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Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in *Forastero* cocoa beans

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ABSTRACT

The practice of pod storage (PS) has been applied in many cocoa producing countries, especially by Ghanaian farmers over the years. However, the study of PS has not received much attention, hence, until now, its potential impact on specific flavor precursor development and implications on the flavor of cocoa beans still remains uncovered. The study was therefore aimed at exploring this possibility through physico-chemical and flavor precursor analyses, carried out on equally fermented and dried pod stored (0, 3 and 7 days) Ghanaian cocoa beans. Flavor analysis was also conducted on equally roasted pod stored cocoa beans. Through visual assessment of the pods, pulp and beans, the compelling impact of PS on fermentation index (FI) and nib acidity could be demonstrated by the various biochemical and physical changes such as respiration, moisture reduction, and cellular degradation, occurring during the process. Whereas the entire reaction of sugar degradation may be deemed complex, a clear relationship between the FI, nib acidity and the glucose content was observed. Also, PS was found to increase with marginal increase in total reducing sugars (glucose and fructose). Although the concentration of free amino acids was directly proportional to the duration of PS, within the framework of this study, a significant difference ($p < .05$) was only observed in the case of extended duration (7 days). Overall, 7 PS seemed to have enhanced the formation of more volatiles. This was followed by 0 PS and finally 3 PS. Suggestively, these findings could provide some indications in explaining the typical flavor profiles of the Ghanaian cocoa beans, considering the fact that 87.8% of Ghanaian farmers adhere to this practice. Meanwhile, for the chocolate industry, the surging demand for cocoa/chocolate products exhibiting unique flavors, could be partly addressed by adopting PS as a tool for varietizing the flavor capacity of "bulk" cocoa through the expression or suppression of specific flavor precursors in the raw material on the farm level, which comes with almost no additional cost.

1. Introduction

The aroma potential of cocoa (*Theobroma cacao* L.) is highly essential for flavor development in chocolates and other derived products. Among others, key influential factors such as the origin, genotype, fermentation, drying, roasting and conching have been greatly investigated and linked to the flavor of the final product (Aculey et al., 2010; Afoakwa, 2010; Afoakwa, Paterson, Fowler, & Ryan, 2008; Aprotosoaie, Luca, & Miron, 2015; Beckett, 2009; Kongor et al., 2016). Of these, factors such as the origin and genotype may clearly differentiate cocoa based on the proportions of various inherent storage molecules (proteins, carbohydrates, phenolic substances), the further

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degradation of which, yield various flavor precursors. However, the actual flavor precursor generation has often been attributed to the complex biochemical reactions initiated following the harvesting of mature ripe pods, until the end of fermentation (Afoakwa, 2014). As a result, the fermentation of cocoa beans has remained a central focus for several studies (Amin, Jinap, & Jamilah, 1998; Beckett, 2009; Camu et al., 2008; Lima, Almeida, Nout, & Zwietering, 2011). Among others, the result of the fermentation process includes; reducing sugars and amino acids, which are prerequisites for flavor volatile formation, in the Maillard reaction, during subsequent roasting. This is a complex chemical reaction which is initiated following an attack by the free amino group of the amino acids on the reactive carbonyl groups of reducing sugars such as glucose and fructose. Here, the end product of the first phase; Amadori compounds, are necessary for the formation of 3-deoxyhexuloses and 2,3-enediol with dehydroreductone intermediates under acidic and basic/neutral conditions respectively. The latter is particularly important for the formation of the α-dicarbonyl compounds from which all kinds of volatiles such as aldehydes, ketones, pyrazines, pyrroles and pyridines may emerge (Afoakwa et al., 2008; Aprotosoaie et al., 2015; Kongor et al., 2016).

In spite of the present knowledge in these post-harvest processes and their various contributions toward flavor improvement in cocoa beans, the need for further studies into other possibilities still remains crucial. Since a freshly harvested pod remains a living system, metabolic processes which are predominant, prior to harvesting, may still continue to persist afterwards (Aroyeun, Ogunbayo, & Olaiya, 2006; Wills, McGlasson, Graham, & Joyce, 1998). For this reason, the possible occurrence of various biochemical changes within the pod - from the time of harvest until their opening ahead of fermentation - cannot be overlooked. Suggestively, the duration of storage, temperature, relative humidity, variety of cocoa, age of tree/pods, state of maturation or ripening, among others, may play key roles in determining the extent to which these biochemical changes occur within the pod (Afoakwa, 2014; Meyer, Biehl, Said, & Samarakoddy, 1989; Sulaiman, Yang, & Ariffin, 2017). This practice of keeping harvested pods for a specified duration of time under specific conditions preceding their opening is referred to as pod storage (PS).

Although the said biochemical changes may prove to be beneficial, they must be curtailed to prevent the early onset of senescence (Aroyeun et al., 2006; Wills et al., 1998). Unfortunately, research into the implications of cocoa PS has not been equivocally established in spite of preliminary indications existing as far back as the 80's (Biehl et al., 1989; Meyer et al., 1989; Said, Meyer, & Biehl, 1987). The few existing studies, however, have attributed this phenomenon to various physical and biochemical changes including; water evaporation, the inversion of sucrose, changes in acidity as well as the modification of phenolic substances in cocoa beans (Afoakwa, 2014; Afoakwa, Kongor, Takrama, & Budu, 2013b; Afoakwa, Kongor, Takrama, Budu, & Mensah-Brown, 2013; Afoakwa, Quao, Takrama, Budu, & Saalia, 2011; Biehl et al., 1989; Meyer et al., 1989). Notably, interpretations of these findings were not independent of the aforementioned factors influencing the mechanism of PS.

Arguably, this idea of PS first emerged as intervention to curbing the problem of acidity identified in dried fermented Malaysian cocoa beans. However, Duncan (1984), highlighted that Ghanaian farmers adopted the technique of PS as a means of using family labor to gather enough pods into piles before organizing friends and neighbors to assist in opening them. To the best of our knowledge, the potential impact of PS on either the expression or suppression of flavor precursors is still shrouded in mystery, in spite of previous fundamental studies by Afoakwa et al. (2011), Afoakwa, Kongor, Takrama, and Budu (2013b), Afoakwa, Kongor, Takrama, Budu, and Mensah-Brown (2013). Through proximal analyses, they demonstrated that both fermentation and increasing PS resulted in a significant decrease in ash, crude protein, fat and total polyphenol content of the beans while carbohydrate content increased with both treatments. Additionally, increasing PS and fermentation significantly increased the copper content of the beans whereas reductions in magnesium and potassium occurred. In dealing with Ghanaian cocoa beans, they highlighted that storage of cocoa pods between 3 and 7 days with 6 days of fermentation led to appreciable reductions in nib acidification, sugars (non-reducing and total sugars) and proteins with consequent increases in reducing sugars and acceptable free fatty acid levels. This study therefore explored the possibilities of PS on a much deeper level through chromatographic analyses with specific focus on sugar and free amino acid profiles of pod stored cocoa beans and the possible consequences on some Maillard reaction related volatiles in the beans.

2. Materials and methods

2.1. Field survey.

This Section was part of a field study aimed at investigating the determinants of cocoa productivity and profitability by smallholder farmers in Ghana (Kongor et al., 2017). This study included the assessment of the practice of PS among cocoa farmers in Ghana. A four-stage sampling approach was used to select a representative number of cocoa farmers as comprehensively described in the report of Kongor et al. (2017). From this, a total of 731 farmers covering the six major cocoa growing regions in Ghana (Brong Ahafo = 158, Western = 110, Eastern = 126, Ashanti = 115, Central = 112, and Volta region = 110) were interviewed. Among other questions relating to demographic characteristics, farm characteristics and farm management practices (data not shown), farmers were also asked whether (or not) they practiced PS. For those who did, the number of days of this practice was also inquired.

2.1. Harvesting, pod storage and visual assessment

Ripe cocoa pods were harvested during the September–October peak season from a cocoa farm in Jachere, Ghana (N7.088525, W2.110127833333333). This was representative of a cluster of farms demonstrating high potential for quality cocoa beans following an earlier survey in this region (Kongor et al., 2017). Cocoa trees were approximately 31 years old. The pods were stored in small heaps (ca 80–100 pods) on the farm for three different storage times (0, 3, and 7 days), at ambient temperature (28–30 °C) and relative humidity of 77–85%. At the end of each duration of PS, the state of the pods, husks, pulp and beans were examined visually and with digitally captured images (Sony cyber-shot, Minato, Tokyo, Japan) in order to ascertain the impact of PS on their physical appearance. Here, pictures were taken before and after opening the pods, as well as, the extracted beans with surrounding pulp prior to their fermentation.

2.2. Fermentation, drying and sample preparation

After the visual assessment and pod opening, beans were gathered in heaps (ca 45 kg) according to their respective PS durations and covered with fresh banana leaves to begin the spontaneous heap fermentation process. This occurred for six days with two turnings at 48 h intervals as recommended for local farmers by the Cocoa Research Institute of Ghana (CRIG). After this, the fermented cocoa beans were sun-dried on raised platforms until the required moisture content of < 7% was achieved, as described by Afoakwa (2010). The dried beans were then stored in jute bags of 64 kg gross weight. They were then air-freighted to the Faculty of Bioscience Engineering, Ghent University, Belgium. Hereto, the bags were stored in a cool well-ventilated room on odorless wooden racks. Random sampling of the beans was done by mixing beans from the top, middle and bottom sections of the jute bags. The sampled beans were manually deshelled of which half was ground into very fine powder and the other half milled into liquor prior to analyses. For the powder, a Moulinex grinder (AF11, China) was used, whereas the ECGC-12SLTA melanger (CocoaTown,

USA) was used to make the liquors. All analyses were done in triplicate.

2.3. Reagents and chemicals

DL-Norvaline (99%), L-cysteine (99.5%), L-4-hydroxyproline (99%), DL-valine (99%), DL-alanine (99%), L-tryptophan (99.5%), L-citrulline (99%), sarcosine (99%), DL-histidine (99%), Lisoleucine (99.5%), DL-leucine (99%), and glutamine (99,5%) were purchased from Fluka (Sigma − Aldrich, Bornem, Belgium). DL-Lysine monohydrochloride (98%), DL-methionine (99%), glycine (99.5%), L-arginine hydrochloride (99%), L-phenylalanine (99%), L-glutamic acid (99%). L-Lysine (97%), arginine (98%), proline (99%), asparagine monohydrate (99%) and trichloroacetic acid (99%) were purchased from Acros Organics, Thermo-Fisher Scientific (Erembodegem, Belgium). Aspartic acid (99%), DL-threonine (99%), and L-tyrosine (99%) were purchased from Merck (Darmstadt, Germany). DL-Serine were purchased from Janssen Chimica (Geel, Belgium).

For the gas chromatographic profiling of the sugars, the following aqueous solutions were prepared: Carrez I [14% $K_4Fe(CN)_6$, Merck, Germany] and Carrez II (30% $ZnSO_4$, Chem-lab, Belgium). Furthermore, hexamethyldisilazane, trifluoroacetic acid from Chem-lab (Belgium) and an oximation reagent; 2.5 g of hydroxylaminehydrochloride (UCB, Belgium) in 100 mL of dry pyridine (Merck, Germany). Others such as sodium hydroxide and hydrochloric acid were purchased from Chem-Lab (Zedelgem, Belgium). Methanol was purchased from Fischer Scientific (Aalst, Belgium).

2.4. Bean cut test and fermentation index (FI)

The bean cut test was performed as described by Afoakwa (2010). A batch of 50 cocoa beans were cut lengthwise to expose the maximum cut-surface of the cotyledons using a cocoa guillotine (MAGRA). Both halves were examined under full daylight and categorized as slaty, deep purple, pale purple or brown. Defects assessed include flat, mouldy and germinated beans. The procedure was repeated thrice following independent sampling and their means and standard deviations recorded. FI was determined spectrophotometrically by extracting cocoa color pigments from ground nibs (0.5 g) with 50 mL methanol: HCl solution in the ratio of 97:3 as described by Gourieva and Tserevitinov (1979). The mixture was homogenized for one minute using ultra-turrax, refrigerated at 8 °C for 19 h and then vacuum filtered. The filtrate volume was adjusted to 50 mL with the methanol: HCl solution, after which the absorbance at 460 nm and 530 nm were measured using a UV–visible spectrophotometer (Cary Bio 50). The FI was calculated as a ratio of the absorbance at 460 nm to that at 530 nm.

2.5. Moisture content

Moisture content was determined gravimetrically according to the AOAC (2005) method 931.04. 5 g of finely ground cocoa nibs was mixed with ashed sea sand in an aluminum weighing pan. The moisture content was calculated based on weight loss after drying the samples in a 105 °C air oven for 4 h. Before weighing the dried sample, it was cooled down in a desiccator for 60 min, to avoid the risk of moisture re-absorption from the environment.

2.6. Nib acidity

The method stated by Afoakwa, Kongor, Takrama, and Budu (2013a) was used with slight modification. Finely ground cocoa nibs (10 g) were homogenized in 90 mL hot boiling deionized water. The homogenate was filtered with a No. 2 Whatman filter paper and cooled to 20–25 °C. The pH of the emerging filtrate was measured using a pH meter (Schott Instruments, LAB 850). Thereafter, a 50 mL aliquot was titrated to an end point pH of 8.1 with 0.1 N NaOH. The titratable acidity was expressed as milli equivalent of NaOH per g of sample.

2.7. Sugar profile

Mono- and disaccharides were determined by gas chromatography (GC) as described by De Wilde et al. (2005). An internal standard was prepared: 6 mg/mL phenyl-β-D-glucopyranoside (Sigma-Aldrich, USA) in distilled water. 10 g of sample was added to a 100 mL volumetric flask and mixed with 10 mL internal standard and 50 mL distilled water. The flask was incubated in a warm water bath (60 °C) for 30 min. Subsequently, 5 mL Carrez I and 5 mL Carrez II were added, as a cleanup step. The volumetric flask was filled with distilled water and swirled. The solution was filtered over a filter paper (130 mm, Novolab, Belgium), then 0.5 mL of this solution was pipetted into a 2 mL vial and evaporated under nitrogen.

The residue was derivatized in two steps, first an oximation with 500 µL of oximation reagent (30 min at 60 °C), second to trimethylsilylesters with 500 µL of hexamethyldisilizane and 50 µL of trifluoraceticacid (10 min at 20 °C). Thereafter, the solution was allowed to sediment for 2 h after which the supernatant was decanted into 2 mL vials.

Sugar profile analysis was performed by means of GC FID (Varian 3380, Varian Instrument Group, Walnut Creek, CA) equipped with a column containing (5%-phenyl)-methylpolysiloxane (0.25 µm film thickness) as stationary phase (30 m × 0.32 mm i.d., VF1-ms, Agilent Technologies, Palo Alto, CA, USA). Helium was used as carrier gas (flow = 1 mL/min). 1 µL of sample was injected using 1/40 split and the injector temperature was 250 °C. The temperature program of the column was as follows: 180 °C for 1 min, ramp at 15 °C per min to 290 °C. The conditions of the FID were as follows: temperature = 340 °C, operated with hydrogen and air at 30 and 300 mL/min, respectively, as well as helium at 20 mL/min as makeup gas. Since various sugars respond differently to ionization, a standard curve was established for each cocoa sugar relative to the internal standard. Additionally, a vial of standard sugar containing a mixture of known sugars (fructose, glucose, sucrose, lactose and maltose) with known concentrations was prepared and analyzed under the same procedure. From these, identification of the different cocoa sugars in the samples was done by comparing their retention times (RT) to those of the known sugars. Finally, concentrations of the different sugars could be recalculated from the individual response factors (RF) determined in reference to the respective sugars identified from the standard sugar.

2.8. Free amino acid profile

2.8.1. Defatting of cocoa nibs

Cocoa nibs were ground into liquor using the ECGC-12SLTA melanger (CocoaTown, USA) during a period of 40 min at room temperature. To every 1 g of the cocoa liquor, 25 mL petroleum ether was added. It was then homogenized with an ultra-turrax and subsequently centrifuged (9000 *g* for 10 min). Afterwards, the layer of petroleum ether was decanted and the process repeated twice. The defatted residue (cocoa powder) was then air-dried and stored at 4 °C.

2.8.2. Analysis

Samples of defatted cocoa were treated with an aqueous solution of 15% trichloroacetic acid, further centrifuged, cooled to 4.0 °C during 15 min and then centrifuged at 11750 *g* for 10 min in a Spectrafuge 16 M centrifuge (Labnet International, NJ, USA). Additionally, the samples were filtered on a Millex LCR 0.45 µm low protein binding filter (Merck Millipore LTD, Cork, Ireland), and analyzed by HPLC with fluorescence detection following the methods described by Schuster (1988) and Henderson, Ricker, Bidlingmeyer, and Woodward (2000) and Mestdagh, Kerkaert, Cucu, and De Meulanaer (2011). Further, the free amino acids were separated with a reversed phase HPLC apparatus after they had been online converted to ortho-phthalaldehyde (OPA) derivatives in an Agilent 1100 autosampler (Agilent Technologies,

Table 1
Survey report showing typical practice of PS among Ghanaian farmers in the six major cocoa growing regions in Ghana. Results have been indicated as percentage of farmers.

Pod storage	Brong-Ahafo [n = 158]	Western [n = 110]	Eastern [n = 126]	Ashanti [n = 115]	Central [n = 112]	Volta [n = 110]	Total [n = 731]
Yes	84.2	100.0	81.0	93.9	92.9	77.3	87.8
No	15.8	0.0	19.1	6.1	7.1	22.7	12.2
No. of days of PS							
1–3	27.8	36.4	53.9	46.3	63.5	71.8	48.1
4–7	66.9	59.1	46.1	51.9	36.5	27.1	49.5
> 7	5.3	4.6	0.0	1.9	0.0	1.2	2.3

Diegem, Belgium). Agilent ZORBAX Eclipse Plus C18 column was used for separation with precolumn. Standard solutions for calibration contained 10, 20, 40, 80, 200, 400 and 800 pmol/μL of each amino acid plus a blank containing only 50 μL internal standard solution, norvaline and sarcosine, with a concentration of 10 nmol/μL.

2.9. Aroma profiling of roasted pod stored cocoa beans

Prior to analysis, each batch of pod stored beans (1 kg) was roasted in the same manner at 135 °C for 35 min in a conventional oven (Termaks, Lien 79, N-5057 Bergen, Norway). Afterwards, each batch was allowed cool down at room temperature. They were then manually deshelled and ground into liquor using the ECGC-12SLTA melanger (CocoaTown, USA) as stated above.

The aroma analysis was carried out using Headspace-solid phase micro extraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS) according to the method described by Tran et al. (2015) with slight modifications. The isolation of the volatiles released was performed in a multi-purpose sampler (Gerstel, Mülheim an der Rur, Germany) equipped with an HS-SPME unit as follows: 2 g of the cocoa liquor was mixed with 2 μL internal standard 1-octen-3-ol at a concentration of 0.396 mg/mL MeOH in hermetically sealed 20 mL vials and incubated for 10 min at 60 °C in a thermostatic agitator. Next, a well-conditioned 50/30 μm CAR/DVB/PDMS SPME fibre (Supelco, Sigma–Aldrich N.V., Bornem, Belgium) was exposed to the headspace for 25 min at 60 °C.

The volatiles were then analyzed by GC–MS, using splitless injection, helium as a carrier gas (1 mL/min), and a Zebron 7 HG-G007–11 ZB-WAX column of 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness (Zebron, Phenomenex, Macclesfield, UK). The following time-temperature program was applied: 35 °C (5 min), from 35 °C to 182 °C (4 °C/min) and from 182 °C to 240 °C (7 °C/min). Injector and transfer lines were maintained at 250 °C and 280 °C, respectively. The total ion current (70 eV) was recorded over the mass range from m/z 40 to 230 (scan mode) using no solvent delay and a threshold of 50.

Identification of volatile organic compounds (VOC's) in the headspace was performed by comparing the MS-spectrum of each peak to those from the Wiley 275 library. However, another verification was carried out by determination of Kovat indices (KI's) after injection of a series of n-alkane homologues (C5-C13). From these, the experimental Kovat indices; KI (exp), of the confirmed aroma compounds were calculated based on their respective retention times and compared with Kovat indices from literature; KI (lit).

The semi-quantitative concentrations of the identified volatile compounds were expressed as nanograms of the internal standard equivalents per gram of cocoa liquor and calculated as the area of the compound of interest divided by the response factor of the internal standard. For each liquor, isolation, separations, identifications and quantifications of the VOCs were performed on three independent samples.

Finally, in order to evaluate a volatile's contribution to the overall flavor profile of the cocoa liquor, odor activity values (OAV's) were calculated using odor threshold values (OTV's) documented in literature. Here, the OTV in oil media were used since cocoa liquor is a fat continuous dispersion (ca 55% fat). These values were sourced from Van Gemert (2011). From these, OAV's were calculated by dividing the detected headspace concentration by their respective OTV's. Hitherto, a VOC with OAV ≥ 1 may be considered as an odor-active volatile and *vice versa*. It is worth mentioning that depending on its OTV, a volatile with a relatively higher headspace concentration may not necessarily contribute to the overall flavor profile of a particular liquor. Moreover, as can be seen from Table 6, not all OTV references were found. Hence, not all potentially odor-active VOC's could be elucidated. Thus, even though the calculated OAV's may provide some insight, they must be interpreted with caution.

2.10. Statistical analysis

Statistical analysis was performed with Minitab 18 (Minitab Inc., USA). A one-way ANOVA ($\alpha = 0.05$) was used to test for significant effects of PS on the various parameters under study. Assumptions of normality and equality of variance were tested prior to the analysis using Kolmogorov-Smirnov test and Modified Levene's test, respectively. Where assumptions were fulfilled, a post-hoc Tukey's test was used to investigate significant differences between the different levels of the predictors. However, when assumption was not fulfilled, non-parametric alternatives namely Welch was used along with Games Howell post-hoc test. Thereafter, principal component analysis (PCA) was used to study the relationships between the samples in terms of their flavor profiles using XLSTAT 2014.5.03 (Addinsoft, USA).

3. Results and discussion

3.1. Field survey

Results from the survey are summarized in Table 1. As can be seen, on a national level, 87.8% of farmers stored their pods prior to fermentation. Only 12.2% of farmers however, split their pods and fermented their beans on the same day of harvest. Of those who stored their pods, 48.1% stored their pods for up to three days whereas 49.5% did for 4–7 days. Differences were seen among regions. For instance, in the Eastern, Central and Volta regions, harvested cocoa pods were mostly stored between 1 and 3 days, whereas, in Brong-Ahafo, Western and Ashanti regions, pods could be stored up to seven days. On a whole, only 2.3% of farmers stored their pods for an extended duration beyond 7 days. The findings from this survey clearly revealed the popularity and practice of PS among farmers from all growing regions across the country. For many years, Ghana has maintained her reputation in the cocoa sector, both in terms of production capacity and quality. Notably, her cocoa has served as the benchmark for quality *Forastero* worldwide (Afoakwa, 2016). For these reasons, an exposition of how the practice of PS may affect the flavor potential of these cocoa beans will not only be relevant in explaining the typical flavor profiles of the Ghanaian cocoa, specifically from this region under study, but will also set the pace for further studies in these aspects.

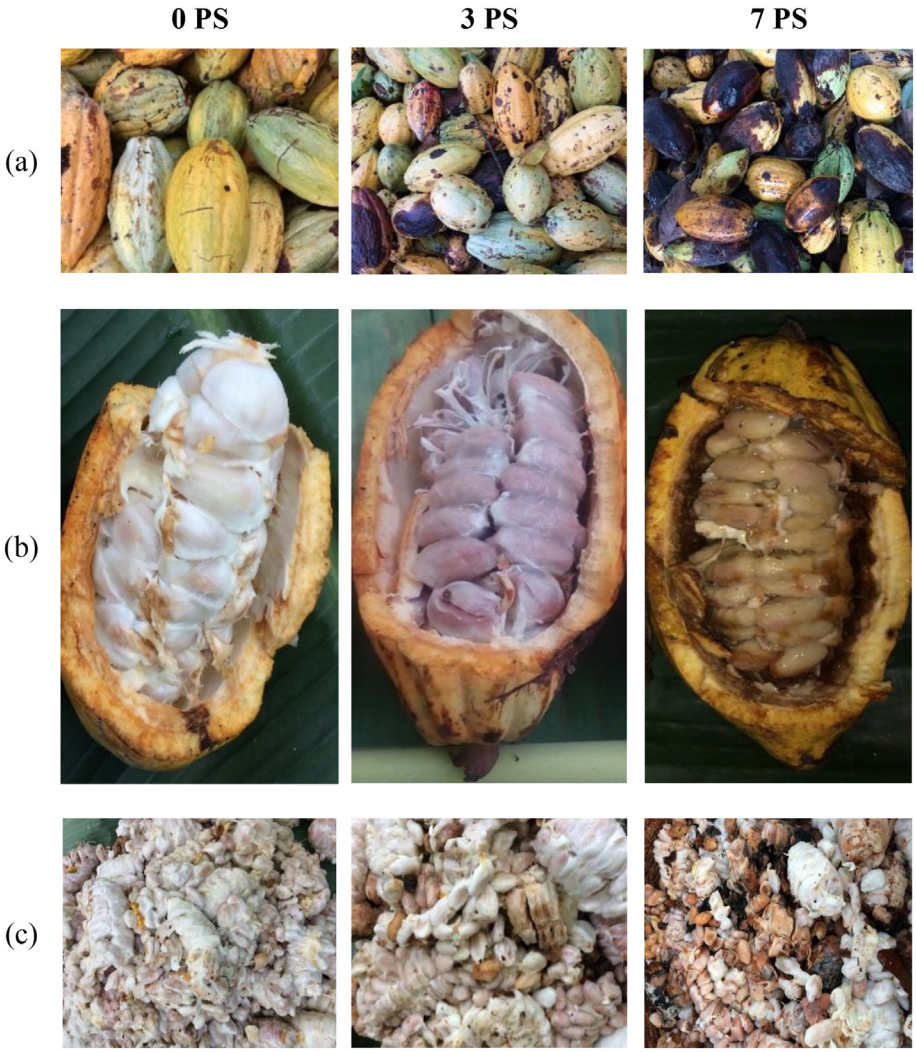

Fig. 1. Physical appearance of (a) stored cocoa pods, (b) opened pods exposing pulp and beans, and (c) extracted beans engulfed with pulp prior to fermentation.

3.2. Visual assessment

In order to understand the various physical and biochemical changes occurring during the process, a visual assessment was first carried out on the stored pods, husks, pulp and beans (Fig. 1).

3.2.1. Examination of pod and husk

First and foremost, an external examination of the stored pods (Fig. 1a) revealed the natural state of deterioration (senescence) of cellular components of the pod husks. From these, we observe an increasing trend of deterioration with PS duration. It can be suggested that the natural tendency for a biological decomposition of cells along with microbial spoilage of the pod material could be responsible for the observed change in color from bright yellow/orange in 0 PS to dark and spotted in 3 PS, and in many cases, even black colored pods, as was dominant among the samples of 7 PS. As described earlier, typically in Ghana, the practice of PS involves gathering of the freshly harvest pods and piling them up in heaps for a specified period of time. During this period, the pods may be subjected to various microbial attack from the environment, resulting in the observed loss of freshness, deterioration and consequent (dis)coloration of the pods. It can be suspected that the role of certain microorganisms may be key over others in promoting the observed degradation just like in the case of spontaneous fermentation. However, attributing specific biochemical changes to specific microorganisms at this stage may require further studies.

3.2.2. Examination of pulp and beans

A visual examination of the pulp affirmed decreasing pulp volume with increasing PS (Fig. 1b).Suggestively, the loss of moisture to the surrounding along with respiration of the inherent sugars in the pulp could have accounted for this trend. Of the known constituents of the cocoa pulp, water and sugars have been identified as the most abundant, ranging between 82 and 87% and 10–15%, respectively (Afoakwa, 2016). Thus, given the specific PS conditions, it is possible to lose copious amount of water from the moisture-rich pulp to the surrounding. Likewise, the sugar, present in the pulp could serve as an ideal substrate for energy production (through respiration) for the still-living cells. Wills et al. (1998) and Aroyeun et al. (2006) noted that even after harvesting, the cocoa pod still remains a living system for a period of time, during which, respiration persists in order to maintain energy supply for the constituent cells. Results of Biehl et al. (1989) also attested to some biochemical changes in both pod husk and pulp during PS. However, they observed that the extent of these biochemical changes were significantly higher in the pulp than in the pod husk. From Fig. 1b, a careful examination of both pod husks and pulp provided some indication in support of this earlier observation by Biehl et al. (1989). It is not a wonder, therefore, that the remaining pulp surrounding the beans of 3 PS looked somewhat dry and greatly minimized as compared to that of 0 PS. Comparatively, the study of Biehl et al. (1989) observed a drastic reduction (ca 40–50%) in the amount of moisture and dry matter per seed. To this, they also

attributed the effect of moisture evaporation and respiration of the inherent sugars (mainly the inversion of sucrose) in the pulp.

In addition to various environmental factors, the initial quantity/volume of pulp surrounding the beans (often depending on the harvesting season), state of ripeness of the pods (determining the amount of fermentable sugars), as well as the cultivar of the cocoa (determining the concentration and composition of bean and pulp constituents), may suggestively act as factors influencing the dynamics of PS during the process.. Importantly, the age of the pods, which also determines the specific quantities of fermentable sugars (Pakiyasothy, Jansz, Senanayake, Wijesundara, & Wickremasinghe, 1981; Pettipher, 1986) available as substrate for metabolism in the pulp, and possibly, the concentration and characteristics of pectin and other complex cell wall polysaccharides may ultimately determine the cellular stability and consistency of the pulp during this process. In this study, a slight modification in pulp volume (due to moisture evaporation and respiration of sugars) was observed between 0 PS and 3 PS. However, the difference between 3 PS and 7 PS is clear. Hitherto, an additional degradation of both pod husk and pulp were observed. Suggestive of an onset of a 'fermentation-like' process, the less attached beans in 7 PS predominantly appeared darker, soaked in some amount of 'sweating' (due to the partially liquefied pulp) and possessed a strong alcoholic smell within its surrounding (Fig. 1b, c).

3.3. Physico-chemical assessment

3.3.1. Bean cut test and fermentation index (FI)

The cut test results of the pod stored beans (Table 2) showed no significant difference ($p > .05$) in terms of slaty, deep purple, brown, germinated and flat beans among the pod stored beans. Meanwhile, the amount of pale purple beans for 0 PS was significantly lower ($p < .05$) than 3 PS and 7 PS. Takrama, Aculey, and Aneani (2006) emphasized that pale purple beans are not defective beans and their presence in a batch does not present any serious problem as these are almost turning into brown color. Hence, they tend to change color to brown upon further storage. Despite 3 PS producing the highest pale purple beans (22.67% ± 4.62), the value does not exceed the range 30–40% acceptable on the trade market (Takrama et al., 2006). This also indicates that the beans had undergone adequate fermentation and will not induce bitter and astringent flavors. A similar observation was made about the proportion of brown beans for each sample where they were distinctly higher than the acceptable level (60%). Despite observing a mouldy bean proportion of 0.67% ± 1.15 in 0 PS, a significant increase ($p < .05$) was recorded as PS duration increased from 3 to 7 days. This concurs with findings of Meyer et al. (1989) who asserted that the amount of mouldy beans significantly increases with PS with a consequential increase in production waste. Although 7 PS recorded the highest proportion of mouldy beans, it did not exceed the limit of 4% according to Wood and Lass (1985).

Unlike the cut test which is based on human visual assessment, the FI rather presents a more objective tool for the estimation of the quality and extent of fermentation. Here, since both unstored and stored samples were subjected to the same fermentation conditions, the impact of PS may clearly be elucidated. Gourieva and Tserevitinov (1979) highlighted that well-fermented beans should have an FI ≥ 1 after the required fermentation time. Just like in the case of the cut test, the FI range of 1.04–1.22 again confirmed that all three samples were fully-fermented. Even though the cut test results showed no significant impact of PS on the proportion of brown beans, it was interesting to observe a relationship between the results of the cut test and FI values, remarkably for 0 PS which recorded the highest FI and percentage of brown beans. Nonetheless, 3 PS had FI significantly lower ($p < .05$) than both 0 PS and 7 PS. According to Meyer et al. (1989), in the case of sufficiently dry stored pods (with a pulp volume per gram of seed ≤ 0.6 mL), the anaerobic phase of fermentation may be suppressed compared to those of unstored pods. Under such conditions, the extent of fermentation is limited, leading to a relatively high pH or low acidity. This reason could have been responsible for the significantly lower ($p < .05$) FI, accompanied with the least nib acidity, as recorded in 3 PS (Tables 2 & 3). More so, earlier work by Sols, Gancedo, and DelaFuente (1971) also allude to this possibility. However, from a different view point, they demonstrated that, in the case of an increased concentration of rapidly fermentable sugars such as glucose, respiration of yeast may be limited even under aerobic conditions during fermentation, thus, limiting the course of fermentation as observed in 3 PS. This, is congruent with the *'Crabtree effect'* according to Sols et al. (1971). Interestingly, the significantly lower ($p < .05$) FI (Table 2) and higher glucose concentration (Table 4) in 3 PS suggests this possibility.

Howbeit, the significant increase ($p < .05$) in FI from 3 PS to 7 PS could be as a consequence of the additional impact of the degradation of both pod and pulp material, suggestive of an onset of a "fermentation-like" process, as observed in Fig. 1. In the study of Kongor, Takrama, Budu, Mensah-Brown, and Afoakwa (2013), a marginal increase in FI with increasing PS was likewise observed. However, they attributed this to the reduction in pulp volume per seed and an improved micro-aeration of the fermenting mass, which may have augmented the activities of polyphenol oxidase (PPO) in the production of brown pigments in the beans, hence, the consequential increase in FI. However, they did not show the physical state of the pods or pulp prior to the fermentation process. Meanwhile, it is clear from Fig. 1 the physical state and condition of the pod or pulp may have some implications on the dynamics of the fermentation process and consequently the FI.

It is clear from this study that the extent of fermentation (as

Table 2
Results of cut test and fermentation index for 0, 3 and 7 days pod stored beans.

	0PS	3PS	7PS
Bean cut test (%)			
Slaty	0.00 ± 0.00^{a}	0.67 ± 1.15^{a}	0.00 ± 0.00^{a}
Deep purple	3.33 ± 3.06^{a}	1.33 ± 1.15^{a}	2.00 ± 2.00^{a}
Pale purple	12.00 ± 0.00^{b}	22.67 ± 4.62^{a}	20.00 ± 2.00^{a}
Brown	84.00 ± 4.00^{a}	75.33 ± 5.03^{a}	75.33 ± 2.31^{a}
Germinated	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Mouldy	0.67 ± 1.15^{ab}	0.00 ± 0.00^{b}	2.67 ± 1.15^{a}
Flat	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Fermentation index (FI)	1.22 ± 0.04^{a}	1.04 ± 0.02^{b}	1.16 ± 0.03^{a}

For each row different alphabets represent significant differences (α = 0.05) among samples.

Table 3
Nib acidity of 0, 3 and 7 days pod stored beans.

	0PS	3PS	7PS
pH (20 °C)	5.52 ± 0.02^{b}	5.61 ± 0.01^{a}	5.45 ± 0.01^{c}
TA (meq NaOH/g)	4.42 ± 0.01^{b}	4.03 ± 0.01^{c}	4.94 ± 0.03^{a}

For each row different alphabets represent significant differences (α = 0.05) among samples.

Table 4
Sugar profile of 0, 3 and 7 days pod stored cocoa beans.

	0PS	3PS	7PS
Glucose (%)	0.264 ± 0.001^{b}	0.300 ± 0.003^{a}	0.211 ± 0.001^{c}
Fructose (%)	0.672 ± 0.004^{b}	0.671 ± 0.004^{b}	0.769 ± 0.002^{a}
Sucrose (%)	0.021 ± 0.001^{c}	0.135 ± 0.001^{a}	0.086 ± 0.001^{b}
Total reducing (%)*	0.935 ± 0.005^{b}	0.971 ± 0.007^{a}	0.980 ± 0.003^{a}

For each row different alphabets represent significant differences (α = 0.05) among samples.
(*) – total reducing sugar is sum of glucose and fructose.

indicated by the FI) may have been hindered from 0 PS to 3 PS due to the impact of pulp volume reduction. Meanwhile, the onset of a "fermentation-like" process in 7 PS appears to have played an additional role in determining the brownness of the beans, thereby explaining the rise in FI. However, compared to the results from the cut test, the observed significant differences in FI among the samples may rather be deemed minimal from a practical point of view.

3.3.2. Nib acidity

The trend in FI was somewhat inversely mirrored by the trend in nib acidity (Table 3). However, acidity was significantly high ($p < .05$) in 7 PS, followed by 0 PS and finally 3 PS. Notably, the extensive degradation of pulp occurring in 7 PS may have yielded more ethanol for more acetic acid production, accompanied with other organic acids and pulp constituents, which may have diffused into the nibs during the period of extended PS and subsequent during the fermentation process. This can be seen from the significantly high ($p < .05$) amount of titratable acids in 7 PS (Table 3) compared to 0 PS which also yielded considerable amount of acids. However, the significantly low ($p < .05$) nib acidity in 3 PS can be explained by its low pulp volume. Meyer et al. (1989), proposed that the reduced pulp volume may enhance mass aeration during fermentation, which in turn, elevates the ratio of respiration to ethanol fermentation as well as its consequent oxidation to acetic acid. The shift in this ratio, may have therefore served as the inhibiting factor to the bean acidification in 3 PS during subsequent flavor precursor formation following bean death.

As already remarked in Section 3.2.2, the advanced state of cellular degradation in both pod and pulp material in 7 PS, was also accompanied with a partial degradation/liquefaction of pectin in the pulp, thus, leading to the formation of some amount of 'sweating' inside the pods, within which, the beans appeared to be soaked. Interestingly, analysis of the initial moisture content of all equally dried beans also revealed a significantly higher ($p < .05$) moisture content in 7 PS (5.53% ± 0.04) compared to 3 PS (5.19% ± 0.03) and 0 PS (5.19% ± 0.06). Thus, considering the initial moisture content, together with the remarkably high amount of titratable acids in 7 PS (Table 3), one can suggest the possible transfer of moisture along with other dissolved pulp components (sugars and organic acids) proceeding the onset of pulp component degradation during an extended duration of PS such as in the case of 7 PS. Interestingly, some studies have already speculated the phenomenon of mass transfer between the pulp and the bean through the radicle of the bean as well as the semi-permeable testa (seed coat enclosing the bean) under similar circumstances of extensive cellular disintegration during the fermentation process. Through these channels, it has been demonstrated; the transfer of water, ethanol, acetic acid, lactic acid and some volatile organic compounds (such as monoterpenes) originating from the pulp into the bean (Biehl & Voigt, 1999; Kadow, Bohlmann, Phillips, & Lieberei, 2013; Reineccius, Andersen, Kavanagh, & Keeney, 1972; Wood & Lass, 1985). We suggest this to be the reason accounting for the observed significantly higher ($p < .05$) amount of titratable acids in 7 PS than the reference (0 PS).

3.4. Precursor assessment

3.4.1. Sugar profile

In this Section, the quantities of different sugars as affected by PS in fermented and dried cocoa beans have been elucidated. Chromatograms showing peaks corresponding to different sugars in cocoa bean samples with reference to standard sugar are shown in Fig. A.1. Of the peaks identified, the concentrations of glucose, fructose and sucrose have been represented in Table 4. Here, the concentration of glucose increased marginally from 0 PS (0.264% ± 0.001) to 3 PS (0.300% ± 0.003) and finally decreased in 7 PS (0.211% ± 0.001). Particularly, this trend was seen to be complementary to the degree of fermentation (Table 2) and inversely related to the acidity of the nibs (Table 2). It is known, that the acidification of nibs, during the process of fermentation is crucial for both bean death and the initiation of various enzyme-substrate interactions. From this, various reducing sugars emerge (Beckett, 2009). It can therefore be suggested that the extent of nib acidification conceivably played a role in determining the concentrations of glucose released in the different samples. The abundance of fructose over glucose in the samples was not surprising as this was also observed by Reineccius et al. (1972). They attributed this to the preferential metabolism of glucose moieties as sucrose was hydrolyzed during the fermentation process. However, the significantly higher ($p < .05$) concentration of fructose in 7 PS (0.769% ± 0.002) as compared to 0 PS (0.672% ± 0.004) and 3 PS (0.671% ± 0.004) could be indicative of the impact of the "fermentation-like" process initiated in 7 PS prior to the fermentation process (Fig. 1). Moreover, the apparent correlation between nib acidity and the measure to which fructose dominated the reducing sugar fraction in the samples was also compelling. Here, the respective fructose-glucose ratio for 0 PS, 3 PS and 7 PS were approximately 3:1, 2:1 and 4:1. This again confirms the role of PS in influencing the dynamics of sugar degradation through nib acidification during fermentation. As suggested by Reineccius et al. (1972) and Beckett (2009), these ratios can be suspected to play a key role in channeling the outcome of the Maillard reaction after subsequent roasting of these beans.

Compared to 0 PS (0.021% ± 0.001), 3 PS (0.135% ± 0.001) had the highest amount of sucrose, followed by 7 PS (0.086% ± 0.001). Generally, the low levels of sucrose in these samples support with the fact that sucrose is hydrolyzed to release fructose and glucose by native invertase amidst fermentation (Afoakwa, Kongor, Takrama, & Budu, 2013b; Puziah, Jinap, Sharifah, & Asbi, 1998). Hitherto, the significantly higher ($p < .05$) sucrose content of 3 PS corresponds with its significantly lower ($p < .05$) FI (Table 2) and nib acidity (Table 3). In accordance with their findings, Biehl et al. (1989) reported that several yeasts isolated from fermenting cocoa mass had an increased invertase activity in more acidic media. Thus, affirming the relationship between sucrose inversion, on one hand, and the degree of fermentation along with nib acidification on the other. However, the fact that 7 PS was significantly higher ($p < .05$) than 0 PS still remains unclear. Meanwhile, as previously suspected, it can be suggested that the overall sucrose content, among other sugars, may have been partly derived from the pulp and not only from the cotyledons. Based on their findings, Reineccius et al. (1972) emphasized that absorbed and occluded water-soluble constituents from the pulp could indeed contribute to the overall amount of sugars within the beans. In this scenario, sucrose concertation in 7 PS would ultimately be expected to exceed that of 0 PS, due to the extensive cellular degradation and the extended duration of storage in the former. Thus, this may create a greater possibility for these dissolved pulp components (including sucrose) to penetrate the beans during PS, and ultimately, during fermentation. Nonetheless, the extent to which this phenomenon may affect the original quantities of residual sucrose endemic in the different beans still requires further investigation.

Unknown sugars identified in the samples (Fig. A.2), can be as a result of various primary or secondary reactions triggered under the fermentation conditions. In their report Aprotosoaie et al. (2015) largely attributed this to microbial activity. Of these, unknown sugars 1–4 (suspected to be sugar alcohols) are likely products from various side reactions following sugar degradation during the fermentation process. However, the different quantities of substrates present and conditions of fermentation as influenced by PS could be responsible for the observed differences. Similarly, studies conducted by Reineccius et al. (1972) also revealed additional sugars such as pentitol, mannitol, inositol and sorbose in fermented cocoa beans.

Compared to the standard sugar, two peaks (RT = 17.684 min and 18.049 min) corresponded with those of lactose (RT = 17.977 min and 18.186 min, Fig. A.1). However, to the best of our knowledge, lactose is not a native sugar in cocoa beans. Its occurrence (unknown 5, Fig. A.2),

however, may be suggestive of a glucose-galactose bond occurring as a secondary product following polyphenol degradation at some point during the fermentation process. Research has revealed the hydrolytic activity of glycosidases on anthocyanins (mainly cyaniding-3-α-L-arabinoside and cyaniding-3-β-galactoside) in yielding products such as anthocyanidins and reducing sugars; arabinose and galactose (Camu et al., 2008; Lima et al., 2011). Whilst this is still unclear, given the presence of glucose and the breakdown of anthocyanins (results not shown) during PS and fermentation, a likely interaction of these products in a secondary condensation reaction leading to the formation of this glucose-galactose bond can be suggested. However, in this case, the presence of lactose could be effective in the Maillard reaction along with other reducing sugars during subsequent roasting process. Evidence of lactose (glucose-galactose bond) involvement in the possible formation of some typical cocoa-related flavor volatiles through the Maillard reaction has been reported by Gerrard, Newton, Fairbanks, Golding, and Andrewes (2012). Here, the reaction of lactose with a lysine group of protein – leading to the formation of an imine and subsequently lactulosyllysine (an amadori product) has been proposed. From these and other series of complex reactions, various furfurals and furanones can be produced.

Altogether, these finding clearly exemplify the enormous complexity encompassing the phenomenon of sugar degradation during PS and subsequent fermentation of cocoa beans. Nonetheless, it is evident from Table 4, that the duration of PS may have contributed to a marginal increase (0 PS = 0.935% ± 0.005; 3 PS = 0.971% ± 0.007; 7 PS = 0.980% ± 0.003) in the total amount of reducing sugars in the beans, even without the consideration of lactose. Interestingly, this agrees with findings reported by Afoakwa (2014). It must however be noted, that, the assertion of continuous sucrose inversion in the pulp during PS (Biehl et al., 1989; Meyer et al., 1989) should not be confused for the mechanism occurring within the bean since each occur under different enzymatic conditions.

3.4.2. Free amino acid profile

The free amino acid profiles of 0 PS, 3 PS and 7 PS have also been evaluated (typical chromatogram in Fig. A.3). From Table 5, the total free amino acid content of the different cocoa beans occurred between 11.35 and 19.70 mg/g fat-free sample. From the various fractions, a gradual increase in free amino acid content in function of PS duration is seen. Even though 0 PS and 3 PS showed no clear significant difference, the extent of proteolysis after 7 days was undisputable.

In their view, Voigt, Biehl, and Heinrich (1994), Voigt, Heinrichs, Voigt, and Biehl (1994) maintain that nib acidification during fermentation remains crucial for the flavor quality of cocoa beans. Thus, the distinct pH optima of proteases (being ca pH 3.8 and ca pH 5.8 for aspartic endopeptidase and serine exopeptidase respectively) play an important role in determining their performance and the outcome of proteolysis. From Table 3, the pH range of 5.45–5.61 - being close to the pH optimum of the enzyme serine exopeptidase - could be dully associated with the high production of hydrophobic amino acids in the different cocoa beans. However, the proximity of these pH values to each other could have been the reason why a direct link was not found between the quantity of free amino acids and the trend in nib acidity. It could be suggested that, the duration of PS and the advanced state of cellular deterioration (Fig. 1) and proteolysis, instead of the pH, could have played a more important role in accounting for the significantly high ($p < .05$) amount of free amino acids in the 7 PS sample. This finding was also in agreement with that of Afoakwa et al. (2011), who attributed the decreases in protein content with PS to its breakdown, releasing free amino acids and other peptides.

Generally, various studies conducted on cocoa beans have also identified similar amino acids profiles (Adeyeye, Akinyeye, Ogunlade, Olaofe, & Boluwade, 2010; Kirchhoff, Biehl, Ziegeler-Berghausen, Hammoor, & Lieberei, 1989; Marseglia, Palla, & Caligiani, 2014; Rohsius, Matissek, & Lieberei, 2006; Tran et al., 2015). However, our study revealed two amino acids; citrulline and ornithine, which have not yet been reported in cocoa beans – at least to the best of our knowledge. Whereas, the former showed no significant change ($p < .05$) with respect to PS, the latter increased significantly ($p < .05$) with the duration of PS. In the review of Kerler, Van der Ven, and Weenen (1997), these amino acids have been said to act as precursors for the thermal production of 2-acetyl-1-pyrroline, through the Maillard reaction in food products such as popcorn, roasted sesame, wheat and rye bread.

Table 5
Free amino acid profiles of 0, 3 and 7 days pod stored cocoa beans.

	AA mg/g fat-free sample		
	0PS	3PS	7PS
Aspartate	0.27 ± 0.04^{b}	0.30 ± 0.05^{ab}	0.42 ± 0.01^{a}
Glutamate	1.14 ± 0.12^{a}	1.33 ± 0.23^{a}	1.58 ± 0.03^{a}
Asparagine	0.38 ± 0.01^{c}	0.59 ± 0.10^{b}	0.83 ± 0.01^{a}
Glutamine	0.12 ± 0.01^{b}	0.23 ± 0.04^{a}	0.24 ± 0.00^{a}
Acidic (total)	1.91 ± 0.16^{b}	2.46 ± 0.42^{ab}	3.07 ± 0.05^{a}
Histidine	0.09 ± 0.01^{a}	0.16 ± 0.03^{b}	0.25 ± 0.00^{c}
Arginine	0.76 ± 0.14^{b}	0.84 ± 0.13^{b}	1.36 ± 0.02^{a}
Lysine	0.60 ± 0.06^{b}	0.77 ± 0.13^{b}	1.15 ± 0.01^{a}
Basic (total)	1.46 ± 0.19^{b}	1.77 ± 0.29^{b}	2.76 ± 0.03^{a}
Alanine	0.84 ± 0.14^{b}	1.00 ± 0.17^{ab}	1.32 ± 0.02^{a}
Tyrosine	0.64 ± 0.07^{b}	0.77 ± 0.12^{b}	1.13 ± 0.02^{a}
Valine	0.60 ± 0.08^{b}	0.75 ± 0.13^{ab}	0.95 ± 0.02^{a}
Phenylalanine	0.06 ± 0.01^{c}	0.09 ± 0.01^{b}	0.14 ± 0.00^{a}
Isoleucine	0.88 ± 0.11^{b}	1.04 ± 0.16^{b}	1.53 ± 0.02^{a}
Leucine	1.37 ± 0.07^{b}	1.75 ± 0.30^{b}	2.59 ± 0.04^{a}
Hydrophobic (total)	4.39 ± 0.48^{b}	5.40 ± 0.89^{b}	7.66 ± 0.11^{a}
Serine	0.40 ± 0.09^{b}	0.46 ± 0.08^{ab}	0.65 ± 0.01^{a}
Glycine	0.15 ± 0.02^{b}	0.18 ± 0.03^{b}	0.31 ± 0.00^{a}
Threonine	0.30 ± 0.04^{b}	0.36 ± 0.06^{b}	0.52 ± 0.01^{a}
Citrulline	0.19 ± 0.03^{a}	0.16 ± 0.03^{a}	0.23 ± 0.00^{a}
Methionine	0.07 ± 0.00^{c}	0.11 ± 0.02^{b}	0.20 ± 0.00^{a}
Tryptophane	0.46 ± 0.04^{b}	0.60 ± 0.10^{ab}	0.79 ± 0.01^{a}
Ornithine	1.64 ± 0.19^{b}	2.14 ± 0.35^{ab}	2.86 ± 0.04^{a}
Proline	0.38 ± 0.03^{b}	0.60 ± 0.11^{a}	0.65 ± 0.01^{a}
Other (total)	3.59 ± 0.44^{b}	4.61 ± 0.78^{ab}	6.21 ± 0.09^{a}
Grand total	11.35 ± 1.26^{b}	14.23 ± 2.38^{b}	19.70 ± 0.28^{a}

For each row different alphabets represent significant differences (α = 0.05) among samples.

3.5. Aroma profiling of roasted pod stored cocoa beans

The Maillard reaction is the most important chemical reaction responsible for the transformation of various flavor precursors (free amino acids, oligopeptides, and reducing sugars) into key volatile organic compounds that characterize the flavor of cocoa. According to Aprotosoaie et al. (2015), the reaction starts when the free amino group of an amino acid attacks the reactive carbonyl group of glucose or fructose to form the Schiff base. From here, a variety of successive chemical reactions occur leading to the formation of the α-dicarbonyl intermediates. These are necessary for the Strecker degradation reaction to occur following an attack of an amino acid. From this various volatile aldehydes and ketones are generated. The same reaction is also responsible for the formation of various pyrazines, pyrroles and pyridines through subsequent polymerization and heterocyclization reactions (Afoakwa et al., 2008; Beckett, 2009). Table 6 provides an overview of some groups of volatiles (aldehydes, ketones, pyrazines, furans, furanones, pyrans and others) identified from the roasted pod stored beans.

The aldehydes are marked for their impact on the development of good cocoa flavor. As can be seen, 0 PS exhibited the highest headspace concentration with an insignificant decline ($p > .05$) observed as the duration of PS increased from 3 to 7 days. Acetaldehyde was the least

Table 6
Mean semi-quantitative concentrations and odor activity values of some Maillard reaction related volatiles identified in roasted pod stored (0, 3 and 7 days) cocoa beans.

No.	VOC	Concentration (ng/g)			OTV (ng/g)	OAV			Description*	KI (exp)	KI (lit) [+]
		0PS	3PS	7PS		0PS	3PS	7PS			
Aldehydes											
1	Acetaldehyde	21.29 ± 2.69^{A}	13.82 ± 4.03^{B}	21.06 ± 1.39^{A}	0.22	96.78	62.83	95.72	Bitter pungent	678.82	714
2	2-Methylbutanal	117.39 ± 7.25^{B}	95.30 ± 6.11^{C}	132.43 ± 1.38^{A}	2.2–152	≤53.36	≤43.32	≤60.19	Cocoa, chocolate, almond	935.01	880–961
3	3-Methylbutanal	428.35 ± 36.53^{A}	343.99 ± 22.65^{B}	404.56 ± 1.86^{A}	5.4–80	5.35–79.32	4.30–63.70	5.06–74.92	Cocoa, chocolate	937.37	884–943
4	2-Isopropylbut-2-enal	83.29 ± 3.20^{A}	46.97 ± 4.89^{B}	54.93 ± 4.38^{B}						1174.10	
5	Benzaldehyde	1084.56 ± 45.37^{A}	985.69 ± 34.22^{B}	654.31 ± 34.35^{C}	60	18.08	16.43	10.91	Bitter, almond	1498.67	1495
6	Benzeneacetaldehyde/phenyl acetaldehyde	53.08 ± 13.79^{B}	56.99 ± 4.13^{AB}	75.01 ± 6.26^{A}	22–154	≤2.41	≤2.59	≤3.41	Honey, floral	1609.13	1592–1689
7	2-Phenyl-2-butenal	491.98 ± 24.52^{A}	189.32 ± 8.20^{C}	238.06 ± 5.13^{B}					Cocoa, sweet, roasted, rum	1862.03	1896–1972
8	Methyl phenyl pentenal	36.48 ± 2.55^{A}	44.08 ± 1.83^{A}	36.51 ± 10.32^{A}					Cocoa	1872.29	
9	5-Methyl-2-phenyl-2-hexenal	158.44 ± 5.89^{B}	182.45 ± 6.40^{A}	173.02 ± 17.12^{AB}					Cocoa, chocolate	1978.05	
	Total	2474.85 ± 125.49^{A}	1958.61 ± 57.76^{B}	1789.8 ± 55.27^{B}							
Ketones											
10	2-Pentanone	89.42 ± 19.49^{B}	109.33 ± 5.68^{AB}	141.51 ± 34.50^{A}	288,000	< 1	< 1	< 1	Sweet, fruity, banana, fermented	978.82	943–1023.72
11	2,3-Pentanedione	31.37 ± 0.47^{A}	31.68 ± 6.54^{A}	22.47 ± 6.17^{A}					Bitter pungent	1055.50	1054
12	2-Heptanone	88.06 ± 4.58^{B}	125.80 ± 9.33^{A}	116.47 ± 1.43^{A}	1500–34,000	< 1	< 1	< 1	Fruity, floral	1250.15	1145–1216
13	3-Hydroxy-2-butanone	823.65 ± 59.51^{B}	466.33 ± 2.65^{C}	1694.56 ± 16.57^{A}					Butter, cream	1282.81	1236–1333
14	1-Hydroxy-2-propanone	178.31 ± 7.97^{B}	197.58 ± 2.14^{A}	193.45 ± 1.43^{A}						1295.70	1272–1340
15	3-Hydroxy-2-pentanone	42.16 ± 1.89^{B}	34.09 ± 2.81^{C}	91.61 ± 3.90^{A}						1338.31	1338–1368
16	2-Nonanone	38.84 ± 7.21^{C}	191.99 ± 9.20^{A}	82.34 ± 15.38^{B}	100	< 1	1.92	< 1		1387.94	1347–1420
17	1-(2-furanyl)-ethanone	36.25 ± 4.48^{B}	32.42 ± 3.48^{B}	76.35 ± 9.54^{A}						1485.78	1457–1536
18	Acetophenone	212.67 ± 2.16^{B}	307.68 ± 9.38^{A}	344.07 ± 28.73^{A}	5629	< 1	< 1	< 1	Must, floral, almond	1616.18	1600–1695
	Total	1540.74 ± 60.10^{B}	1496.90 ± 26.58^{B}	2762.84 ± 56.08^{A}							
Pyrazines											
19	Methylpyrazine	46.48 ± 4.52^{C}	101.76 ± 1.70^{B}	139.55 ± 7.41^{A}	27,000	< 1	< 1	< 1	Nutty, chocolate, cocoa, roasted nuts	1265.68	1235–1312
20	2,5-Dimethyl pyrazine	156.01 ± 9.50^{B}	244.94 ± 14.42^{A}	170.25 ± 6.82^{B}	2600–17,000	< 1	< 1	< 1	Chocolate, nutty	1323.20	1274–1358
21	2,6-Dimethyl pyrazine	74.74 ± 0.89^{C}	101.91 ± 11.07^{B}	123.51 ± 5.15^{A}	1021–8000	< 1	< 1	< 1	Nutty, herbal	1328.75	1280–1370
22	2,3-Dimethyl pyrazine	178.46 ± 6.54^{B}	187.85 ± 6.60^{B}	530.59 ± 8.85^{A}	123	1.45	1.53	4.31	Caramel, cocoa	1345.60	1306–1377
23	2-Ethyl-6-methyl pyrazine	65.81 ± 4.84^{A}	51.83 ± 3.76^{A}	75.32 ± 33.39^{A}					Cocoa, roasted, green	1384.17	1491–1521
24	Trimethyl pyrazine	474.96 ± 32.26^{B}	408.77 ± 13.10^{C}	1499.51 ± 18.38^{A}	290	1.64	1.41	5.17	Cocoa, rusted nuts, peanut	1403.82	1395
25	2,3-Dimethyl-5-ethylpyrazine	177.17 ± 19.68^{B}	121.12 ± 41.52^{B}	324.70 ± 23.16^{A}	60	2.95	2.02	5.41	Cocoa, roasted nuts, peanut	1453.70	
26	Tetramethyl pyrazine	753.35 ± 43.78^{B}	385.54 ± 11.35^{C}	4658.96 ± 65.28^{A}	38,000	< 1	< 1	< 1	Cocoa, chocolate, roasted	1465.81	1438–1485
27	2-Methyl-6-vinyl pyrazine	27.24 ± 2.28^{C}	38.48 ± 4.15^{B}	48.20 ± 3.12^{A}						1474.46	
28	3,5-Diethyl-2-methyl pyrazine	53.85 ± 3.95^{A}	22.81 ± 1.24^{B}	40.28 ± 11.82^{AB}					cocoa, chocolate, rum, sweet, roasted	1483.03	
	Total	2008.07 ± 105.37^{B}	1665.01 ± 60.64^{C}	7610.88 ± 106.13^{A}							
Furans, furanones, pyrans											
29	2-Pentyl furan	28.92 ± 5.30^{B}	37.10 ± 4.36^{AB}	45.49 ± 6.62^{A}	100–2000	< 1	< 1	< 1		1233.13	1193–1265
30	3-Methyl-2-(2-methyl-2-butenyl)-furan	24.00 ± 9.11^{AB}	19.60 ± 1.21^{B}	30.73 ± 2.95^{A}						1399.47	
31	Dihydro-2(3H)-furanone	173.96 ± 9.79^{A}	191.17 ± 3.16^{A}	183.16 ± 10.90^{A}					Sweet aromatic, buttery	1591.76	1630
32	Furfural alcohol	262.86 ± 7.33^{B}	273.74 ± 4.89^{B}	461.22 ± 13.32^{A}					Mild, oily, “burnt”, caramel	1633.39	
33	2,5-Dimethyl-4-hydroxy-3[2H]-furanone	57.70 ± 4.30^{C}	64.21 ± 1.11^{B}	136.81 ± 0.98^{A}	1.6–50	1.15–36.06	1.28–40.13	2.74–85.51	Sweet, fruity caramel, burnt pineapple aroma	1949.68	
34	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	412.65 ± 42.62^{B}	373.62 ± 41.42^{B}	573.27 ± 31.32^{A}					Roasted	2117.10	
	Total	960.09 ± 24.93^{B}	959.44 ± 32.33^{B}	1430.68 ± 49.44^{A}							

(*continued on next page*)

Table 6 (*continued*)

No.	VOC	Concentration (ng/g)			OTV (ng/g)	OAV			Description*	KI (exp)	KI (lit)[+]
		0PS	3PS	7PS		0PS	3PS	7PS			
Others											
35	Dimethyldisulfide	44.12 ± 2.75^{B}	31.33 ± 7.46^{B}	59.89 ± 4.70^{A}	12	3.68	2.61	4.99	cabbage, sulfur, gasoline	1061.10	1071
36	2-Butene oxide	440.09 ± 18.50^{B}	335.96 ± 10.39^{B}	1951.32 ± 125.85^{A}						1544.36	
37	Benzonitrile	73.50 ± 9.42^{B}	72.98 ± 12.15^{B}	111.41 ± 13.20^{A}					Nutty, almond-like	1571.57	1583–1637
38	2-Methoxy phenol	68.12 ± 9.21^{B}	60.09 ± 3.26^{B}	123.72 ± 3.86^{A}	10–70	≤6.81	≤6.01	1.77–12.37	Smoky	1804.22	1815–1910
39	2-Acetylpyrrole	208.54 ± 8.54^{B}	196.59 ± 8.96^{B}	342.42 ± 15.51^{A}					cocoa, chocolate, hazelnut, roasted	1896.55	1930–2020
40	Pantoic lactone	45.14 ± 4.47^{B}	51.60 ± 1.00^{B}	76.54 ± 14.32^{A}						1941.43	2034&2070
	Total	879.50 ± 17.44^{B}	748.57 ± 8.11^{B}	2665.29 ± 133.28^{A}							
	Grand total	7863.25 ± 282.23^{B}	6828.53 ± 86.62^{C}	16,259.56 ± 52.95^{A}							

For each row different alphabets represent significant differences ($\alpha = 0.05$) among samples; (*) odor description from Afoakwa et al. (2008), Aprotosoaie et al. (2015) and Tran et al. (2015); ($^{+}$) KI (lit) sourced from webbook. nist.gov or pherobase.com.

abundant aldehyde with its lowest headspace concentration observed in 3 PS, withal, it was the most odor-active aldehyde identified in the three samples. Other odor-active volatiles included 2/3-methylbutanal, benzaldehyde and phenyl acetaldehyde.

There was an insignificant decline in the total headspace concentration of ketones as PS increased from 0 PS to 3 PS, proceeded by a sharp increase in 7 PS. Ketones were the least odor-active group with only 2-nonanone being identified. 3-hydroxy-2-butanone was the most abundant ketone with the highest concentration identified in 7 PS, followed by 0 PS and then 3 PS, however, its contribution to the overall flavor was uncertain as its OTV in oil media was unknown.

Although a significant decline ($p < .05$) was observed as PS increased to 3 days, the total pyrazine concentration increased about 4 folds as PS increased to 7 days. This was mirrored by the predominance of eight out of the ten pyrazines identified in the liquor of 7 PS. In retrospect, we could suggest that the lower concentration of aldehydes in 7 PS may be due to their conversion to these heterocyclic pyrazines which further contributed to their higher concentration. Tetramethylpyrazine which was the most predominant pyrazine in all samples has been reported as one of the main components of cocoa aroma (Rodriguez-Campos et al., 2012). Nevertheless, the contributing effect of this volatile to the flavor quality of the cocoa was negligible due to its low OTV (OAV < 1). Also identified was the odor-active trimethypyrazine, another important pyrazine. Its headspace concentration decreased significantly ($p < .05$) from 0 PS to 3 PS and then increased sharply to 7 PS. Among the pyrazines, 2,3-dimethyl-5-ethylpyrazine was the most odor-active and likely to impart its typical cocoa and nutty notes (Bonvechi, 2005).

According to Miralles-Garcia (2008), the formation of high quantities of the furans, furanones and pyranones groups occur mainly by the caramelisation of the sugars. Though furfural and furans are well-known caramelisation products from C5 sugars, they can also be formed through the Maillard reaction pathways involving nitro compounds (Miralles-Garcia, 2008). The total headspace concentration of this group of volatiles was the highest in 7 PS although 0 PS and 3 PS showed no significant difference. Furans such as furfural alcohol which imparts caramel flavor note was found to increase sharply as PS increased to 7 days. It is however unclear if this volatile contributes to the overall flavor of the cocoa as its OTV in oil media is unknown. The only odor active furan identified was 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Doornbos, Van Den Ouweland, and Tjan (1981) revealed that, Amadori rearrangement products derived from rhamnose and proline are important precursors for the generation of this potent sweet, fruity caramel-like volatile.

Other volatiles such as dimethyldisulfide, 2-methoxy phenol and 2-acetylpyrrole were also present in the liquors. Odor-active dimethyldifisude and 2-methoxy-phenol which are associated with undesirable flavor notes were predominant in 7 PS, followed by 0 PS, and then 3 PS. 2-acetyl-1-pyrrole, although not odor-active, was obtained in fairly high concentrations in the three samples. It is derived from proline through Maillard reaction and imparts many important notes such as caramel, chocolate and roasted flavor, which are desirable in roasted cocoa (Mottram, 2007). Together with the others, this group of volatiles was the highest in 7 PS, whereas the concentrations in 3 PS and 0 PS showed no significant difference among each other.

On the whole, 7 PS seemed to have enhanced the formation of more Maillard reaction related volatiles. This was followed by 0 PS and finally 3 PS. Even though the total reducing sugars increased significantly from 0 PS to 3 PS and remained indifferent until 7 PS, the respective fructose-glucose ratio for these were approximately 3:1, 2:1 and 4:1. As suggested by Reineccius et al. (1972) and Beckett (2009), this ratio can be expected to play a key role in determining the outcome of volatile generation in the Maillard reaction during roasting of the beans. Hence, the observed trend in the total volatile concentrations. Additionally, the significantly high amounts of free amino acids in 7 PS may have contributed to the formation of most volatiles, especially, pyrazines, whose

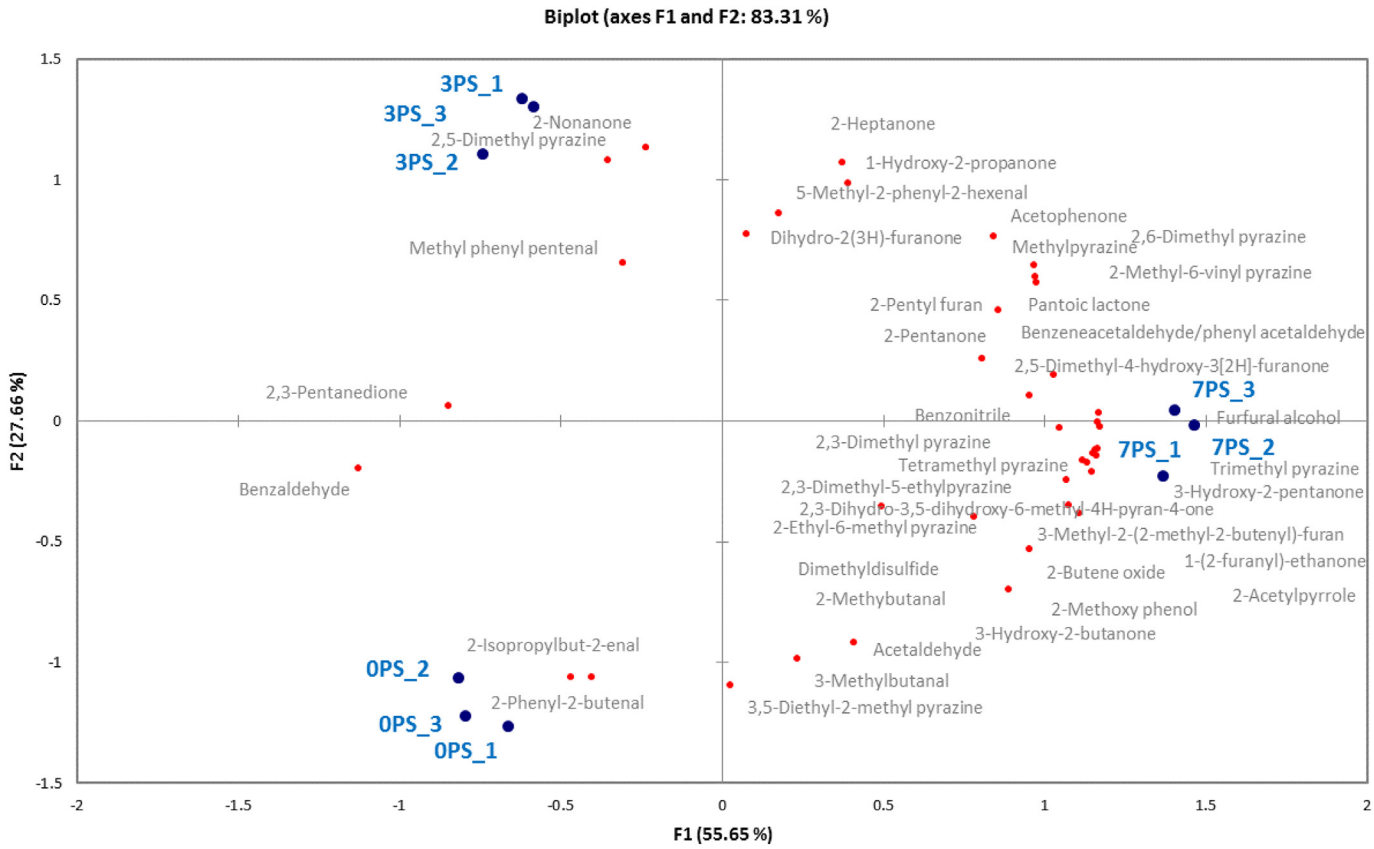

Fig. 2. PCA showing volatile composition of roasted cocoa beans as affected by 0, 3 or 7 days of pod storage.

abundance in roasted cocoa beans have been closely associated with the hydrophobic class amino acids (Voigt, Biehl, & Heinrich, 1994; Voigt, Heinrichs, et al., 1994).

The possible influence of PS on different flavor volatile concentrations was also investigated through principal component analysis (PCA). A biplot of the first two principal components (PC1 = 55.65% and PC2 = 27.66%) is shown in Fig. 2, where a total of 83.31% of the variability is explained. Here, the clustering of volatiles around 7 PS also depicted the predominance of these volatiles in the sample compared to 0 PS and 3 PS. From these, one may expect an intensity of diverse flavor notes to be perceived in the liquor from 7 PS. Although 0 PS was highly associated with volatiles such as 2-isopropylbut-2-enal, 2-phenyl-2-butenal and benzaldehyde, 3 PS was dominated by volatiles such as 2-nonanone, 2,5-dimethyl pyrazine and methyl phenyl pentenal. Although several reasons could account for the lower total volatile concentration in 3 PS, within the framework of this study, the renowned effect of pulp volume reduction through moisture evaporation and respiration of inherent sugars leading to the suppression of the anaerobic phase during the initial stages of fermentation could be suggested. The results indicate that storing the cocoa pods for 3 days may be too short a duration to cause any pronounced effect on the volatile formation in the cocoa beans. Meanwhile, one could suggest that the early onset of a cellular degradation ("fermentation-like" process) in 7 PS may have yielded a substantial amount of flavor precursors resulting in the higher concentration of these Maillard reaction related volatiles as identified.

4. Conclusions

There is no doubt about the convenience and practice of PS among Ghanaian cocoa farmers. However, the knowledge gap between this practice and the consequential impact on precursor and flavor modification rather possess a limitation on the possibility of harnessing its benefits. Among other findings, the significant impact of PS on FI and nib acidity can be explained by the biochemical changes leading to pulp modification (*e.g.* moisture reduction) and cellular degradation within the pods as visualized. Whilst the entire reaction of sugar degradation may be deemed complex, a clear relationship between the FI, nib acidity and the glucose content was observed. Also, a significantly high ($p < .05$) fructose concentration in 7 PS was possibly due to the advanced state of cellular degradation as visualized from the opened pods. Whilst sucrose is suspected to have been involved in other side reactions, the significantly high ($p < .05$) concentration in 3 PS could be accounted for by its significantly low ($p < .05$) FI. Even though the presence of a glucose-galactose bond (lactose) is highly suspected as a secondary product, the mystery surrounding its occurrence in cocoa beans still needs to be elucidated. The studies revealed that, whereas a short duration of PS may not significantly contribute to substantial amount of free amino acids in cocoa beans, the significant increase ($p < .05$) of these at extended duration (7 PS) may be crucial in boosting the flavor potential of cocoa beans. Overall, 7 PS seemed to have enhanced the formation of more volatiles. This was followed by 0 PS and finally 3 PS. Even though a marginal increasing in total reducing sugars (glucose and fructose) was associated with increasing PS, the fructose-glucose ratio of the samples as affected by PS seemed to have also contributed to this outcome as also suggested in some studies.

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Declaration of interest

The authors declare that they have no conflict of interest.

Appendix A. Detailed profiles of sugars and free amino acids

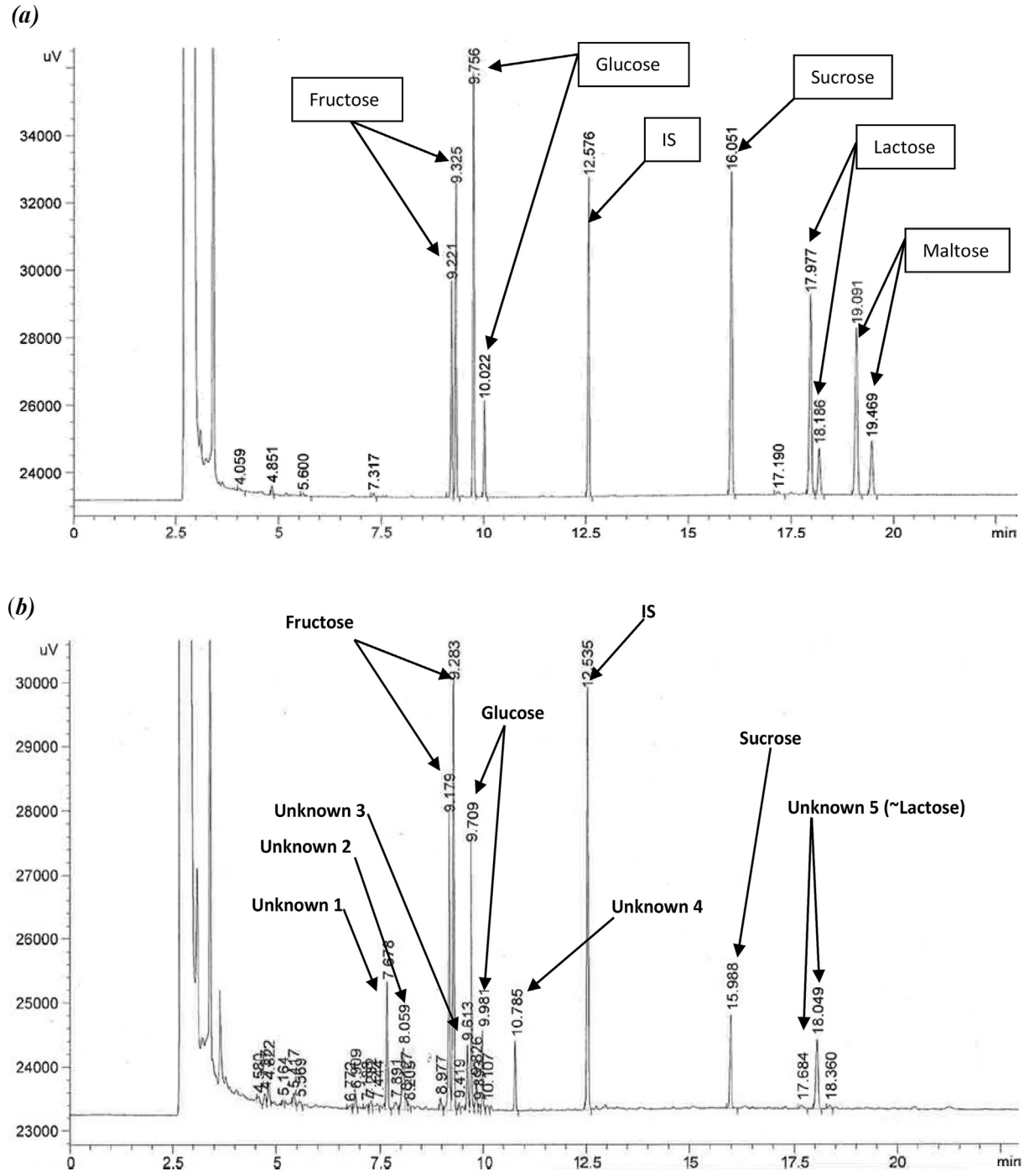

Fig. A.1. Chromatograms showing sugar profiles of (a) standard sugar and (b) pod stored cocoa bean.

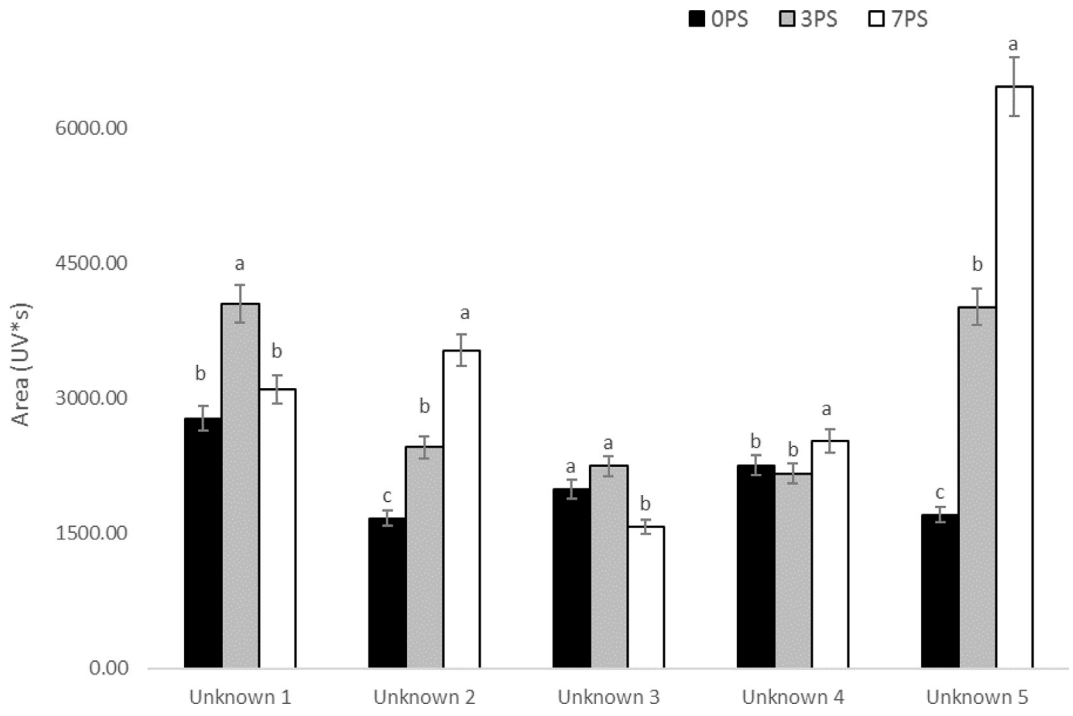

Fig. A.2. Abundances of unknown sugars identified in 0, 3 and 7 days pod stored cocoa beans.

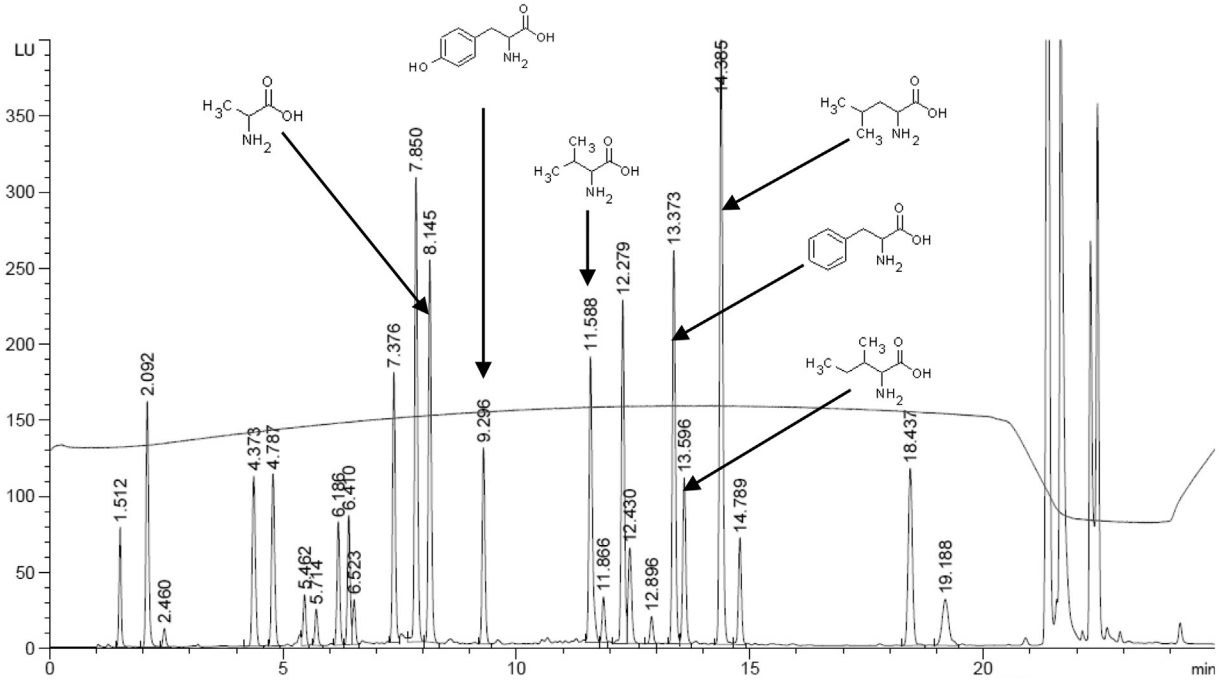

Fig. A.3. Chromatogram showing typical free amino acid profile of pod stored cocoa bean.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.05.064.

References

Aculey, P. C., Snitkjaer, P., Owusu, M., Bassompiere, M., Takrama, J. S., Nørgaard, L., & Nielsen, D. S. (2010). Ghanaian cocoa bean fermentation characterized by spectroscopic and chromatographic methods and chemometrics. *Journal of Food Science, 75*(6), 300–307.

Adeyeye, E. I., Akinyeye, R. O., Ogunlade, I., Olaofe, O., & Boluwade, J. O. (2010). Effect of farm and industrial processing on the amino acid profile of cocoa beans. *Food Chemistry, 118*, 357–363.

Afoakwa, E. O. (2010). *Chocolate science and technology* (1st ed.). UK: Wiley-Blackwell Publishers3–82.

Afoakwa, E. O. (2014). *Cocoa production and processing technology* (1st ed.). Boca Raton, Fl: CRC Press.

Afoakwa, E. O. (2016). *Chocolate science and technology* (2nd ed.). Ltd, UK: John Wiley & Sons.

Afoakwa, E. O., Kongor, J. E., Takrama, J., & Budu, A. S. (2013a). Changes in acidification, sugars and mineral composition of cocoa pulp during fermentation of pulp

pre-conditioned cocoa (Theobroma cacao) beans. *International Food Research Journal, 20*(3), 1215–1222.
Afoakwa, E. O., Kongor, J. E., Takrama, J., & Budu, A. S. (2013b). Changes in nib acidification and biochemical composition during fermentation of pulp pre-conditioned cocoa (Theobroma cacao) beans. *International Food Research Journal, 20*(4), 1843–1853.
Afoakwa, E. O., Kongor, J. E., Takrama, J., Budu, A. S., & Mensah-Brown, H. (2013). Effects of Pulp pre-conditioning on total polyphenols, *O*-diphenols and anthocyanin concentrations during fermentation and drying of cocoa (*Theobroma cacao*) beans. *Journal of Food Science and Engineering, 3*, 235–245.
Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2008). Flavor formation and character in cocoa and chocolate: A critical review. *Critical Reviews in Food Science and Nutrition, 48*(9), 840–857. http://dx.doi.org/10.1080/10408390701719272.
Afoakwa, E. O., Quao, J., Takrama, J., Budu, A. S., & Saalia, F. K. (2011). Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *Journal of Food Science and Technology, 47*(1), 3–11.
Amin, I., Jinap, S., & Jamilah, B. (1998). Proteolytic activity (aspartic Endoproteinase and Carboxypeptidase) of cocoa bean during fermentation. *Journal of the Science of Food and Agriculture, 76*, 123–128.
AOAC (2005). *Official method 931.04 moisture in cocoa products* (18th ed.). Washington, DC: Association of Official Analytical Chemists.
Aprotosoaie, A. C., Luca, S. V., & Miron, A. (2015). Flavor chemistry of cocoa and cocoa products-an overview. *Comprehensive Reviews in Food Science and Food Safety, 15*, 73–91.
Aroyeun, S. O., Ogunbayo, J. O., & Olaiya, A. O. (2006). Effect of modified packaging and storage time of cocoa pods on the commercial quality of cocoa beans. *British Food Journal, 108*(2), 141–151. http://dx.doi.org/10.1108/00070700610644951.
Beckett, S. T. (2009). *Industrial chocolate manufacture and use* (4th ed.). York, UK: Wiley-Blackwell.
Biehl, B., Meyer, B., Meyer, B., Crone, G., Pollmann, L., & Said, M. B. (1989). Chemical and physical changes in the pulp during ripening and post-harvest storage of cocoa pods. *Journal of the Science of Food and Agriculture, 48*, 189–208.
Biehl, B., & Voigt, J. (1999). Biochemistry of cocoa flavour precursors. *Proceedings of the 12th international cocoa research conference* (pp. 929–938). Salvador, Brazil: Cocoa Producers Alliance.
Bonvechi, J. S. (2005). Investigation of aromatic compounds in roasted cocoa powder. *European Food Research and Technology, 221*, 19–29.
Camu, N., De Winter, T., Addo, S. K., Takrama, J. S., Bernaert, H., & De Vuyst, L. (2008). Fermentation of cocoa beans: Influence of microbial activities and polyphenol concentrations on the flavour of chocolate. *Journal of the Science of Food and Agriculture, 88*(13), 2288–2297.
De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., ... Verhé, R. (2005). Influence of storage practices on acrylamide formation during potato frying. *Journal of Agricultural and Food Chemistry, 53*, 6550–6557.
Doornbos, T., Van Den Ouweland, G. A. M., & Tjan, S. B. (1981). Amadori compounds, derived from 6-deoxy sugars, as flavour precursor. In C. Eriksson (Ed.). *Progress in food and nutrition science, Maillard reactions in food* (pp. 57–63). Oxford: Pergamon Press.
Duncan, R. J. E. (1984). A survey of Ghanaian cocoa farmers' fermentation and drying practices and their implication for Malaysian practices. *Proceedings of International Conference on Cocoa and Coconut, October 15–17, Malaysia*.
Gerrard, J. A., Newton, A. E., Fairbanks, A. J., Golding, M., & Andrewes, P. (2012). The role of the Maillard reaction in the formation of flavour compounds in dairy products – Not only a deleterious reaction but also a rich source of flavour compounds. *Food & Function, 3*, 1231–1241.
Gourieva, K.B., & Tserevitinov, O.B. (1979). Methods of evaluating the degree of fermentation of cocoa beans. USSR Patent No. 64654.
Henderson, J. W., Ricker, R. D., Bidlingmeyer, B. A., & Woodward, C. (2000). Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids. *Application note 5980-1193E Agilent Technologies*.
Kadow, D., Bohlmann, J., Phillips, W., & Lieberei, R. (2013). Identification of main fine or flavour components in two genotypes of the cocoa tree (Theobroma cacao L.). *Journal of Applied Botany and Food Quality, 86*, 90–98.
Kerler, J., Van der Ven, J. G. M., & Weenen, H. (1997). α-Acetyl-N-heterocycles in the Maillard reaction. *Food Reviews International, 13*, 553–575. http://dx.doi.org/10.1080/87559129709541140.
Kirchhoff, P. M., Biehl, B., Ziegeler-Berghausen, H., Hammoor, M., & Lieberei, R. (1989). Kinetics of the formation of free amino acids in cocoa seeds during fermentation. *Food Chemistry,* (3), 161–179.
Kongor, J. E., De Steur, H., Van de Walle, D., Gellynck, X., Afoakwa, E. O., Boeckx, P., & Dewettinck, K. (2017). *Constraints for future cocoa production in Ghana.* Springer; Agroforest Syst.http://dx.doi.org/10.1007/s10457-017-0082-9.
Kongor, J. E., Hinneh, M., Van de Walle, D., Afoakwa, E. O., Boeckx, P., & Dewettinck, K. (2016). Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile — A review. *Food Research International, 82*, 44–52.
Kongor, J. E., Takrama, J. F., Budu, A. S., Mensah-Brown, H., & Afoakwa, E. O. (2013). Effects of fermentation and drying on the fermentation index and cut tests of pulp pre-conditioned Ghanaian cocoa (Theobroma cacao) beans. *Journal of Food Science and Engineering, 3*, 625–634.
Lima, L. J., Almeida, M. H., Nout, M. R., & Zwietering, M. H. (2011). Theobroma cacao L., "The food of the Gods": Quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. *Critical Reviews in Food Science and Nutrition, 51*(8), 731–761.
Marseglia, A., Palla, G., & Caligiani, A. (2014). Presence and variation of γ-aminobutyric acid and other free amino acids in cocoa beans from different geographical origins. *Food Research International, 63*, 360–366.
Mestdagh, F., Kerkaert, B., Cucu, T., & De Meulanaer, B. D. (2011). Interaction between proteins and lipids during light-induced oxidation. *Food Chemistry, 126*, 1190–1197.
Meyer, B., Biehl, B., Said, M., & Samarakoddy, R. J. (1989). Postharvest pod storage: A method for pulp preconditioning to impair strong nib acidification during cocoa fermentation in Malaysia. *Journal of the Science of Food and Agriculture, 48*, 285–304.
Miralles-Garcia, J. (2008). *Chemical composition and flavour development of cocoa products by thermal and enzymatic technologies. PhD Thesis presented to School of Environment and Life Science.* University of Salford.
Mottram, D. S. (2007). The maillard reaction: Source of flavour in thermally processed foods. In R. G. Berger (Ed.). *Flavours and fragrances: Chemistry, bioprocessing and sustainability* (pp. 269–284). Hannover, Germany: Springer-Verlag.
Pakiyasothy, E. V., Jansz, E. R., Senanayake, U. M., Wijesundara, R. C., & Wickremasinghe, P. (1981). Effect of maturity on some chemical components of cocoa. *Journal of the Science of Food and Agriculture, 32*, 873–876.
Pettipher, G. L. (1986). Analysis of cocoa pulp and the formulation of a standardised artificial cocoa pulp medium. *Journal of the Science of Food and Agriculture, 37*, 297–309.
Puziah, H., Jinap, S., Sharifah, K. S. M., & Asbi, A. (1998). Effect of mass and turning time on free amino acids, peptide-N, sugar and pyrazine concentration during cocoa fermentation. *Journal of the Science of Food and Agriculture, 78*, 543–550.
Reineccius, G. A., Andersen, D. A., Kavanagh, T. E., & Keeney, P. G. (1972). Identification and quantification of the free sugars in cocoa beans. *Journal of Agriculture and Food Chemistry, 20*, 199–202.
Rodriguez-Campos, J., Escalona-Buendia, H. B., Contreras-Ramos, S. M., Orozco-Avila, I., Jaramillo-Flores, E., & Lugo-Cervantes, E. (2012). Effect of fermentation time and drying temperature on volatile compounds in cocoa. *Food Chemistry, 132*(1), 277–288.
Rohsius, C., Matissek, R., & Lieberei, R. (2006). Free amino acid amounts in raw cocoas from different origins. *European Food Research and Technology, 222*, 432–438.
Said, M., Meyer, B., & Biehl, B. (1987). Pulp preconditioning – A new approach towards quality improvement of Malaysian cocoa beans. *Perak Planters Association Journal, 63*, 35–47.
Schuster, R. (1988). Determination of amino acids in biological, pharmaceutical, plant and food samples by automated pecolumn derivatization and high performance liquid chromatography. *Journal of Chromatography-Biomedical Applications, 431*, 271–284.
Sols, A., Gancedo, C., & DelaFuente, G. (1971). Energy-yielding metabolism in yeasts. In A. H. Rose, & J. S. Harrison (Eds.). *Physiology und Biochemistry of Yeasts* (pp. 271–307). New York: Academic Press.
Sulaiman, B. K., Yang, A. T., & Ariffin, F. (2017). Colour and antioxidant properties of cocoa beans from pods storage and fermentation using shallow box. *MATTER: International Journal of Science and Technology, 3*(2), 295–307.
Takrama, J. F., Aculey, P. C., & Aneani, F. (2006). Fermentation of cocoa with placenta: A scientific study. *Proceedings of 15th International Cocoa Research Conference, Costa Rica. vol. II. Proceedings of 15th International Cocoa Research Conference, Costa Rica* (pp. 1373–1379).
Tran, P. D., Van de Walle, D., De Clercq, N., De Winne, A., Kadow, D., Lieberei, R., ... Van Durme, J. (2015). Assessing cocoa aroma quality by multiple analytical approaches. *Food Research International, 77*, 657–669.
Van Gemert, L. J. (2011). *ODOUR THRESHOLDS: Compilations of odour threshold values in air, water and other media* (Second ed.). BV, Utrecht: Oliemans, Punter & Partners.
Voigt, J., Biehl, B., & Heinrich, H. (1994). In vitro formation of cocoa-specific aroma precursors: Aroma-related peptides generated from cocoa-seed protein by co-operation of an aspartic endoprotease and a carboxypeptidase. *Food Chemistry, 49*, 173–180.
Voigt, J., Heinrichs, H., Voigt, G., & Biehl, B. (1994). Cocoa-specific aroma precursors are generated by proteolytic digestion of the vicilin-like globulin of cocoa seeds. *Food Chemistry, 50*, 177–184.
Wills, R., McGlasson, W. B., Graham, D., & Joyce, D. (1998). *Post-harvest: An introduction to the physiology and handling of fruits, vegetables and ornamentals.* Wallingford: CAB International262.
Wood, G. A. R., & Lass, R. A. (1985). *Cocoa* (4th ed.). London: Longman.