

# Effects of Fermentation and Drying on the Fermentation Index and Cut Test of Pulp Pre-conditioned Ghanaian Cocoa (*Theobroma cacao*) Beans

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**Abstract:** Investigations were conducted to establish effects of fermentation and drying on the fermentation index (FI) and cut test of pulp pre-conditioned Ghanaian cocoa beans using a 4 × 3 full factorial experimental design with the principal factors being pod storage (0, 3, 7 and 10 d) and fermentation time (0, 3 and 6 d) to study the changes occurring during the fermentation process. The study also used a 4 × 3 full factorial design with pod storage (0, 3, 7 and 10 d) and drying time (0, 3 and 7 d) being the principal factors investigated to study the changes occurring during the drying process. FI and cut test of the beans were studied during fermentation as well as the drying process. FI of the beans increased significantly with pod storage and fermentation but decreased slightly during drying. FI of the unfermented beans increased slightly from 0.674 for the unstored pods to 0.763 after 10 days of pod storage. The FI of the fermented beans (six days fermentation) also increased from 1.390 for the unstored pods to 1.424 for pods stored for 10 days. It decreased from 1.389 at the start of drying for the unstored pods to 1.105 for pods stored for 10 days at the end of drying (seven days). FI of all the beans were however, above 1.0 at the end of fermentation and drying for all pod storage treatments. Cut test revealed that storage of pods for 3, 7 and 10 days increased the percentage of brown beans by 66%, 94% and 72%, respectively, by the sixth day of fermentation. Percentage of brown beans decreased to 61%, 76% and 63%, respectively, for pods stored for 3, 7 and 10 d at the end of drying (seven days). Cocoa pods can be stored for up to 10 days, fermented for six days and dried for seven days with the necessary formation of brown pigments characteristics of well fermented and dried cocoa beans.

**Key words:** *Theobroma cacao*, pod storage, pulp pre-conditioning, fermentation, drying, fermentation index, cut test.

## 1. Introduction

Indicators of well-fermented and dried quality cocoa beans are good brown colour, low astringency and bitterness and an absence of off-flavours such as smoky notes and excessive acidity [1]. The development of good brown cocoa bean colour as well as chocolate flavour begins with the chemical and biochemical changes occurring within the cocoa bean during

fermentation and drying. Fermentation is essential for development of appropriate flavours from precursors. The mode of fermentation and drying provides the necessary conditions for complex biochemical reactions to occur [1-4].

Flavour potential of cocoa is measured by the fermentative quality and this in turn is measured by a colour index called the fermentation index (FI) [5]. FI is a measure of brownness of cocoa nibs and it is measured to ascertain the degree of fermentation of the beans [6]. It is determined spectrophotometrically as the ratio of absorbance of cocoa pigments at 460 nm to

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that at 530 nm [7]. Fermented cocoa beans with FI values of less than one indicate under fermentation while fermented beans with FI values of one and above are considered to be well fermented [8]. FI is a more objective measure of fermentation compared to the simple cut test but has no practical application on the field [5].

At a visual level, the overall colour of cocoa beans is measured as part of a cut test [9, 10]. The cut test is the simplest and still the most widely used method to assess the quality of a random sample of beans from a batch by visual evaluation of the cut beans [11]. It is used for the evaluation of sanitary quality of beans [12] and also assesses the degree of fermentation by counting the fully brown, brown/purple and purple coloured beans [13]. For the determination, 300 beans are opened or cut lengthwise through the middle, so as to expose the maximum cut surface of cotyledons. Both halves of each bean are visually examined in full daylight or equivalent artificial light. Each defective type of bean is counted separately, and the result for each kind of defect expressed as a percentage of the 300 beans examined. The test identifies beans that are visibly mouldy, slaty (i.e., unfermented), infested, germinated, flat (i.e., containing no nib or cotyledon) and purple or brown [13]. It is however, extremely subjective, and Lopez and Dimick [11] noted that it is at best limited to the measurement of bean defects and colour.

Slaty beans are beans in which more than 50% of the cotyledon is grey or slaty in colour [13], and have rubbery cotyledon and resistance to cutting [14]. These beans have not undergone fermentation and they have a low level of cocoa flavour with high levels of astringency. Germinated beans are those where the seed has started to grow before being killed during the fermentation or drying process and the shell has been pierced by the growth of the first root [11]. In the dry germinated bean, the root usually drops out, leaving a hole, which makes the bean more easily attacked by insects and moulds. Purple beans occur when the fermentation has been terminated prematurely [14].

Flat beans are those which have begun to form, but have not developed or filled out. There is no useful cotyledon in them so they simply add to the shell content, which is waste. Insect-damaged beans are those which have been penetrated by insects that feed on the cotyledon. These should not be present. Any number will involve loss of material and a risk of contamination with fragments of the insect. Defective beans are the sum of germinated beans, infested beans and flat beans while fully brown beans are well-fermented beans. Results are expressed as a percentage. A batch of cocoa beans with more than 60% fully brown colour beans is considered as good-quality product [15]. The grading criteria and quality categories of commercial cocoa beans are specified in the International Cocoa Ordinance [10]. International cocoa trading bodies define quality in terms of degree of fermentation and the extent of defects present [9, 10]. Other aspects of quality include fat percent, cocoa butter hardness, level of acidity, low shell percent, absence of off odours [9, 16] and flavour.

Cocoa fermentation and drying are crucial for the development of quality beans and are reported to be influenced by factors such as pod storage [17]. Pod storage as a means of pulp pre-conditioning cocoa beans prior to fermentation has been reported to cause decreases in pulp volume per seed due to water evaporation during fermentation [3, 17, 18] which in turn influenced the activity of polyphenol oxidase during fermentation and drying, with resultant modifications in the polyphenolic and anthocyanin concentrations [19]. The modifications in the polyphenolic and anthocyanin concentrations are expected to influence the fermentative quality of the beans. Thus, this study was aimed at investigating effects of fermentation and drying on the FI and cut test of pulp pre-conditioned Ghanaian cocoa beans.

## **2. Materials and Methods**

### *2.1 Materials*

Ripe cocoa pods (mixed hybrids) were obtained

from the Cocoa Research Institute of Ghana (CRIG), Tafo-Akim, Eastern Region. Cocoa pods of uniform ripeness were harvested during the major harvesting season (September-February). The harvesting was done by traditional methods (under ambient temperature during the day, 28-30 °C) and the pods transported to the fermentary 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 d, respectively. The respective pods were then split after these predetermined storage time 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 d 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% and 8%. The dried beans were then bagged in airtight black plastic bags and stored at ambient temperature (25-28 °C) in a dark room free from strong odours and used for analyses. Random sampling was done at the same time of the day and depth in the mass (40 cm to 80 cm from upper surface).

#### 2.1.1 Drying of Fermented Cocoa Beans

The fermented cocoa beans were dried in the open sun on raised platforms using the traditional process described by Afoakwa [20]. Drying started at 8 am and ended at 5 pm each day for seven days. The relative humidity during drying was between 85% and 100% each day. The beans were stirred four times each day and were covered with palm mats in the evening till the next morning. Samples were taken at 0 (undried samples or immediately after fermentation), 3 and 7 d of drying. The samples were then packaged in air tight plastic bags and taken to the laboratory for analysis. All the treatments were conducted in duplicates.

#### 2.1.2 Experimental Design

A 4 × 3 full factorial experimental design with the principal factors being pod storage (0, 3, 7 and 10 d) and fermentation time (0, 3 and 6 d) was used to study the changes occurring during the fermentation process. The study also used a 4 × 3 full factorial design with pod storage (0, 3, 7 and 10 d) and drying time (0, 3 and 7 d) being the principal factors investigated to study the changes occurring during the drying process. FI and cut test of the beans were studied during fermentation as well as the drying process.

### 2.2 Methods

#### 2.2.1 FI

FI was determined using the method described by Gourieva and Tserrevitinov [8] with slight modifications. About 0.1 g ground cocoa nibs was extracted with 50 mL of 97:3 mixture of methanol:HCl. The homogenate was allowed to stand in refrigerator (8 °C) for 20 h and then vacuum filtered. The filtrate was read in a Spectrophotometer (LKB Biochrom Novaspec II UV Spectrometer, Birmingham, UK) at 460 nm and 530 nm absorbance. The FI of the sample was obtained by calculating the ratio of absorbance at 460 nm to the absorbance at 530 nm. Three replicate readings were obtained for each sample and the mean values reported.

#### 2.2.2 Measurements of Cut Test

The cut test was performed using the international method described by Guehi et al. [12]. A total of 300 beans were cut lengthwise through the middle in order to expose the maximum cut surface of the cotyledons. Both halves were examined in full daylight and placed in one of the following categories: purple, pale purple, brown, slaty, germinated and mouldy.

### 2.3 Statistical Analyses

Statgraphics software version 3.0 (STSC, Inc., Rockville, MD, USA) was used to analyze the data for analysis of variance (ANOVA). Least significant difference (LSD) was used to separate and compare the

means, and significance was accepted at 5% level ( $p < 0.05$ ). Again, the combined effects of pulp preconditioning, fermentation time and drying time on the studied parameters were studied using the response surface methodology. Models were developed to relate pulp preconditioning and fermentation time and also pulp preconditioning and drying time on the studied parameters. The coefficients of the variables in the models and their contribution to the model's variation were reported. The  $R^2$  values were used to judge the adequacy of the models. The  $R^2$  of a model refers to the proportion of variation in the response attributed to the model rather than random error. For a good fit of a model, an  $R^2$  of at least 60% was used. All analyses were conducted in triplicates and the mean values reported.

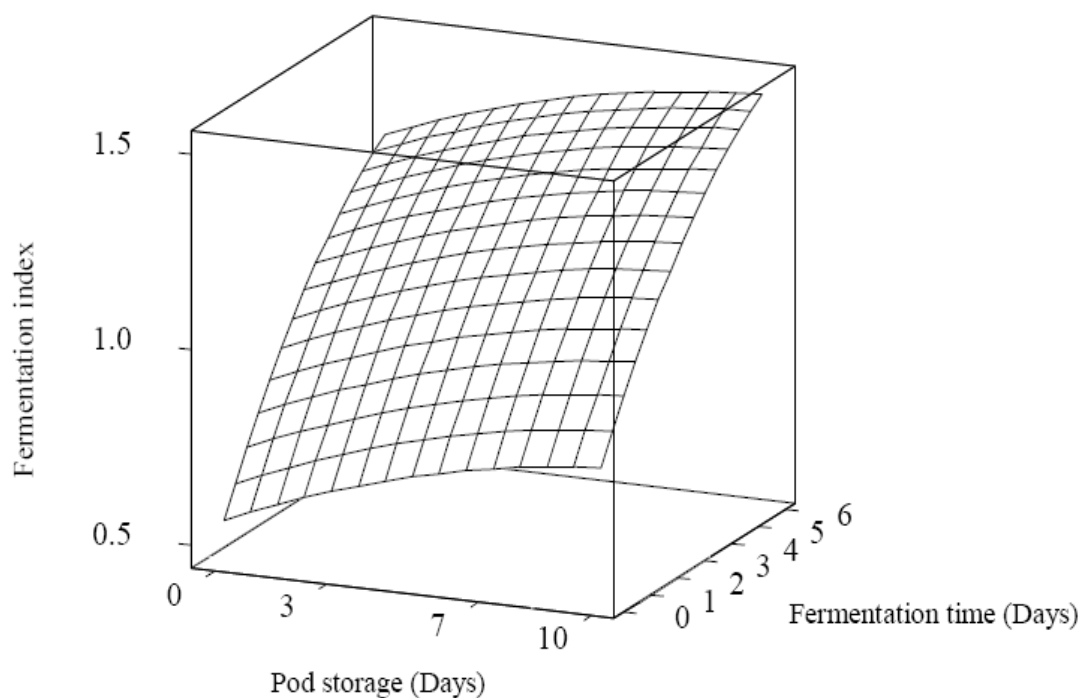
### 3. Results and Discussion

#### 3.1 Changes in FI during Fermentation

FI, as explained by Takrama et al. [6] is a measure of brownness of cocoa nibs and it is measured to ascertain the degree of fermentation of the beans. Polyphenol compounds such as anthocyanins responsible for the

characteristic purple colour of unfermented cocoa beans [21] are hydrolyzed to anthocyanidins during cocoa fermentation. Anthocyanidins then polymerize along with simple catechins to form complex tannins. Anthocyanins usually disappear rapidly during fermentation process, e.g., 93% were reportedly lost after four days fermentation [22] and colour of the beans changes from slaty over purple to brown [23]. Thus, anthocyanin content has been considered as a good index for determination of the degree of cocoa bean fermentation [24, 25]. Fermented cocoa beans with FI values below one indicate under fermentation while fermented beans with FI values of one and above are considered to be well fermented [8].

Response surface plot (Fig. 1) showed the FI of the beans during fermentation for all pod storage treatments. FI increased from 0.674 at the start of fermentation to 1.390 for the unstored pods. It also increased from 0.675 to 1.389 for pods stored for three days, 0.675 to 1.423 for pods stored for seven days and from 0.763 to 1.424 for pods stored for 10 days. The increased in FI as fermentation progressed could be due to the formation of more and more condensation



**Fig. 1** Response surface plot showing effect of pod storage and fermentation time on the FI of cocoa beans.

products of anthocyanin, such as cyanidin-3-β-D-galactosid and cyanidin-3-α-L-arabinosid [26], as a result of the breakdown of anthocyanin pigments. Afoakwa et al. [27] also observed similar trend of increasing FI with fermentation time for all pod storage treatments except for pods stored for 21 days which recorded a decrease in FI by the sixth day of fermentation.

Pod storage, however, caused only marginal increases in FI at all fermentation time. FI of the unfermented beans increased slightly from 0.674 for the unstored pods to 0.763 after 10 days of pod storage. The FI of the fermented beans (six days fermentation) also increased from 1.390 for the unstored pods to 1.424 for pods stored for 10 days. Pod storage reduces the pulp volume per seed due to water evaporation and inversion of sucrose and increases micro-aeration within the pulp as well as within the fermenting mass. This might served to enhance the activity of polyphenol oxidase resulting in the oxidation of polyphenol compounds resulting in the formation of brown pigment thus increasing the FI of the cocoa nibs.

Regression analysis of the data showed significant ( $p < 0.05$ ) influence of the linear factor of fermentation time and pod storage and quadratic factor of fermentation time on the FI of the nibs. There was no significant ( $p > 0.05$ ) interaction between pod storage and fermentation time on the FI of the nibs. The model

developed could explain about 87% of the variations in the FI of the nibs, suggesting that 13% of the variations were due to other factors not investigated in this work (Table 1).

*3.2 Cut Test of Unfermented and Fermented Cocoa Beans*

Results of cut test of unfermented beans as well as beans fermented for three and six days for all the pod storage treatments are shown in Table 2. Generally, there were increases in brown beans with increasing fermentation time for all the pod storage treatments. The brown beans increased from 0.33% to 57% and from 5% to 66% for the unstored pods and pods stored for three days, Respectively, at the end of fermentation.

**Table 1 Regression coefficients and their  $R^2$  values in the models for FI of cocoa beans during fermentation and drying.**

Variables	FI <sup>a</sup>	FI <sup>b</sup>
Constant	1.17296*	1.02127*
$X_1$	0.11583*	0.04485*
$X_2$	0.35506*	-0.15509*
$X_1^2$	-0.04400	0.04965
$X_2^2$	-0.09588*	0.17896*
$X_1 \times X_2$	-0.00884	0.04022*
$R^2$	87.2%	81.7%
$R^2$ (adjusted)	85.7%	79.5%

\* significant at  $p < 0.05$ ;  $X_1$  = pod storage;  $X_2$  = fermentation/drying time; <sup>a</sup> FI during fermentation; <sup>b</sup> FI during drying.

**Table 2 Cut test result for unfermented and fermented cocoa beans.**

Pod storage (days)	Fermentation time (days)	Deep purple (%)	Pale purple (%)	Brown (%)	Slaty (%)	Mouldy (%)	Germinated (%)	Other defects (%)
0	0	6.67	1.67	0.33	91.33	0	0	0
	3	7.67	59.0	22.33	11.0	0	0	0
	6	3.33	40.0	56.67	0	0	0	0
3	0	10.67	3.33	5.33	89.67	0	0	0
	3	6.34	74.33	14.33	5.0	0	0	0
	6	2.37	31.67	65.96	0	0	0	0
7	0	25.0	19.0	22.67	33.33	0	0	0
	3	0.67	20.0	78.33	1.0	0	0	0
	6	0	6.33	93.67	0	0	0	0
10	0	10.68	23.0	11.33	48.33	3.33	3.33	0
	3	0	44.67	52.0	0	0	3.33	0
	6	0	27.34	72.33	0	0	0.33	0

It also increased from 23% to 94% and 11% to 72% for pods stored for seven and 10 days, respectively, at the end of fermentation. Changes in anthocyanins and oxidation products of the polyphenol oxidase might have contributed to the brown pigments formation in the cocoa beans during the fermentation period [1]. Anthocyanins are rapidly hydrolyzed to anthocyanidins and sugars (galactose and arabinose) by glycosidase during cocoa fermentation [28]. The decreasing of polyphenols and anthocyanins content normally lead to the changes of cocoa beans' colour from purple to brown [12]. Again, Shamsuddin and Dimmick [29] suggested that the brown pigments might also be produced from complexation of condensed tannin, a high molecular weight product of flavonoid polymerization, with protein, via hydrogen bonding.

Results also showed that increasing pod storage increased the percentage of brown beans. Afoakwa et al. [27] reported that cocoa flavours developed best when the degree of fermentation (% fully brown beans) was above 60%. Beans stored for 3, 7 and 10 d produced brown beans above 60%. This is because pod storage reduced the pulp volume per seed and increased micro-aeration within the pulp and the fermenting mass. This served to enhance the activity of polyphenol oxidase resulting in the oxidation of polyphenols. There were also reductions in the deep purple beans with increasing fermentation for all pod storage time. Purple beans are reported to contain high polyphenols and anthocyanins content [12] and the reduction in purple beans with fermentation suggested that polyphenols and anthocyanins were being degraded. The percentage of pale purple beans at the end of fermentation for all pod storage treatment ranged from 6% to 40%. Pale purple beans are not defective beans as they change to brown upon storage [6] and the trade accepts up to 30%-40% but samples containing over 50% are unacceptable [10]. Results showed that the proportion of pale purple beans did not exceed 50% for all the pod storage days and this gave an indication that the beans were adequately fermented.

The percentage of slaty beans was higher in the unfermented beans for all pod storage treatments but reduced drastically at the end of the fermentation. Germination occurred in beans stored for 10 days prior to fermentation (Table 2) and this was as a result of the prolonged storage of the pods which resulted in the rotting of pods and consequently penetration of oxygen into the pods creating optimum conditions for growth of the beans. However, during fermentation, heat was produced and coupled with the diffusion of some metabolites (ethanol and acetic acid) into the beans resulted in the death of the beans hence arresting germination. This was evident as the percentage of germinated beans reduced from 3.33% to 0.33% by the end of the fermentation process. Earlier work by Afoakwa et al. [27] also reported the occurrence of germinated beans of 2% and 11% in the pods stored for 14 days and 21 days, respectively, prior to fermentation due to prolonged storage. Germinated beans are considered a defect because the hole left by the emerging radical provides an easy entrance for insects and moulds [27]. They are also considered lacking good chocolate flavour [10].

About 3% mouldy beans were detected in the beans whose pods were stored for 10 days before fermentation and this could be ascribed to the invasion of mould species *Phytophthora palminovora* and *Botryodiplodia theobrommae* during the prolonged pod storage. Wood and Lass [10] reported that internal moulds were the major causes of off-flavours during cocoa processing, and samples of beans with as little as 4% of internal moulds can produce off-flavours in their finished products. The beans of the pods stored for 10 days however, did not exceed this limit. Moulds inside the beans are also reported to increase the free fatty acid (FFA) content of the cocoa butter [10] and this might have accounted for the high FFA value of 0.52% for pods stored for 10 days at the end of fermentation [30].

### 3.3 Changes in FI during Drying

FI of the cocoa beans decreased during drying for

all the pod storage treatments (Fig. 2). It decreased from 1.389 at the start of drying to 1.023 at the end of drying for the unstored pods. It also decreased from 1.390 to 1.025, 1.423 to 1.053 and 1.424 to 1.105 for pods stored for 3, 7 and 10 days, respectively. The decrease in FI during the drying process may be due to anthocyanin oxidation, which occurs in the presence of oxygen and high temperature [6]. Wrolstad [31] observed that the enzyme, anthocyanase degrades anthocyanin pigments to unstable cyanidin and sugar groups during fermentation and early periods of drying, and with time, the unstable cyanidin caused fading of colour. This might have accounted for the reduction in FI with increasing duration of drying.

Pod storage caused slight increase in FI during drying. The FI of the dried beans (seven days of drying) increased from 1.023 for the unstored pods to 1.105 for pods stored for 10 days. Even though FI decreased with increasing drying time, the FI values recorded for all the beans in this study at the end of the drying process were above 1.0. This suggests that cocoa pods can be stored for up to 10 days, fermented for six days and dried for seven days with the necessary formation of brown pigments characteristics of well fermented and dried cocoa beans.

Regression analysis of the data showed significant ( $p$

$< 0.05$ ) influence of the linear factor of pod storage and drying time and quadratic factor of drying time on the FI of the cotyledons. There was also significant ( $p < 0.05$ ) influence of the interaction between pod storage and drying time on the FI of the cotyledons. The model developed could explain about 82% of the variations in the FI of the cotyledons, suggesting that 18% of the variations were due to other factors not investigated in this work (Table 1).

### 3.4 Cut Test of Dried Fermented Cocoa Beans

Table 3 showed the results of cut test of fermented beans during the drying for all the pod storage treatments. Generally, there was a decrease in brown beans with increasing drying time for all the pod storage treatments. The brown beans decreased from 57% to 40% and 66% to 50% for the unstored pods and pods stored for three days, respectively, at the end of drying.

It also decreased from 94% to 66% and 72% to 54% for pods stored for seven and 10 days, respectively, at the end of drying. The fall in brown beans during the drying process may be due to anthocyanin oxidation, which occurs in the presence of oxygen and high temperature [6]. This is seen in the decrease of FI of the beans during drying (Fig. 2).

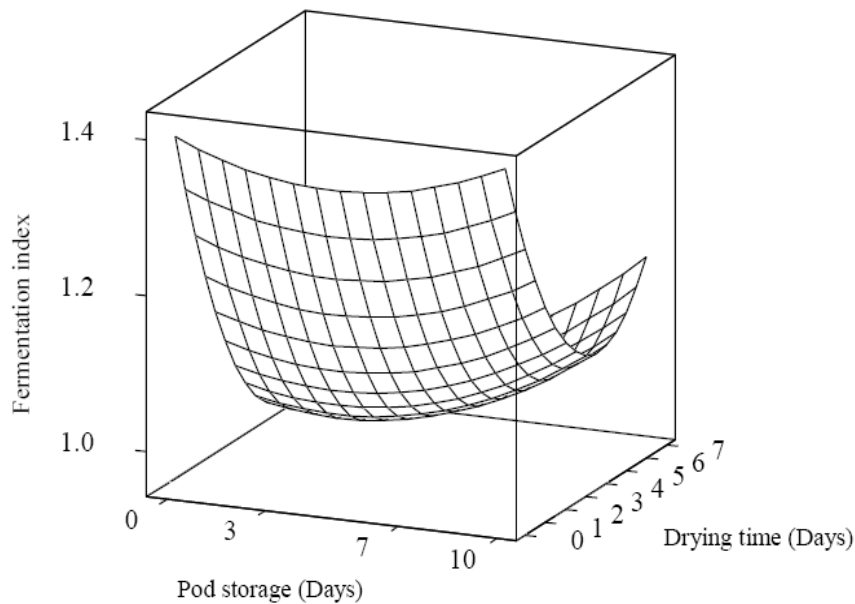


Fig. 2 Response surface plot showing effect of pod storage and drying time on the FI of cocoa beans.

**Table 3** Cut test of dried fermented cocoa beans.

Pod storage (days)	Drying time (days)	Deep purple (%)	Pale purple (%)	Brown (%)	Slaty (%)	Mouldy (%)	Germinated (%)	Other defects (%)
0	0	3.33	40.0	56.67	0	0	0	0
	3	15.0	41.67	43.33	0	0	0	0
	7	11.0	48.67	40.33	0	0	0	0
3	0	2.37	31.67	65.96	0	0	0	0
	3	2.67	33.0	64.33	0	0	0	0
	7	0.33	39.0	60.67	0	0	0	0
7	0	0	6.33	93.67	0	0	0	0
	3	0.33	16.62	83.05	0	0	0	0
	7	0	23.67	76.33	0	0	0	0
10	0	0	27.34	72.33	0	0	0.33	0
	3	0.33	31.33	68.34	0	0	0	0
	7	0.33	36.67	63.0	0	0	0	0

The pale purple beans were noted to increase with increasing drying time. Table 3 showed increasing pale purple beans from 40% to 49% for the unstored pods, 32% to 45%, 6% to 34% and 27% to 44%, for the beans from pods stored for 3, 7 and 10 d, respectively. Pale purple beans are not defective beans as they change to brown beans upon storage [6] and the trade accepts up to 30%-40% but samples containing over 50% are unacceptable [10]. Results (Table 3) showed that the proportion of pale purple beans did not exceed 50% for all the pod storage days and this gave an indication that the beans were adequately fermented and dried. Slaty beans were absent in all beans during drying. This was because all the beans were properly fermented (six days) prior to the drying process.

#### 4. Conclusions

FI of the beans increased with increasing pod storage and fermentation time due to the breakdown of anthocyanins. FI of all the beans at the end of fermentation were above 1.0 indicating that all the beans were well fermented. Increasing pod storage and fermentation caused reductions in percentage of slaty beans and deep purple beans but increased the percentage of brown beans. Beans stored for 3, 7 and 10 d produced brown beans above 60%. Prolonged pod storage (10 days) caused 3% mouldy beans and 3% germinated beans.

FI of the beans decreased during drying for all the

pod storage treatments. The fall in FI during the drying process may be due to anthocyanin oxidation, which occurs in the presence of oxygen and high temperature. Pod storage however, caused slight increase in FI during drying. FI values recorded for all the beans in this study at the end of the drying process were above 1.0. This suggests that cocoa pods can be stored for up to 10 days, fermented for six days and dried for seven days with the necessary formation of brown pigments characteristics of well fermented and dried cocoa beans. Increasing duration of drying decreased the percentage of brown beans at all pod storage time. The percentage of brown beans was highest for pods stored for seven days (66%).

#### Declaration of Interest

The authors have no conflict of interest and are responsible for the content of the manuscript.

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