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Changes in acidification, sugars and mineral composition of cocoa pulp during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans

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Abstract

Changes in acidification, sugars and mineral composition of cocoa pulp during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans were investigated using a 4 x 3 full factorial experimental design with pod storage and fermentation time as principal factors. pH, non-volatile (titratable) acidity, reducing sugars, total solids and mineral composition of cocoa pulp were studied using standard analytical methods. pH of the pulp increased with increasing pod storage and fermentation with consequential decrease in non-volatile acidity. Contrary, both pod storage and fermentation decreased the reducing sugars and total solids of the pulp. The most abundant mineral in unfermented cocoa pulp was calcium, followed by potassium and sodium with values of 316.92 mg/100 g, 255.12 mg/100 g and 103.26 mg/100 g respectively. Zinc was the mineral with the least concentration of 1.04 mg/100 g, whilst iron and magnesium had appreciable values of 4.26 mg/100 g and 32.52 mg/100 g respectively. Pod storage and fermentation however showed variable effects on the studied minerals. Pod storage caused reductions in acidity, fermentable sugars and some minerals in cocoa pulp during fermentation.

Introduction

Cocoa (*Theobroma cacao* L.), generally known to have originated from Central and Southern America is an important agricultural export commodity in the world and forms the backbone of the economies of some countries in West Africa, such as Cote d'Ivoire and Ghana (Afoakwa *et al.*, 2011a). Cocoa beans are the seeds obtained from cocoa pods and is made up of three main parts namely, the testa (seed coat), the embryo and the cotyledon (Thompson *et al*., 2001; Afoakwa, 2010). Attached to the testa is the sugary, white mucilaginous pulp which is formed during pod development from an endocarp meristem (Biehl and Ziegleder, 2003).

Cocoa pulp is reported to be rich in fermentable sugars, such as glucose, fructose and sucrose, and has a low pH of 3.0–3.5, mainly because of the presence of citric acid (Guehi *et al.*, 2010) making it a rich medium for microbial growth (Lefeber *et al*., 2010). Proteins, free amino acids, vitamins, and minerals are also present in the pulp. Work done by Lefeber *et al.* (2010) showed that the ratio of sucrose, glucose, and fructose present in the pulp varies with the age of the pod. The pulp is also reported to contain high amount of pectin and other polysaccharides which make the pulp viscous, limiting diffusion of air through the beans during fermentation (Schwan *et al.*, 1995).

During cocoa bean fermentation, microorganisms grow in the pulp and produce a diversity of metabolites such as organic acids, along with substantial heat. The organic acids and heat diffuse into the cocoa seeds, killing them and disrupting their cellular integrity (Biehl *et al*., 1990, Voigt *et al*., 1994). Production of acids in the pulp is important in cocoa fermentation as these acids diffuse into the beans and subsequently induce the important biochemical reactions leading to well fermented cocoa beans. However, high acid production in the pulp is detrimental as it leads to excessive acid diffusing into the beans resulting in the production of acidic beans, thus changes in acidity during fermentation of cocoa is crucial to the final bean quality. Ostovar and Keeney (1973) reported that the pulp is the substrate metabolized by a sequence of microorganisms during fermentation, and Afoakwa *et al.* (2011b) stated that since the properties of the substrate determine microbial development and metabolism, changes in the pulp may affect the production of acids by lactic acid bacteria, yeasts and acetic acid bacteria.

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Pulp pre-conditioning involves changing the properties of the pulp prior to the development of microorganisms in fermentation (Afoakwa *et al*., 2011b). The technique can be employed in three ways prior to fermentation - pod storage, mechanical or enzymatic depulping and bean spreading (Wood and Lass 1985; Biehl *et al*., 1989; Schwan and Wheals 2004; Afoakwa *et al*., 2012), and these lead to varying modifications in the moisture content, sugar concentration, volume of pulp per seed as well as pH and acidity of the pulp during cocoa bean fermentation.

Cocoa fermentation is crucial for the development of quality beans and it is reported to be influenced by factors such as pod storage (Afoakwa *et al*., 2011b). Previous reports have explained that pod storage results in decreases in pulp volume per seed due to water evaporation during fermentation (Biehl *et al*., 1989; Nazaruddin *et al*., 2006; Afoakwa *et al*., 2011b). However, the extent to which pod storage would influence the mineral composition in cocoa pulp, changes in pulp sugars with consequential production of organic acids in the pulp during fermentation still remains unclear. Thus, this study was aimed at investigating changes in acidification, sugars and mineral composition of cocoa pulp during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans.

Materials and Methods

Material

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

About 30 kg of extracted cocoa beans were placed in woven baskets lined with banana leaves. The surface were also covered with banana leaves and fermented for six days with consecutive opening and turning every 48 h. 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 to 80 cm from upper surface).

Preparation of the pulp samples

The pulp was manually separated from the beans by rubbing the beans (with adhering pulp) between fingers and squeezing the pulp into clean sample bag. The pulp was then stored at -20°C prior to analyses.

Experimental design

A 4 x 3 full factorial experimental design was used for the study. The principal factors investigated were pod storage (0, 3, 7, 10 days) and fermentation time (0, 3, 6 days). The pH, non-volatile (titratable) acidity, reducing sugars, total solids and mineral composition of the pulp were studied.

pH and non-volatile (titratable) acidity

pH and non-volatile (titratable) acidity of the pulp were determined using the method described by Nazaruddin *et al.* (2006) with slight modifications. Ten grams of the pulp was homogenized in 90 ml of hot distilled water, stirred manually for 30 s and filtered using Whatman No. 4® filter paper and cooled to 20–25°C. Twenty five (25) ml aliquot of the resulting filtrate was pipetted into a beaker and the pH was measured using a pH meter (model MP230 Mettler Toledo MP 230, Mettler Company Limited, Geneva, Switzerland) calibrated with buffers at pH 4.01, 7.00 and 9.21. A further 10 ml aliquot was used to determine acidity by titration to an end point pH of 8.1with 0.1 N NaOH solution and the values reported as moles of sodium hydroxide per 100 g sample. The analysis was conducted in triplicates and the mean values reported.

Determination of reducing sugars

Reducing sugars of the pulp was determined using the phenol sulphuric acid method as described by Brummer and Cui (2005) with slight modifications. About 0.5 g of the pulp was boiled in 30 ml 80% ethanol under reflux for 30 minutes. The supernatant decanted into another round bottom flask. The collected supernatant was concentrated (not to dryness) under reduced pressure using the rotary evaporator. After the removal of ethanol, the extract was then clarified using 7.2 ml of 5% $ZnSO_4$ and 10 ml of 0.3 N barium hydroxide octahydrate [$Ba(OH)_2.8H_2O$] to precipitate proteins, colour, and other organic substances out of the solution and allowed to stand for about 5 minutes and then filtered.

A mixture of Zeokarb 225 (H^+), a cation and anion exchange resin and deactivated $Fe(OH)_2$ was added to the filtrate to rid it of ions, shaken and filtered. 1ml phenol and 5 ml H_2SO_4 reagents were added to 1ml of the extract and allowed to stand for an hour and absorbance read at 480 nm. A standard glucose solution of 20, 40, 60, 80, and 100 ppm was prepared and the absorbance read at 480 nm and a standard curve drawn. From the standard graph, the amount of reducing sugars present in the samples was calculated and results expressed as mg/g of cocoa pulp. The analysis was conducted in triplicates and the mean values reported.

Determination of total solids of cocoa pulp

Total solids of the pulp were determined using the method described by Javaid *et al*. (2009) with slight modifications. Two (2) grams of pulp sample was weighed into a pre-weighed flat bottom dish and transferred to a hot air oven at 101°C for 2 hours. Dried samples were transferred to a desiccator having silica gel as desiccant. After 1 h, the dish was weighed and kept in an oven for further drying (30 mins). The heating, cooling, and weighing processes were repeated until constant weight was achieved. Total solids content was calculated by the following formula: Total solids (%) = weight of dried pulp sample/ weight of fresh pulp sample x 100.

Mineral analyses

Wet digestion

Mineral analyses were determined using AOAC (2005) methods with slight modifications. About 0.5 g of the sample was weighed into a 250 ml beaker. Twenty five ml (25 ml) of concentrated nitric acid was added and the beaker covered with a watch glass. The sample was digested with great care on a hot plate in a fume chamber until the solution was pale yellow. The solution was cooled and 1 ml perchloric acid (70% $HCLO_4$) added. The digestion was continued until the solution was colourless or nearly so (the evaluation of dense white fumes was regarded to be indicative of the removal of nitric acid). When the digestion was completed, the solution was cooled slightly and 30 ml of distilled water added. The mixture was brought to boil for about 10 min and filtered hot into a 100 ml volumetric flask using a Whatman No. 4® filter paper. The solution was then made to the mark with distilled water.

Determination of Ca, Mg, Zn, Fe, Na and K

The concentrations of Ca, Mg, Zn, Fe, Na and K of the pulp were determined using Spectra AA 220FS Spectrophotometer (Varian Co., Mulgrave, Australia) with an acetylene flame. One (1) ml aliquots of the digest was used to determine the Ca, Mg, Zn, Fe, Na and K content of the samples.

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 ($p < 0.05$).

Results and Discussions

Changes in pH profile of cocoa pulp

The pH of unfermented cocoa pulp has been reported to range between 3.3–4.0, primarily due to a high concentration of citric acid (Thompson *et al*., 2001; Ardhana and Fleet, 2003; Schwan and Wheals, 2004). The pH of the freshly harvested unfermented cocoa pulp was highly acidic (3.88) which increased gradually to 4.02 after 10 days of pod storage (Figure 1). The gradual increase in pH of the pulp during pod storage might be due to the breakdown of the pulp sugars, which has been reported to reduce the pulp volume per seed leading to the decrease in citric acid concentrations (Biehl *et al*., 1989; Sanagi *et al*., 1997) hence, increasing the pH of the pulp.

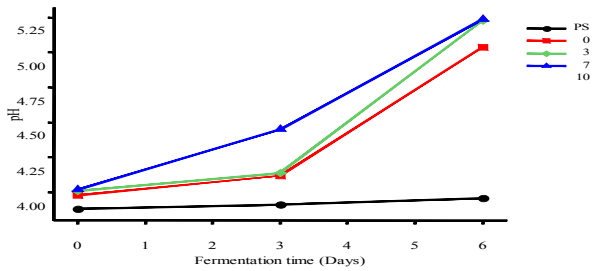

Figure 1. Changes in pH of cocoa pulp during fermentation and pod storage (PS)

Fermentation caused consistent increases in the pH of the pulp at all the pod storage days (Figure 1). During fermentation, pH increased from 3.88–3.96 for the unstored pods, 3.98–5.04 for pods stored for 3 days, 4.01–5.23 for 7 days pod storage, and 4.02–5.24 for pods stored for 10 days at the end of the sixth day of fermentation. These findings were in agreement with reports by previous investigators. Yusep *et al*. (2002) found the pH of cocoa pulp to increase progressively from 3.80 to 4.80 at 3 days of fermentation. Ardhana and Fleet (2003) found the pH of cocoa pulp to increase from between 3.7–3.9 at the start of fermentation to between 4.8–4.9 by the end of fermentation. Nielsen *et al*. (2007) also recorded an increase in pH of the pulp from the starting

value of 3.94–4.12 to 4.28–4.69 after 96 hours of fermentation. These gradual increases in pH of the pulp during fermentation are suspected to be due to the reported decline in citric acid concentration (Schwan and Wheals, 2004; Jespersen *et al*., 2005). During fermentation, yeasts and lactic acid bacteria breakdown the citric acid in the pulp to metabolize the pulp sugars leading to an increase in the pH from 3.5 to 4.2 (Schwan *et al*., 1995; Schwan, 1998; Schwan and Wheals, 2004; Jespersen *et al*., 2005). Nielsen *et al*. (2007) reported a decline in the citric acid concentration to low or even non-detectable levels during the first 12 h of fermentation. Statistical analysis on the data showed that both pod storage and fermentation significantly ($p < 0.05$) affected the pH of the pulp (Table 1).

Table 1. ANOVA summary showing F-ratios of the pH, non-volatile (titratable) acidity, reducing sugars and total solids of cocoa pulp during fermentation and pod storage

Variables	pH	Titratable acidity	Reducing sugars	Total solids
Pod storage (PS)	15.10*	179.18*	807.78*	4.58*
Fermentation (FT)	54.02*	686.74*	25545.21*	9.71*
Interaction (PS X FT)	5.51*	18.47*	291.80*	0.42

*Significant at $p<0.05$

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

During fermentation of cocoa beans, microorganisms breakdown the sugars in the pulp resulting in the production of alcohols and organic acids, predominantly acetic acid which then diffuse into the beans. Production of acids in the pulp is important in cocoa fermentation as these acids diffuse into the beans and subsequently induce the important biochemical reactions leading to well fermented cocoa beans. However, high acid production in the pulp is detrimental as it leads to excessive acid diffusing into the beans resulting in the production of acidic beans.

Fermentation caused significant ($p < 0.05$) increases in the acidity levels in the pulp reaching a maximum at day 3 of fermentation after which the titratable acidity decreased considerably till the end of fermentation, and this was noted at all pod storage treatments (Figure 2). The acidity level was highest at 3 days of fermentation as majority of the pulp sugars were probably degraded into alcohols which was then oxidized to acetic acid by acetic acid bacteria within 3 days of fermentation. Ardhana and Fleet (2003) reported high concentration of 10 mg/g acetic acid in cocoa pulp at 72 h (3 days) of fermentation. Acidity levels decreased after day 3 of fermentation because at that time, most of the acid produced diffused into the beans. Again, as the pulp volume reduced, there was improvement in aeration in the fermenting mass leading to the evaporation of volatile acids like acetic acid. Even though pH of the pulp increased within 72 h of fermentation for all pod storage periods, TA also increased within 72 h of fermentation for all pod storage periods. This is because the predominant acid in the unfermented pulp is citric acid and as fermentation progress (after 48 h), the predominant acid in the pulp becomes acetic acid due to the oxidation of alcohol by acetic acid bacteria. Hence the pH of the pulp recorded at 72 h of fermentation is that of acetic acid and that recorded at the onset of fermentation is citric acid and these two acids have different pKa values.

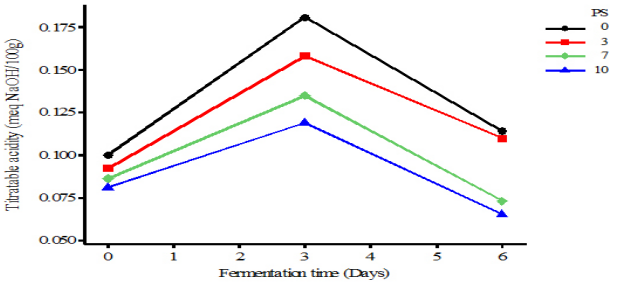

Figure 2. Changes in titratable acidity of cocoa pulp during fermentation and pod storage (PS)

Increasing pod storage (PS) significantly ($p < 0.05$) reduced titratable acidity of the pulp (Table 1). The interaction between fermentation time and pod storage also had significant ($p < 0.05$) effect on the acidity levels of the pulp (Table 1). Pulp from the unstored pods had the highest titratable acidity at day 3 of fermentation whiles pulp from pods stored for 10 days had the least titratable acidity at day 3 of fermentation. This might be due to the fact that pod storage reduced pulp volume per seed, reduced pulp sugar content and thus, increased micro-aeration within the pulp. This decreased the sugar metabolized by yeasts during subsequent fermentation and eventually reduced alcohol fermentation and acetic acid formation in the pulp (Said *et al*., 1987; Biehl *et al*., 1989; Sanagi *et al*., 1997). This suggests that pod storage as a means of pulp preconditioning could be effectively employed to reduce acidity levels in cocoa pulp during fermentation.

Changes in reducing sugars of cocoa pulp

Cocoa pulp is reported to be rich in fermentable sugars notably glucose and fructose and has a relatively low initial pH (3.3–4.0), primarily due to a high concentration of citric acid (Pettipher, 1986; Thompson *et al*., 2001; Ardhana and Fleet, 2003). Results showed that both pod storage and fermentation

time significantly ($p < 0.05$) affected the reducing sugars of the pulp (Table 1). Reducing sugars in the freshly harvested unfermented cocoa pulp decreased drastically from 75.72 ± 1.93 mg/g to 48.13 ± 0.69 mg/g after 10 days of pod storage (Figure 3). This might be due to the breakdown of reducing sugars in the pulp into energy for the physiological and metabolic activities of the beans.

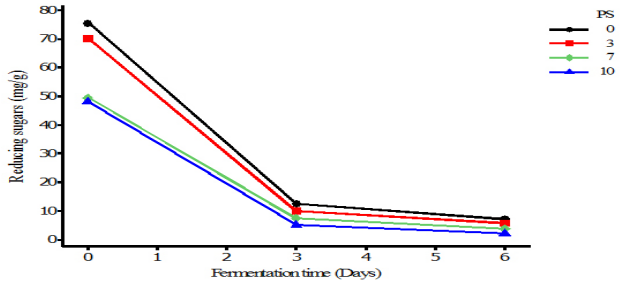

Figure 3. Changes in reducing sugars of cocoa pulp during fermentation and pod storage (PS)

Similarly, increasing fermentation time decreased the concentrations of the fermentable sugars (reducing sugars) in the pulp at all pod storage periods. The concentrations of the reducing sugars decreased from 75.72 ± 1.93 mg/g at the onset of fermentation to 7.29 ± 0.49 mg/g at the end of the 6 days of fermentation for the unstored pods (Figure 3). Similar decreasing trends were observed for all the pods that were preconditioned. The decrease in the concentrations of reducing sugars in the pulp during fermentation was probably due to the activities of yeasts and lactic acid bacteria. The high sugar content, the low pH and low oxygen tension in the pulp favour the growth of yeasts at the onset of fermentation. The yeasts metabolize the fermentable sugars in the pulp to ethanol which in turn is oxidized by acetic acid bacteria to acetic acid (Thompson *et al.*, 2001). Ardhana and Fleet (2003) observed similar trend of decrease in the concentrations of pulp fermentable sugars during fermentation. They reported a decrease in the concentrations of fructose and glucose from 62–11 mg/g and 41–7 mg/g respectively after 120 h (5 days) of fermentation for Forastero cocoa and from 42–9 mg/g and 24–5 mg/g respectively after 72 h (3 days) of fermentation for *Trinitario* cocoa. There was also significant ($p < 0.05$) interaction between pod storage and fermentation on the reducing sugars of the pulp (Table 1).

Changes in total solids of cocoa pulp

Results showed that fermentation significantly ($p < 0.05$) influenced the total solids in the cocoa pulp at all pod storage periods (Table 1). Total solids decreased drastically within the first 3 days of fermentation from 20.5–16.6%, 19.9–16.2%, 17.6–15.5% and 16.6–14.3% respectively for pods stored for 0, 3, 7 and 10 days (Figure 4). The decrease in total solids during the first 3 days of fermentation might largely be to the breakdown of sugars in the pulp by yeasts and lactic acid bacteria. Yeasts and lactic acid bacteria have been reported to metabolize the fermentable sugars in cocoa pulp during fermentation to produce ethanol and lactic acid respectively (Thompson *et al.*, 2001; Ardhana and Fleet, 2003). Total solids however, increased slightly towards the end of fermentation for all pod storage treatments. This slight increase in total solids might probably be due to the accumulation of some microbial metabolites.

Total solids in the pulp decreased significantly ($p < 0.05$) with increasing pod storage (Figure 4). Total solids in the unfermented pulp decreased from 20.5% for the non preconditioned pulp to 16.6% for pulp preconditioned for 10 days. Similar trend of decrease was observed for all fermentation times. At the end of fermentation, total solids of the fermented pulp decreased from 16.6% for the non preconditioned pulp to 14.3% for pulp preconditioned for 10 days. The observed changes might be due to changes in the fermentable sugars and other constituents of the pulp thereby reducing the total solid content in the pulp during pod storage. There was however, no significant ($p > 0.05$) interaction between pod storage and fermentation on the total solids in the pulp (Table 1).

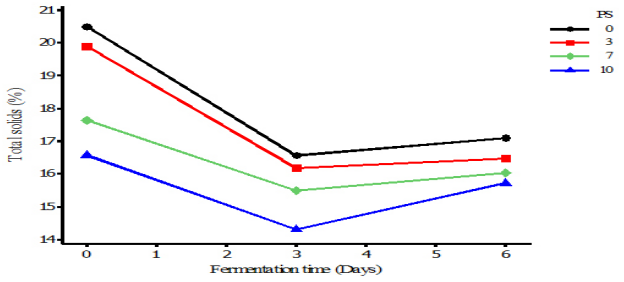

Figure 4. Changes in total solids of cocoa pulp during fermentation and pod storage (PS)

Changes in mineral composition of cocoa pulp

Changes in mineral composition of cocoa pulp during fermentation for all pod storage periods are presented in Table 2. The results showed that the most abundant minerals in the freshly harvested and unfermented cocoa pulp was calcium, followed by potassium and sodium with values of 316.92 mg/100 g, 255.12 mg/100 g and 103.26 mg/100 g respectively (Table 2). Zinc is the mineral with the least concentration of 1.04 mg/100 g, whilst iron and magnesium had appreciable values of 4.26 mg/100 g and 32.52 mg/100 g respectively. Pod storage and fermentation however showed variable trends in the

Table 2. Changes in mineral composition (mg/100 g) of cocoa pulp during fermentation of pulp pre-conditioned cocoa beans

Pod storage (Days)	Fermentation time (Days)	Na	Fe	Ca	Mg	Zn	K
0	0	103.26±1.00	4.26±1.00	316.92±0.47	32.52±1.08	1.04±0.04	255.12±1.97
	3	48.12±2.00	3.56±0.58	347.50±0.50	33.98±1.03	2.22±0.89	226.06±2.65
	6	17.48±2.08	2.58±0.43	395.80±1.15	148.32±0.84	4.58±0.47	643.36±0.57
3	0	40.60±1.15	3.42±0.58	248.42±2.08	35.44±0.58	2.44±0.53	155.46±1.10
	3	33.60±0.58	2.90±0.57	275.78±2.08	134.46±0.50	2.68±0.57	408.64±0.45
	6	9.54±2.08	1.42±0.57	374.50±1.92	156.70±2.08	2.78±0.48	569.06±1.00
7	0	20.18±1.00	3.58±0.36	190.12±1.00	29.56±0.48	0.64±0.00	252.52±0.50
	3	12.70±1.53	2.04±1.00	260.00±0.58	97.96±0.91	1.16±0.20	507.16±0.92
	6	9.66±1.53	1.90±0.57	341.14±1.00	144.42±0.54	1.20±0.10	621.10±1.00
10	0	14.15±2.00	5.26±0.87	307.68±1.53	36.10±0.00	0.30±0.01	289.62±1.38
	3	11.72±5.63	2.96±0.46	301.40±1.49	71.02±1.00	0.56±0.02	392.36±1.53
	6	8.68±0.58	2.30±0.00	189.20±1.65	165.66±0.58	0.98±0.02	815.06±2.00

Mean values ± standard deviation

studied mineral content (Table 2).

Both pod storage and fermentation caused consistent decreases in the composition of sodium and iron. Sodium concentration decreased drastically from 103.26 ± 1.00 mg/100 g at the start of fermentation to 17.48 ± 2.08 mg/100 g at the end of fermentation (6 days) for the unstored pods. Similar decreasing trends in sodium concentration were observed for all pod storage treatments (Table 2). Pod storage also led to decreases in iron concentrations at all fermentation times with values decreasing from 4.26 ± 1.00 mg/100 g at the start of fermentation to 2.58 ± 0.43 mg/100 g at the end of fermentation for the unstored pods (Table 2). Similar decreasing trends were observed for all pod storage treatments (Table 2). The decrease in the concentration of sodium and iron might be due to the utilization of these minerals by the different microorganisms involved in fermentation for their physiological and metabolic activities.

Increasing fermentation time increased the composition of calcium, magnesium, zinc and potassium at all pod storage periods (Table 2). Potassium levels in the unfermented pulp increased from 255.12 mg/100 g to 643.36 mg/100 g by the sixth day of fermentation for the unstored pods. Similar increasing trends were observed for the different pod storage treatments. Pod storage also increased potassium concentration significantly at all fermentation times (Table 2).

Calcium and magnesium contents in the pulp also increased significantly with increasing fermentation time for all pod storage periods, with values increasing from 316.92 mg/100 g and 32.52 mg/100 g respectively at the onset of fermentation to 395.80 mg/100 g and 148.32 mg/100 g at the end of the 6 days of fermentation for the unstored pods. Similar trends were observed at all the pod storage treatments. Increasing pod storage also caused decreases in the concentrations of Ca at all fermentation times whiles increases in Mg content were noted. Zn composition also increased with fermentation at all pod storage periods (Table 2). The observed increases in Ca, Mg, Zn and K during fermentation might be due to the synthesis of these minerals by the microorganisms involved in the fermentation of the pulp. Statistical analysis on the data indicated that both pod storage and fermentation time significantly ($p < 0.05$) influenced the mineral composition of cocoa pulp with significant interaction observed for sodium, calcium, magnesium, zinc and potassium (Table 3).

Table 3. ANOVA summary table showing F-ratios for variation in mineral content of cocoa pulp during fermentation of pulp pre-conditioned cocoa beans

Variables	Na	Fe	Ca	Mg	Zn	K
Pod storage (PS)	824.25*	6.35*	7956.22*	2375.77*	63.94*	17170.88*
Fermentation (FT)	701.38*	32.67*	5344.20*	49984.73*	31.24*	278743.47*
Interaction (PS X FT)	228.62*	1.64	6371.91*	2064.91*	11.63*	12647.75*

* Significant at $p<0.05$

Conclusion

The pH of unfermented cocoa pulp was acidic ranging from 3.88–4.02. Pod storage and fermentation increased the pH of the pulp with consequent decrease in titratable acidity. At the end of fermentation, the pH of the pulp increased from 3.88–3.96 for the unstored pods, 3.98–5.04 for pods stored for 3 days, 4.01–5.23 for 7 days pod storage, and 4.02–5.24 for pods stored for 10 days. The gradual increase

in pH during fermentation was probably due to the breakdown of citric acid in the pulp by yeasts and lactic acid bacteria. Increasing pod storage and fermentation time also decreased the reducing sugars and total solids in the pulp. Changes in mineral composition of cocoa pulp during fermentation of pulp pre-conditioned (pod storage) cocoa were observed. The most abundant minerals in the freshly harvested and unfermented cocoa pulp was calcium, followed by potassium and sodium with values of 316.92 mg/100 g, 255.12 mg/100 g and 103.26 mg/100 g respectively. Zinc was the mineral with the least concentration of 1.04 mg/100 g, whilst iron and magnesium had appreciable values of 4.26 mg/100 g and 32.52 mg/100 g respectively. Pod storage and fermentation however showed variable effects on the studied minerals. Pod storage led to decreases in sodium and iron concentrations at all fermentation times. Increasing fermentation time increased the composition of calcium, magnesium, zinc and potassium at all pod storage periods.

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