diphenols content suggests the dehydrogenation of *O*-diphenols to *O*-quinones during pod storage. The *O*-diphenol content of the unfermented cocoa beans, however, increased slightly from 21.47 mg g^{-1} (unstored pods) to 23.65 mg g^{-1} on the third day of pod storage and then decreased to 19.56 mg g^{-1} after 10 days of pod storage. This might be due to high monophenol content in the beans of freshly harvested pods prior to storage, hence, the monophenols were first oxidized to *O*-diphenols to increase the content of the *O*-diphenols and then subsequently dehydrogenation to *O*-quinones.

Regression analysis of the data also showed significant ($P < 0.05$) influence of the linear factor of fermentation time (FT) and pod storage (PS) and quadratic factor of PS and FT on the *O*-diphenols of the cotyledons. There was also significant ($P < 0.05$) influence of the interaction between PS and FT on the *O*-diphenols of the cotyledons. The model developed had an *R*-squared of 93% implying that 93% of the variations in the *O*-diphenols of the cotyledons could be explained by the model. This also suggests that 7% of the variations were due to other factors that were not investigated in this work (Table 1).

3.3 Changes in Anthocyanins of Cocoa Beans during Fermentation

Anthocyanins which are responsible for the characteristic purple color of unfermented cocoa beans [31] are located in the specialized vacuoles within the cotyledon and are hydrolyzed by glycosidase to

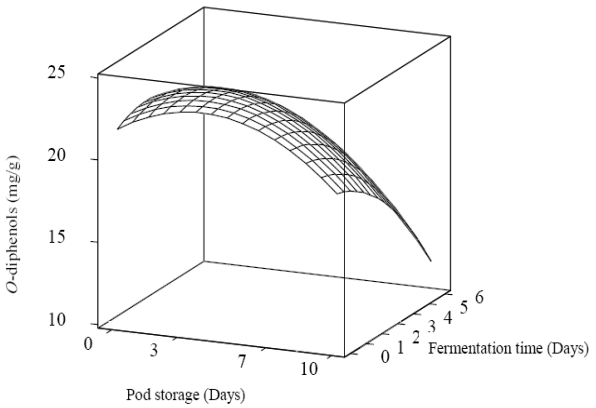

Fig. 2 Response surface plot showing effect of pod storage time and fermentation time on the *O*-diphenols of cocoa beans.

anthocyanidins during cocoa fermentation [1]. Thompson et al. [20] observed that the enzyme cleaves the sugar moieties galactose and arabinose attached to the anthocyanins. These result in the bleaching of the purple color of the beans as well as the release of reducing sugars that can participate in flavor precursor reactions during roasting. Anthocyanins usually disappear rapidly during the fermentation process.

The response surface plot (Fig. 3) showed that the anthocyanins content of the beans decreased significantly ($P < 0.05$) with increasing fermentation time for all pod storage periods. It decreased from 9.53 mg kg^{-1} at the start of fermentation to 5.35 mg kg^{-1}at the end of fermentation for the unstored pods. It also decreased from 8.95-4.07 mg kg^{-1}, 6.57-3.36 mg kg^{-1} and 5.90-3.03 mg kg^{-1} at the end of fermentation for pods stored for 3, 7 and 10 days, respectively. The observed reduction might be due to the activities of glycosidase, which hydrolyzed the anthocyanins in the cotyledons to anthocyanidins [1].

Increasing pod storage consistently reduced the anthocyanin levels at all fermentation times (Fig. 3). The anthocyanins levels of the unfermented cocoa beans decreased significantly ($P < 0.05$) from 9.53 mg kg^{-1} for the unstored pods to 5.90 mg kg^{-1} after 10 days of pod storage. The observed reduction in the anthocyanins levels during pod storage suggests possible breakdown of anthocyanins in the cotyledons to anthocyanidins during the storage period. At the end of fermentation (6 days), anthocyanins decreased by 44% for the unstored pods, 55% for pods stored for 3 days and 60%-70% for pods stored for 7 and 10 days. The breakdown of anthocyanins during pod storage and fermentation is important as this would lead to the formation of more condensation products of anthocyanin, such as cyanidin-3-β-D-galactosid and cyanidin-3-α-L arabinosid [32] and thus, change the color of the beans from slaty over purple to brown [33].

Regression analysis of the data also showed significant ($P < 0.05$) influence of the linear factor of fermentation time (FT) and pod storage (PS) on the anthocyanin content of the cotyledons. There was also significant ($P < 0.05$) influence of the interaction between PS and FT on the anthocyanin content of the cotyledons. The quadratic factor of PS and FT was however not significant ($P > 0.05$). The model developed had an R^2 of 93%. This implies that the model could explain about 93% of the variations in the anthocyanin content of the cotyledons, and that 7% of the variations were due to other factors not investigated in this work (Table 1).

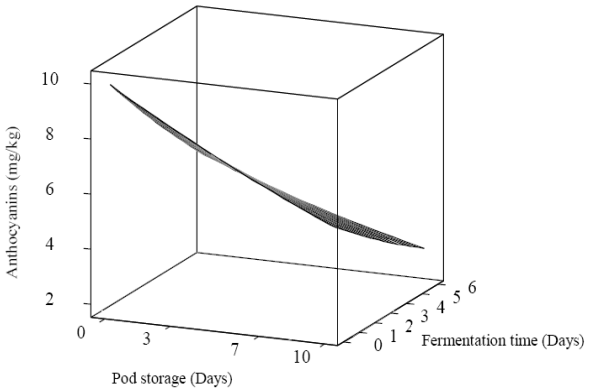

Fig. 3 Response surface plot displaying anthocyanins content of cocoa beans as affected by pod storage and fermentation.

3.4 Changes in Total Polyphenols during Drying of Fermented Cocoa Beans

The enzymatic oxidation reactions that begun during the fermentation process continue during drying and these reactions are accelerated by the increased exposure of the tissue to oxygen [30] and also the presence of sufficient moisture in the beans. However, as moisture is reduced during the drying process, all biochemical reactions eventually stop. During drying, the amount of polyphenol is substantially reduced mainly by enzymatic browning catalyzed by polyphenol oxidase and diffusion outside the beans [19, 32].

Generally, there were decreases in total polyphenols of the fermented cocoa beans during drying (Fig. 4). The polyphenols content of the beans decreased from 153.58 mg g^{-1} at the start of drying to 138.87 mg g^{-1} at the end of the drying process (7 days) for the unstored

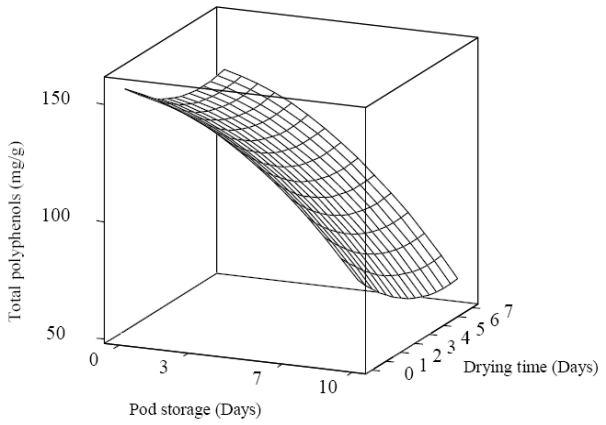

Fig. 4 Response surface plot showing changes in total polyphenols during drying of fermented pulp preconditioned cocoa beans.

pods. It also decreased from 148.77 mg g^{-1} to 123.10 mg g^{-1}, 119.43 mg g^{-1} to 95.05 mg g^{-1} and 83.48 mg g^{-1} to 59.55 mg g^{-1} for pods stored for 3, 7 and 10 days, respectively. Results then suggests that drying of cocoa beans further result in the loss of polyphenols and thus, would aid in reducing the astringency and bitter notes of cocoa beans. Several researchers have also reported decreases in total polyphenols of cocoa beans during drying [19, 34, 35]. Kim and Keeney [34] observed that during cocoa fermentation, (–)-epicatechin polymerizes with (+)-catechin to form complex tannins which then become the major substrate for enzymatic browning during drying. Dimick [35] found that during the drying stage both (–)-epicatechin and procyanidins are oxidized enzymatically and the final result of polyphenol oxidation is the production of polymeric brown pigments. During drying, with the increase in pH and high oxygen uptake, conditions become appropriate for the polyphenol oxidase resulting in phenolic oxidation.

There were drastic reductions in total polyphenols within the first 3 days of drying for all the pod storage treatments. Total polyphenols decreased by 8%, 15%, 16% and 19% by the first three days of drying for the unstored pods, pods stored for 3, 7 and 10 days, respectively. This might be due to the fact that within the first 3 days, there were sufficient amount of moisture in the beans which served to enhance the activity of polyphenol oxidase resulting in the oxidation of polyphenols. Reduction in polyphenolic content of the beans however, slowed down from the fourth day till the end of the drying process (7 days). This was because as the drying process progressed, there was sufficient reduction of moisture in the beans and this as reported by Hii et al. [35] inhibits the activity of the polyphenol oxidase. Polyphenol oxidase activity is also reported to decrease during drying because it is continuously destroyed by tanning as it oxidizes polyphenols [36]. This might have also accounted for the reduced degradation of polyphenols as the drying of the beans progressed to the latter stages. Results (Fig. 4) also showed that increase in pod storage consistently reduced the levels of total polyphenols in the beans at all drying times. Pod storage together with fermentation and drying of cocoa beans decreased the total polyphenol content of the cocoa beans.

The model developed to predict the effect of pod storage and drying on the total polyphenols of fermented cocoa beans had an R^2 of 90% (Table 2). This implies the model could explain 90% of the variations in the polyphenols content of the cotyledons whiles 10% of the variations were due to other factors that were not investigated in this work. Regression coefficients showed significant ($P < 0.05$) influence of both the linear and quadratic terms of pod storage (PS) and drying time (DT) on the total polyphenols of the cotyledons. There was, however, no significant ($P > 0.05$) interaction between PS and DT on the total polyphenols of the cotyledons (Table 2).

3.5 Changes in O-diphenols during Drying of Fermented Cocoa Beans

Response surface plot (Fig. 5) showed that *O*-diphenols content in the beans decreased significantly ($P < 0.05$) with increasing drying time for all pod storage periods. It decreased from 19.70 mg g^{-1} at the start of drying to 13.19 mg g^{-1} by the end of drying (7 days) for the unstored pods. The *O*-diphenols

Table 2 Regression coefficients and their R^2 values in the models for polyphenols, *O*-diphenols and anthocyanins of cocoa beans during drying.

Variables	Total polyphenols	*O*-diphenols	Anthocyanins
Constant	115.083*	14.6845*	2.31883*
X_1	-37.011*	-2.3564*	-0.99139*
X_2	-11.088*	-1.9931*	-0.84828*
X_1^2	-13.559*	-2.7648*	0.42128*
X_2^2	8.010*	1.6271*	0.53855*
$X_1 \times X_2$	-1.891	0.9339*	0.07410
R^2	90.1%	86.0%	86.4%
R^2 (adjusted)	88.9%	84.4%	84.8%

*Significant at $P < 0.05$; X_1 = Pod storage; X_2 = Drying time.

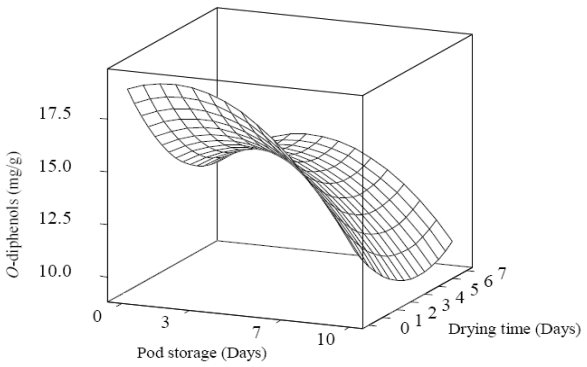

Fig. 5 Response surface plot showing effect of pod storage on *O*-diphenols content drying of fermented cocoa beans.

content also decreased from 18.79 mg g^{-1} to 15.07 mg g^{-1} for pods stored for 3 days, 16.72 mg g^{-1} to 12.78 mg g^{-1} for pods stored for 7 days and from 11.59 mg g^{-1} to 9.83 mg g^{-1} for pods stored for 10 days. The continuous reduction in the levels of *O*-diphenols in the cotyledons with drying time might be due to the dehydrogenation of *O*-diphenols to *O*-quinones catalyzed by polyphenol oxidase [30] during the drying process.

The decrease in the *O*-diphenol content was drastic during the first 3 days of drying for all pod storage periods and then slowed down towards the end of drying. There was 31% decrease in the *O*-diphenol content for the unstored pods during the first 3 days of drying and then slowed down to about 3% reduction towards the end of drying. *O*-diphenols also decreased by 16%, 18% and 9% during the first 3 days of drying for pods stored for 3, 7 and 10 days, respectively which then slowed down towards the end of drying. This might be due to the fact that within the first 3 days of drying, there was sufficient amount of moisture in the beans which served to enhance the activity of polyphenol oxidase resulting in dehydrogenation of *O*-diphenols to *O*-quinones. Towards the latter stages of the drying process, there was sufficient reduction of moisture in the beans and this as reported by Hii et al. [36] inhibits the activity of the polyphenol oxidase. This might have accounted for the reduced dehydrogenation of *O*-diphenols to *O*-quinones as the drying of the beans progressed to the latter stages.

Regression analysis of the data also showed significant ($P < 0.05$) influence of the linear and quadratic factors of pod storage (PS) and drying time (DT) on the *O*-diphenols of the cotyledons. There was also significant ($P < 0.05$) influence of the interaction between PS and DT on the *O*-diphenols of the cotyledons. The model developed could explain about 86% of the variations in the *O*-diphenols of the cotyledons, suggesting that 14% of the variations were due to other factors not investigated in this work (Table 2).

3.6 Changes in Anthocyanins Concentrations during Drying of Fermented Cocoa Beans

Anthocyanins content of the beans decreased significantly ($P < 0.05$) with increasing drying time for all pod storage periods as depicted in the response surface plot (Fig. 6). It decreased from 5.35 mg kg^{-1} at the start of drying to 3.36 mg kg^{-1} at the end of drying for the unstored pods. It also decreased from 4.07-2.42

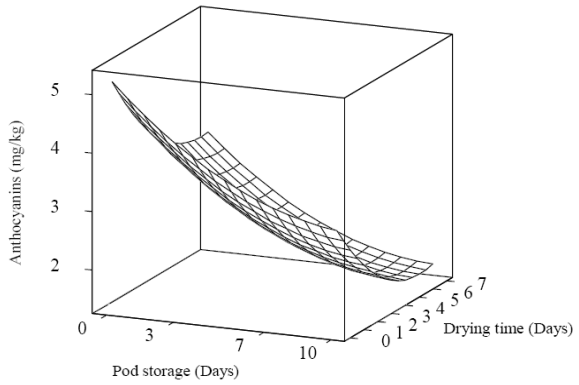

Fig. 6 Response surface plot showing effect of pod storage and drying time on the anthocyanins of cocoa beans.

mg kg^{-1} for pods stored for 3 days, 3.36-1.86 mg kg^{-1} for pods stored for 7 days and 3.03-1.37 mg kg^{-1} for pods stored for 10 days at the end of drying. Reduction in anthocyanins in the beans during drying might be due to the continuous activities of glycosidase which hydrolyzed the anthocyanins in the cotyledons to anthocyanidins [1]. Results from this study also showed that increasing pod storage consistently reduced the anthocyanins levels at all drying times (Fig. 6). The anthocyanins levels of the dried cocoa beans decreased significantly ($P < 0.05$) from 3.36 mg kg^{-1} for the unstored pods to 1.37 mg kg^{-1} for pods stored for 10 days at the end of drying.

Regression analysis revealed significant ($P < 0.05$) influence of the linear and quadratic factors of pod storage (PS) and drying time (DT) on the anthocyanins content of the cotyledons. There was no significant ($P > 0.05$) influence of the interaction between PS and DT on the anthocyanins content of the cotyledons. The model developed could explain about 86% of the variations in the anthocyanin content of the cotyledons, suggesting that 14% of the variations were due to other factors not investigated in this work (Table 2).

4. Conclusions

Total polyphenols, *O*-diphenols and anthocyanins content of freshly harvested unfermented cocoa beans were 171.54, 21.47 and 9.53 mg kg^{-1}, respectively. Pod storage, fermentation and drying caused variable reductions in total polyphenols, *O*-diphenols and anthocyanins content of the cocoa beans. However, the rates of decreases were more pronounced during fermentation than pod storage with only marginal reductions observed during drying. Storage of cocoa pods between 3-7 days with 6 and 7 days of fermentation and drying respectively retained 85%-90% of the total polyphenol and *O*-diphenols of the cocoa beans. The reductions in total polyphenols and *O*-diphenols might be due to polyphenol oxidase activities during pod storage and fermentation. Similarly, anthocyanin content of beans from the 10 days of pod storage decreased by 70% in the sixth day of fermentation. Pod storage decreased the anthocyanin content at all periods of fermentation. These suggest that the post-harvest treatments of pod storage, fermentation and drying all result in variable reductions in polyphenolic content (total polyphenols and *O*-diphenols) and anthocyanins content of cocoa beans.

References

[1] E.O. Afoakwa, Chocolate Science and Technology, Wiley-Blackwell Publishers, Oxford, UK, 2010, pp. 3-82.

[2] J. Wollgast, E. Anklam, Polyphenols in *Theobroma cacao*: Changes in composition during the manufacture of chocolate and methodology for identification and quantification: A review, Food Res Int. 33 (2000) 423-447.

[3] J.S. Bonvehi, F.V. Coll, Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder, Food Chem. 60 (1997) 365-370.

[4] J.S. Bonvehi, F.V. Coll, Evaluation of purine alkaloids and diketopiperazines contents in processed cocoa powder, Euro. Food Res. Technol. 210 (2000) 189-195.

[5] F. Luna, D. Crouzillat, L. Cirou, P. Bucheli, Chemical composition and flavour of Ecuadorian cocoa liquor, J. Agric. Food Chem. 50 (2002) 3527-3532.

[6] J.P. Mcmanus, K.G. Davis, J.E. Beart, S.H. Gaffney, T.H. Lilley, E. Haslam, Polyphenol interactions, Part 1. Introduction: Some observations on the reversible complexation of polyphenols with proteins and polysaccharides, J. Chem. Soc. Perkin Trans 2 (9) (1985) 1429-1438.

[7] C.M. Spencer, Y. Cai, R. Martin, Polyphenol complexation: Some thoughts and observations, Phytochem 27 (1988) 2397-2409.

[8] K.J. Siebert, N.V. Troukhanova, P.Y. Lynn, Nature of polyphenol-protein interactions, J. Agric. Food Chem. 44

(1996) 80-85.

[9] C. Santos-Buelga, A. Scalbert, Proanthocyanidins and tannin-like compounds: Nature, occurrence, dietary intake and effects on nutrition and health, J. Sci. Food Agric. 80 (2000) 1094-1117.

[10] L.J. Romanczyk, J.F. Hammerstone, M.M. Buck, L.S. Post, G.G. Cipolla, C.A. Micceland, et al., Cocoa extract compounds and methods for making and using the same. Patent Cooperation Treaty (PCT), WO 97/36497, 1997.

[11] B. Biehl, G. Ziegleder, Cocoa: Chemistry of processing, in: Encyclopedia of Food Sciences and Nutrition, 2nd ed., Elsevier Science Ltd, 2003, pp. 1436-1448.

[12] E.O. Afoakwa, J. Quao, J. Takrama, A.S. Budu, F.K. Saalia, Changes in total polyphenols, *O*-diphenols and anthocyanin concentrations during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans, Int. Food Res. J. 19 (3) (2012) 1071-1077.

[13] R. Nazaruddin, M.Y. Ayub, S. Mamot, C.H. Heng, HPLC determination of methylxanthines and polyphenols levels in cocoa and chocolate products, Malays J. Anal. Sci. 7 (2001) 377-386.

[14] E.S. de Brito, N.H.P. García, A.C. Amâncio, Effect of polyphenol oxidase (PPO) and air treatments on total phenol and tannin content of cocoa nibs Ciênc, Tecnol. Alimen, Campinas 22 (1) (2002) 45-48.

[15] B. Bartolomé, L.M. Jiménez-Ramsey, L.G. Butler, Nature of the condensed tannins present in the dietary fiber fractions in foods, Food Chem. 53 (4) (1995) 357-362.

[16] L.J. Porter, Z. Ma, B.G. Chan, Cacao procyanidins: Major flavanoids and identification of some minor metabolites, Phytochem. 30 (5) (1991) 1657-1663.

[17] C.E. Hansen, M. del Olmo, C. Burri, Enzyme activities in cocoa beans during fermentation, J. Sci. Food Agric. 77 (1998) 273-281.

[18] K.P. Prabhakaran Nair, Cocoa (*Theoboma cacao* L.), the agronomy and economy of important tree crops of the developing world, Elsevier Inc., 2010, pp. 131-180.

[19] T.M. Kyi, W.R.W. Daud, A.B. Mohammad, M.W. Samsudin, A.A.H. Kadhum, M.Z.M. Talib, The kinetics of polyphenol degradation during the drying of Malaysian cocoa beans, Int. J. Food Sci. Technol. 40 (2005) 323-331.

[20] S.S. Thompson, K.B. Miller, A.S. Lopez, Cocoa and coffee, in: Food Microbiology Fundamentals and Frontiers, ASM Press, Washington DC, 2001, pp. 721-733.

[21] M.M. Sanagi, W.P. Hung, S.M. Yasir, Supercritical fluid extraction of pyrazines in roasted cocoa beans: Effect of pod storage period, J. Chromatogr. A 785 (1997) 361-367.

[22] A. Othman, A. Ismail, N.A. Ghani, I. Adenan, Antioxidant capacity and phenolic content of cocoa beans, Food Chem. 100 (4) (2007) 1523-1530.

[23] Official Methods of Analysis, Association of Official Analytical Chemists, 18th ed., Washington DC, 2005.

[24] S. Misnawi, S. Jinap, B. Jamilah, S. Nazamid, Oxidation of polyphenols in unfermented and partly fermented cocoa beans by cocoa polyphenol oxidase and tyrosinase, J. Sci. Food Agric. 82 (2002) 559-566.

[25] P.E. Aikpokpodion, L.N. Dongo, Effects of fermentation intensity on polyphenols and antioxidant capacity of cocoa beans, Int. J. Sustain. Crop Prod. 5 (4) (2010) 66-70.

[26] R. Nazaruddin, L. Seng, O. Hassan, M. Said, Effect of pulp preconditioning on the content of polyphenols in cocoa beans (*Theobroma cacao*) during fermentation, Indus. Crops Prod 24 (2006) 87-94.

[27] E.S. de Brito, N.H.P. Garcia, M.I. Gallão, A.L. Cortelazzo, P.S. Fevereiro, M.R. Braga, Structural and chemical changes in cocoa (*Theobroma cacao L.*) during fermentation, drying and roasting, J. Sci. Food Agric. 81 (2000) 281-288.

[28] M.B. Said, M.J. Musa, B. Biehl, in: Proceedings of the 10th International Conference on Cocoa on an Integrated Approach towards Quality Improvement of Malaysian Cocoa Beans, 1988, pp. 767-773.

[29] H. Yağar, A. Sağiroğlu, Partially purification and characterization of polyphenol oxidase of quince, Turk. J. Chem. 26 (2002) 97-103.

[30] A.S. Lopez, P.S. Dimick, Cocoa fermentation, in: Biotechnology: A Comprehensive Treatise, Enzymes, Biomass, Food and Feed, 2nd ed., VCH, Weinheim, Vol. 9, 1995, pp. 563-577.

[31] G. Ziegleder, Flavour development in cocoa and chocolate, in: Industrial Chocolate Manufacture and Use, 4th ed., Blackwell Publishing Ltd., 2009, pp. 169-174.

[32] H. Kim, P.G. Keeney, Epicatechin content in fermented and unfermented cocoa beans, J. Food Sci. 49 (1984) 1090-1092.

[33] K.S. Kealey, R.M. Snyder, L.J. Romanczyk, H.M. Geyer, M.E. Myers, E.J. Withcare, et al., Cocoa components, edible products having enhanced polyphenol content, methods of making same and medical uses, Patent Cooperation Treaty (PCT), WO 98/09533, 1998.

[34] H. Kim, P.G. Keeney, Method of analysis for (–)-epicatechin in cocoa beans by high performance liquid chromatography, J. Food Sci. 48 (1983) 548-551.

[35] P.S. Dimick, Pennsylvania State chocolate manufacture short course, University Park, PA: Pennsylvania State University, USA, 1993, pp. 29-465.

[36] C.L. Hii, C.L. Law, S. Suzannah, S. Misnawi, M. Cloke, Polyphenols in cocoa (*Theobroma cacao* L.), As. J. Food Ag-Ind 2 (4) (2009) 702-722.