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Developmental response of tropical warehouse moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae)'s larvae to stored cocoa beans fermented at varied degrees

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Developing larvae of major insect pests like *Ephestia cautella* rapidly degrade stored cocoa beans through feeding and other biological activities on the beans. Completion of developmental stages of insects may be influenced by a number of biochemical factors triggered by the primary post-harvest processing of cocoa beans prior to storage. Hence, this study assessed the development of *E. cautella* from first instar larval stage to adult stage on cocoa beans fermented at varied degrees. Fresh ripening pods of Amelonado variety (N38) were harvested and fermented variedly at 0 - 7 days, and sun-dried to about 5-6% moisture content. The variedly fermented cocoa beans (300 g/treatment) were infested with newly hatched first instar larvae of *E. cautella* (N=10) in a completely randomized design with four replications at 28±2°C and 70±5% relative humidity (RH). Days to adult emergence of the larvae of *E. cautella* on the cocoa beans varied significantly (P>0.05) between 0 day and 76.50 days in 4 days and 7 days-fermented beans, respectively. Significant variations occurred (P>0.05) in the total adult emergents of *E. cautella*, ranging between 0 and 8.25 in 4 days and 1 day fermented beans, respectively. This study reveals that the fermentation period of cocoa beans prior to drying and storage influenced adult emergence of *E. cautella* on stored cocoa beans, with 0 - 3 days fermented beans highly susceptible to *E. cautella*'s development to adult stage. Cocoa beans need to be fermented adequately for 4 to 7 days before drying to prevent insects' damage. Notably, 4-days fermented cocoa beans did not support *E. cautella* development to adult stage at 90 days post larvae-introduction. The 4-days fermented cocoa beans require further investigations for its nutritional suitability and importance in human diets.

Key words: Varied fermentation, *Ephestia cautella*, total adult emergents, days to adult emergence.

INTRODUCTION

Cocoa is a major export and source of income in some countries in West Africa including Nigeria (Tah et al., 2011) and is widely cultivated in the tropical rain forest and adjoining rain-fed ecologies in Nigeria (Opeke, 2005). Nigeria is the fourth largest producer of dried cocoa beans in the world after Cote d'Ivoire, Ghana and Indonesia with an average output of about 225,000 - 245,000 metric tons between 2011 and 2013

(The Statistics Portal, 2014). It is the major component used in production of chocolate, cocoa butter and mucilage, cocoa wine and beverages, cosmetics and many other products (Opeke, 2005). Insect pests infestation is an important production constraint of cocoa on the field and in the store. Over 1500 species of insects are known to feed on cocoa on the field but only 2% is of economic importance (Entwistle, 1972; Wood and Lass, 1989). Fermentation is an essential post-harvest processing of freshly harvested cocoa beans which precedes drying (Hii et al., 2009), in order to attain the desired flavour, physicochemical and/or industrial properties for its acceptability by consumers. Fermentation periods of freshly harvested beans affects the physiological and chemical compositions of the dried cocoa beans in store either positively or negatively, which in turn determines the frequency of development of biotic factors that can degrade and/or reduce the quality and quantity of stored cocoa beans.

There is a microbial succession of wide range of yeasts, lactic acid, and acetic-acid bacteria during which high temperature (about 50°C) and microbial products such as ethanol, lactic acid and acetic acid kill the embryo of the beans (Schwan and Wheals, 2004; Nielsen et al., 2005; Nielsen et al., 2007; Nielsen et al., 2008). In this process of microbial succession, there are flavor precursors production (Schwan and Wheals, 2004; Camu et al., 2008). The fermentation periods of freshly harvested cocoa beans is influenced by the microbial activities on the beans as the epicatechin and theobromine levels decrease, which in turn affects the colour, flavor (aroma) of the beans (Lagunes et al., 2007; Rodriguez-Campos et al., 2011) and the organoleptic properties of the dried cocoa beans in store (Camu et al., 2008). Freshly harvested cocoa beans require fermentation for 5 - 7 days before drying in order to have the desired chocolate flavor (Fowler 1999; Beckett, 2000), but field survey of farmers in some communities in Southwestern Nigeria revealed that some farmers have not been fermenting the cocoa beans for the recommended period of 5 - 7 days before drying, which suggests why insect infestation rate and other degrading biotic agents might be on the increase in stored cocoa beans from such localities. This in turn pollutes other cocoa beans of good quality pooled together with those that were not properly fermented (Oyedokun, 2013). Without proper fermentation, there is no chocolate flavor because during fermentation, precursor compounds for chocolate flavor are formed which will react together during roasting at optimum temperature of 150°C for 30 min (Ramil et al., 2006; Lambert, 2008).

Insect infestations affect the quality and quantity of stored cocoa beans through bioactivity processes, thereby stripping the beans of the desired flavor and other quality parameters that might have been built up during fermentation process. Inadequate fermentation of cocoa beans could be a factor or bio-indicator that is contributing mainly to insect development and eventual infestation of the produce in store. Hence, this study evaluated the influence of fermentation periods on nutritional contents of the freshly harvested cocoa beans and its impacts on some aspects of developmental biology of *E. cautella* by determining the relationships between fermentation periods, some chemical constituents of dried cocoa beans of varying fermentation periods and days to adult emergence of *E. cautella* on cocoa beans at 90 days in store.

MATERIALS AND METHODS

Freshly harvested, ripening cocoa pods of Amelonado variety (genotype-N38) were collected from the Common Fund for Commodities (CFC) Plot at Cocoa Research Institute of Nigeria, Ibadan, Nigeria during the main crop harvest season of 2010. The pods were broken with the aid of a small wooden club and were subjected to different fermentation periods (DFP) of F0, F1, F2, F3, F4, F5, F6 and F7 days using box fermentation method. The wooden boxes (7 cm height, 25 cm width and 35 cm length) were lined with fresh plantain leaves before the fresh beans were poured into the boxes. The whole beans removed from the pods were pooled and divided into eight batches of equal fresh weight (2 kg) and each batch was fermented at different days. Zero-day fermented beans (F_0) were collected from the batches as batch 1, rubbed in fine sawdust and later rinsed thoroughly with clean water to remove the pertinacious mucilage covering the beans and thereafter sun-dried for ten days on a raised platform till well-dried. One-day fermented beans (F_1) were collected after one day of fermentation and subsequent fermentation periods were collected following the number of days assigned per batch up till seven-days. The fermented beans were sun-dried to about 6% moisture content at 10 days of drying. Each fermentation day's batch was replicated three times in completely randomized design in the three fermentation boxes.

Homogenous samples of *E. cautella* Walker (n=10) that were raised on cocoa-wheat-soya diet were collected, sexed, paired for mating and placed in pairs in plastic oviposition cages (18.5 cm top diameter, 13.5 cm base diameter and height of 19.5 cm) lined with black filter papers which provided the contrast needed to collect the milky-white laid eggs. The eggs laid after 24 h were collected and placed in 90 mm diameter Petri dishes lined with black filter paper. The lid of the Petri dishes were perforated to allow for aeration and the Petri dishes were placed on a table with its legs dipped in water polluted with fresh engine oil so as to prevent ants from predating on the eggs. Ten day-old, freshly hatched first instar larvae of *E. cautella* were carefully introduced into each rearing cage (12.5 cm top diameter, 11.5 cm base diameter and height of 6.5 cm) containing 300 g of dried cocoa beans fermented at different days.

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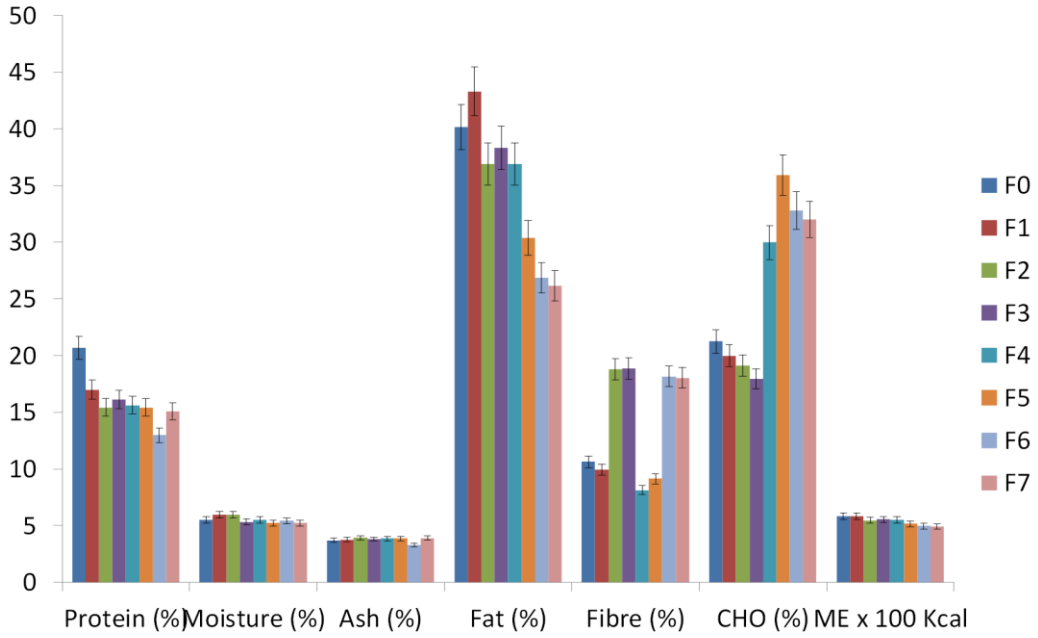

Figure 1. The proximate composition of dried cocoa beans fermented for different days. F0 to F7, fermentations at 0 to 7 days respectively.

Prior to introduction of the day-old larvae onto the beans, the beans were sterilized in a Gallenkamp Oven at 40°C for 4 h to kill any insect eggs that might have been laid on the beans during outdoor drying of the cocoa beans. After cooling, the larvae were introduced into the cages containing sterilized cocoa beans fermented at varied degrees using a soft, pointing-tip camel hair brush dipped in sterile water and adequately drained. The experiment was replicated four times. The rearing cages (cut at the sides) containing the dried cocoa beans and day-old larvae had its sides covered with muslin cloth and were kept undisturbed and observed daily in the laboratory at the ambient tropical temperature (28 ± 2°C) and relative humidity (70 ± 5%) till adults started emerging. Data were collected on the number of days to adult emergence of *E. cautella* on the beans and the total number of adult *E. cautella* that emerged per treatment. The experiment terminated at 90 days after the beans were infested with first instar larvae.

The proximate, some physicochemical properties of the fat extract and some mineral compositions of the variedly fermented beans were determined using standard procedures (AOAC, 1990). Data were analyzed using analysis of variance (ANOVA) and the means separated using Tukey's studentized range (HSD). Also, proximate, physicochemical and mineral compositions of the beans were correlated with total adult emergents, days to adult emergence and cocoa beans fermented for four days using SPSS 17 Version.

RESULTS AND DISCUSSION

Effect of fermentation periods on proximate composition of the cocoa beans and development of *E. cautella*

The proximate composition of cocoa beans (Amelonado-N38) variedly fermented at F_0, F_1 F_2, F_3, F_4, F_5, F_6, and F_7 days is shown in Figure 1. The F_0 cocoa beans had significantly (P>0.05) higher mean value of protein (21.33 ± 0.84) than other cocoa beans of F_1, F_2, F_3, F_4, F_5, F_6 and F_7 that had between 13-17% crude protein (Figure 1). The moisture content of all the variedly fermented cocoa beans ranged between 5.23 and 5.95% which was not significantly different (P < 0.05) in all the fermented cocoa beans. Moreover, significant variations (P = 0.05) were recorded in the percentage ash content of F_0, F_1 and F_3 cocoa beans. There was no significant difference (P < 0.05) in the percentage ash content of cocoa beans fermented at F_2, F_4, F_5, and F_7, that had 3.92, 3.86, 3.87 and 3.89%, respectively. The percentage fat content ranged between 26.15% in F_7 and 43.31% in F_1 cocoa beans. The fat content of the cocoa beans decreased significantly (P>0.05) with increase in fermentation period which may be due to the bioactivities of the microorganisms present in the cocoa pulp (Lagunes-Galvez, 2007; Guehi et al., 2008). The F_4 beans had significantly (P<0.05) lower fibre content of 8.14% while F_3 had the highest (18.89%) fibre content. Carbohydrate was significantly higher (P>0.05) in F_5 beans (Figure 1), while F_3 beans had the lowest percentage carbohydrate composition. Metabolizable energy was lowest in F_7 and highest value was recorded in F_0 cocoa beans.

This study shows that development of *E. cautella* from first instar larva to adult stage was influenced by varied fermentation periods of the cocoa beans prior to drying and storage. This might be as a result of differences in

Table 1. Fermentation levels and some mineral elements of dried cocoa beans.

Fermentation level (days)	Lead (mg/L)	Iron (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)
F_0	$0.15^{c} \pm 0.00$	$2.41^{c} \pm 0.02$	$17.54^{b} \pm 0.02$	$76.51^{d} \pm 0.18$	$163.13^{f} \pm 0.93$
F_1	$0.11^{b} \pm 0.00$	$1.82^{a} \pm 0.01$	$18.01^{b} \pm 0.06$	$83.42^{e} \pm 0.24$	$175.75^{h} \pm 0.47$
F_2	$0.11^{b} \pm 0.01$	$2.60^{d} \pm 0.04$	$19.30^{c} \pm 0.17$	$67.45^{b} \pm 0.63$	$166.38^{g} \pm 1.04$
F_3	$0.10^{b} \pm 0.004$	$1.89^{a} \pm 0.01$	$19.99^{d} \pm 0.04$	$68.67^{b} \pm 0.26$	$136.08^{d} \pm 0.12$
F_4	$0.10^{b} \pm 0.005$	$2.09^{b} \pm 0.031$	$25.55^{g} \pm 0.27$	$71.61^{c} \pm 0.24$	$131.11^{c} \pm 0.29$
F_5	$0.07^{a} \pm 0.001$	$1.81^{a} \pm 0.016$	$24.13^{f} \pm 0.07$	$71.34^{c} \pm 0.15$	$158.05^{e} \pm 0.09$
F_6	$0.03^{a} \pm 0.001$	$2.45^{c} \pm 0.016$	$15.96^{a} \pm 0.04$	$54.56^{a} \pm 0.45$	$98.13^{a} \pm 0.27$
F_7	$0.07^{a} \pm 0.00$	$2.02^{b} \pm 0.01$	$22.25^{e} \pm 0.09$	$75.52^{d} \pm 0.26$	$126.48^{b} \pm 0.21$

Means following the same letter in the same column are not significantly different (P≤0.05) following Tukey's Studentized Range HSD Test. N=3 replicates/treatment. F0 to F7, fermentations at 0 to 7 days respectively.

the biochemical composition (proximate, physicochemical and mineral) of the cocoa beans being influenced by duration of fermentation vis-à-vis microbial activities of microorganisms which produces metabolites that diffuse from the pulp into the beans. Variation in the proximate and mineral composition of the cocoa beans was influenced by the fermentation levels of the bean prior to storage and this influenced the development of *E. cautella* on the cocoa beans that was stored over a period of 90 days. This finding corroborates earlier studies (Jonfia-Essien, 2006; Guehi et al., 2008) that biochemical variations occur in stored cocoa beans which eventually enhance insect pest population build-up. This study also showed that unfermented cocoa beans had higher protein content than other variedly fermented beans which might have predisposed the F_0 cocoa beans to higher infestation rate and earlier developmental period of *E. cautella* on the beans. This is in agreement with the results of Chapman (2003), (House,1969), Schoonhoven et al. (2005) that optimum-high amount of protein is required and/or essential in insect diet for growth and development even as the larvae have the ability to sense the presence of protein in the food substrates through chemoreception. The lowest fibre content and other biochemical contents found in F_4 cocoa beans in which there was no adult emergents of *E. cautella* at 90 days in store suggests a possibility of nutritional imbalance that occurred in the F_4 cocoa beans, which might be due to incomplete microbial actions and/or reactions during fermentation process. This corroborates (Dadd, 1985) that insects may respond to imbalance diet by moving away from the food or abstain from feeding on such food.

Since no alternate or alternative food host was available to the developing larvae of *E. cautella* in this study, attempt by the developing larvae to abstain from such unsuitable diet like F_4 will result into death of the larvae. Hence, as no adult emerged on F_4 beans. The low fibre content and some few other nutritional factors that are bio-indicative suggest why F_4 cocoa beans did not support the development of *E. cautella* from first instar larva to adult stage in this study. It also showed that the ash content varied with duration of fermentation which might be due to the effects of varied microbial activities involved during fermentation process as influenced by the fermentation periods. This indication suggests why cocoa beans that were not properly fermented (0-3 days) were more susceptible to insect attack within a shorter period of storage when compared with the beans fermented between four to seven days. The implication is that inadequately fermented cocoa beans support the development of major insect pests of stored cocoa beans like *E. cautella* with shorter developmental period while adequately fermented cocoa beans (5-7 days) delayed *E. cautella*'s development on such beans except four-day fermented beans that did not support *E. cautella* development within 90 days of storage.

Mineral compositions of the variedly fermented cocoa beans and the effect on *E. cautella* larvae

Table 1 shows some mineral elements composition in the cocoa beans fermented at different days. The lead (Pb) content of the cocoa beans was significantly lower ($P<0.05$) in F_5 to F_7 (0.03 - 0.07 mg/L) cocoa beans while F_0 to F_4 cocoa beans had significantly higher ($P>0.05$) lead content (between 0.10 and 0.15 mg/L) suggesting the role of adequate fermentation as bio-remediation process of reducing the levels of heavy metals in cocoa beans through the physiological and metabolic activities of fermentation microbes.

The iron content of the variedly fermented beans did not follow a regular trend but was significantly different ($P=0.05$) with F_0, F_1, F_3, F_4, F_5, F_6, and F_7 having 2.41, 1.82, 2.60, 1.89, 2.09, 1.81, 2.45 and 2.02 mg/L, respectively. The F_4 fermented cocoa beans had a significantly ($P>0.05$) higher level of calcium content

Table 2. Physicochemical composition of fat extract from cocoa beans fermented at different days.

Fermentation levels (days)	Acid value (mg/g) ($\bar{x}$ ± SE)	Peroxide value (mEq/Kg) ($\bar{x}$ ± SE)	Iodine value (g/100 g) ($\bar{x}$ ± SE)	FFA (g/100 g) ($\bar{x}$ ± SE)	pH ($\bar{x}$ ± SE)
F_0	2.24^{f} ± 0.02	1.58^{e} ± 0.01	42.10^{g} ± 0.12	4.36^{f} ± 0.05	6.40^{e} ± 0.00
F_1	1.88^{e} ± 0.01	1.21^{d} ± 0.01	29.86^{f} ± 0.04	3.76^{e} ± 0.22	6.60^{f} ± 0.00
F_2	1.78^{d} ± 0.01	1.18^{d} ± 0.00	25.28^{d} ± 0.01	3.59^{d} ± 0.00	5.70^{b} ± 0.00
F_3	1.42^{b} ± 0.01	1.05^{c} ± 0.02	24.40^{c} ± 0.00	2.85^{b} ± 0.02	5.80^{c} ± 0.00
F_4	1.12^{a} ± 0.00	2.36^{f} ± 0.01	18.70^{a} ± 0.18	2.26^{a} ± 0.02	6.00^{d} ± 0.00
F_5	1.69^{c} ± 0.01	0.79^{b} ± 0.016	22.54^{b} ± 0.24	3.32^{c} ± 0.02	5.80^{c} ± 0.00
F_6	2.23^{f} ± 0.00	0.59^{a} ± 0.01	25.63^{d} ± 0.06	4.47^{g} ± 0.00	5.80^{c} ± 0.00
F_7	1.68^{c} ± 0.00	1.56^{e} ± 0.00	27.29^{e} ± 0.24	3.38^{c} ± 0.01	5.60^{a} ± 0.00

Means following the same letter in the same column are not significantly different (P≤0.05) following Tukey's Studentized Range HSD Test. N= 3 replicates/treatment. F0 to F7, fermentations at 0 to 7 days respectively.

(25.55 mg/L) while cocoa beans fermented for 5 days (F_5) recorded the lowest calcium content of 15.96 mg/L. The calcium contents increased with fermentation periods till 4 days fermentation and decreased after 4 day fermentation. This might be as a result of synthesis of these minerals by the microorganisms involved in the fermentation process within the earlier days (0-4 days) before the moisture content is relatively low in latter days (5-7 days) which would also affect the biochemical activities of the microorganisms. The magnesium (Mg) content of the variedly fermented cocoa beans varied significantly (P>0.05) from 54.56 mg/L in 6-days fermented beans to 83.42 mg/L in 1-day fermented cocoa beans. The lowest potassium level was recorded in 6-days fermented cocoa beans with 98.13 mg/L which was significantly lower (P>0.05) when compared with F_1 cocoa beans which had 175.75 mg/L. The decreasing trend of Mg and K as fermentation period increases might be as a result of not storing the cocoa pods before processing. This supports earlier study by Afoakwa et al. (2013) that pod storage before processing enhances the concentration of calcium, magnesium, zinc and potassium as fermentation days increases. The higher level of calcium content in F_4 might be among the main factors limiting adults of *E. cautella* from emerging on the F_4 cocoa beans due to nutritional deficiencies and/or unsuitable diet. This corroborates the findings of Nation (2001) that insects require trace amount of calcium and iron for development. The F_0 and F_1 cocoa beans with the higher mean value of magnesium as well as F_0 to F_2 fermented cocoa beans with higher value of potassium had the highest range of *E. cautella* adult emergents with an average of 48 days to emerge. The magnesium and potassium factors might be significant factors in the development of *E. cautella* to adult stage on such cocoa beans.

This supports was earlier studied by Silva et al. (2005) that insects need considerable amount of mineral elements like magnesium, phosphorous and potassium but little calcium, sodium and chlorides are required for insect development. It also explains further that a perfect blend of biochemical composition of food host (fermented cocoa beans) determines the population build-up effect of insect pests on such food host which had been greatly influenced by microbial activities during the various fermentation periods. From the mineral analysis, lead content in all the fermented beans seem to be within the acceptable intake level for *E. cautella* larva development except in Four-day fermented beans in which no adult emerged which may be due to other factors like low fibre and calcium content. This is contrary to the expectation that presence of trace amount of lead will reduce the number of adult emergents because lead is a heavy metal that can reduce pathenogenetic activities of the cells during different stages of larval development.

Some physicochemical properties of the fat content extracted from cocoa beans fermented at different days are shown in Table 2. The mean acid values of the fermented beans was significant (P>0.05) with lowest value of 1.12 mg/g in beans fermented at 4 days while the cocoa beans fermented at zero days had the highest acid value of 2.24 mg/g. Peroxide value in cocoa beans fermented at 4 days was significantly higher (P > 0.05) having 2.36 mEq/kg and significantly lowers in cocoa beans fermented at 6 days with 0.59 mEq/kg. Iodine values ranged between 18.70 g/100 g in 4-days fermented beans and 42.10 g/100 g in cocoa bean with zero fermented days. Free fatty acid (FFA) was lowest in 4-days fermented beans (2.26 g/100 g) and highest in 6-days fermented beans (4.47 g/100 g). The physicochemical values of the butter extract from each fermented beans also revealed that the lower the free fatty acid content of cocoa beans, the lower the development of insects on cocoa beans.

Table 3. Effect of fermentation periods of cocoa beans on days to adult emergence and number of adult emergents of *E. cautella* at 90 days after first instar larvae introduction.

Fermentation period (days)	Days to adult emergence (Mean ± SE)	Total number of adult emergents (Mean ± SE)
0	$47.50^{c} \pm 3.08$	$8.25^{d} \pm 0.75$
1	$49.00^{c} \pm 3.19$	$8.00^{d} \pm 1.00$
2	$48.00^{c} \pm 2.89$	$6.50^{cd} \pm 0.87$
3	$34.25^{b} \pm 13.13$	$1.50^{ab} \pm 0.87$
4	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
5	$68.75^{d} \pm 2.81$	$5.00^{bc} \pm 0.71$
6	$75.75^{e} \pm 2.17$	$3.75^{abc} \pm 0.85$
7	$76.50^{e} \pm 2.95$	$3.50^{abc} \pm 0.96$

N= 10/treatment in four replicates; mean with the same letter is not significantly different at P≤0.05 following Tukey's Studentized Range (HSD). F0 to F7, fermentations at 0 to 7 days respectively.

From this study, it was evident that cocoa beans fermented at four days had the lowest FFA, on which *E. cautella* larvae could not develop to adult stage at 90 days in store and this might be as a result of gamut of factors including low FFA that was present in four-day fermented cocoa beans. This is in consonance with Jonfia-Essien and Navarro (2010) whose report directly correlated relationship between FFA and insect infestation that the higher the FFA, the higher the insect infestation and vice-versa. Meanwhile, the proximate analysis of the fermented beans showed that moisture content in the variedly fermented cocoa beans were at lower levels below 6.5% which should have affected the FFA to be on the increase but this was contrary to the report of Jonfia-Essien and Navarro (2010) that cocoa beans with low moisture content of lower than 6.5% were found to contain high FFA levels. Rather than FFA and moisture, gamut of other compositional factors like percentage fibre content, percentage fat content and mineral composition of the cocoa beans could be factors that would enhance or hinder insect pests' development on cocoa beans in store. For instance from this study, cocoa beans fermented for four days were found to have the highest calcium content, lowest FFA, lowest fibre content, highest peroxide value, lowest acid value and lowest iodine value which suggested why four-day fermented beans did not support the development of *E. cautella* till adult emergence. This agrees with the report of Adeniyi et al. (2011), who stated that fermentation periods of cocoa beans has significant effects on the colour, microbial load as well as the quality and nutrition content of the beans. Variations in the physicochemical composition of cocoa beans fermented at different days as shown in this study accounted for the dynamics in the nutrient base that was made available to the developmental stages of *E. cautella* as this affected the biological parameters like number of adult emergents and days to adult emergence. Imbalanced diets as presented in four days fermented cocoa beans with lowest fibre, lowest acid value, lowest free fatty acid, lowest iodine value, highest peroxide value and highest calcium content suggests the reason for non-emergence of *E. cautella* adults on the beans.

Influence of fermentation periods on days to adult emergence and total adult emergents of *E. cautella*

The effects of fermentation periods on total adult emergents and days to adult emergence at 90 days after introduction of day-old first instar larvae of *Ephestia cautella* is shown in Table 3. There were significant differences (P = 0.05) in the number of days to adult emergence of *E. cautella* on the cocoa beans fermented at different days, ranging from 0.00 days on four days fermented cocoa beans and 58.75 days on five days fermented beans. Adults emerged on differently fermented cocoa beans at the average of 47.50, 49.00, 48.00, 34.24, 0.00, 68.75, 75.75 and 76.7 days on F_0, F_1, F_2, F_3, F_4, F_5, F_6 and F_7, cocoa beans, respectively. Similarly, the total *E. cautella* adult emergents on cocoa beans variedly fermented differed significantly (P = 0.05), with the average of 8.25, 8.00, 6.00, 1.50, 0.00, 5.00, 3.75 and 3.50 adults emerging on, F_0, F_1, F_2, F_3, F_4, F_5, F_6 and F_7 fermented cocoa beans, respectively.

The interactions of fermentation periods, the proximate composition of cocoa beans fermented at different days, adult emergence and total adult emergents is shown in a 2-tailed correlation matrix (Table 4). The increase in fermentation period of the cocoa beans significantly (P > 0.01) reduced the percentage composition of protein, fat, metabolizable energy and also reduced the total number

Table 4. Correlation between total adult emergents, days to emergence of *E*. *cautella* and proximate composition of cocoa beans fermented at different days.

	Fermt.	TAE	DAE	%CP	%Moist	%Ash	% Fat	%Fibre	%CHO	ME
Fermt.	1.00									
TAE	-0.52**	1.00								
DAE	-0.39	0.35	1.00							
%CP	-0.73**	0.27	-0.32	1.00						
%Moist	0.37*	0.21	-0.07	0.20	1.00					
%Ash	0.21	-0.25	-0.11	-0.04	0.06	1.00				
%Fat	-0.94**	0.37	-0.47**	0.78**	0.32	-0.23	1.00			
%Fibre	-0.01	0.05	0.05	-0.16	-0.04	-0.01	-0.05	1.00		
%CHO	0.68**	-0.11	0.56**	-0.42	-0.37*	0.03	-0.69**	-0.52**	1.00	1.00
ME	-0.93**	0.37**	-0.47**	0.82**	0.29	-0.18	0.95**	-0.08	-0.67**	

**Correlation is significant at (p > 0.01), (2 tailed). * Correlation is significant at (p > 0.05), (2 tailed). TAE, total adult emergents; DAE, days to adult emergence; ME, metabolizable energy, CP, crude protein; CHO, carbohydrate; Fermt., fermentation.

Table 5. Correlation between total adult emergents, days to emergence of *E*. *cautella* and some mineral composition of cocoa beans fermented at different days.

	Fermt.	TAE	DAE	Fe	Ca	Mg	Pb	K
Fermt.	1.00							
TAE	-0.52**	1.00						
DAE	-0.39	0.35	1.00					
Fe	-0.08	0.07	-0.02	1.00				
Ca	0.36*	-0.55**	-0.23	-0.40*	1.00			
Mg	-0.39*	0.27	-0.05	-0.45**	0.09	1.00		
Pb	-0.83**	0.34	-0.40*	-0.07	-0.12	0.52**	1.00	
K	-0.83**	0.61**	-0.13	-0.17	-0.14	0.45**	0.77**	1.00

** Correlation is significant at p > 0.01) (2 tailed). * Correlation is significant at p > 0.05 (2 tailed). TAE, Total adult emergents; DAE, days to adult Emergence; Fermt., fermentation.

of *E. cautella* adults that emerged on the cocoa bean samples at 90 days after infestation with day-old larvae. Whereas, percentage composition of carbohydrate significantly (P > 0.01) increased with fermentation period of the cocoa beans. Fermentation period of the cocoa beans significantly (P > 0.05) lengthened the day to adult emergence of *E. cautella* on the beans, even as the moisture content of the beans significantly decreased. At 99% confidence interval, increase in percentage fat significantly reduced the days to adult emergence of *E. cautella,* but increase in percentage carbohydrate content of the beans significantly lengthened the days to adult emergence. However, increase in percentage fat and metabolizable energy content of the beans significantly (P > 0.01) increased the total adult emergents of *E. cautella* on the cocoa beans at 90 days after infestation.

The correlation matrix between total adult emergents (TAE), days to adult emergence (DAE) of *E. cautella* and the mineral values is shown in Table 5. At 99% confidence level, there was a significantly negative correlation between calcium content levels and total adult emergents of *E. cautella* Walker. Also, significantly positive correlation was recorded between the total adult emergents of *E. cautella*, lead content and potassium content of the fermented beans. There was a negative correlation values between the days to adult emergence of *E. cautella* Walker and the mineral values of Fe, Ca, Mg and K which were not significant (P < 0.01; P < 0.05) except in Pb that was significant (P>0.01). There was a positive correlation between Fe content and the total adult emergents and this is contrary to the findings of Ahmed et al. (2010) that iron fortified wheat resulted in decrease in larval weight of *Tribolium castaneum* as compared to low iron content diet. There was significantly negative correlation (P>0.01) between Ca and Fe but positive correlation between Ca and Mg. There were negative correlations between Ca, Pb and K which were not significant (P>0.01; 0.05). Negative correlation existed between Pb, Fe and Ca while significantly positive correlation existed between Pb and Mg (P>0.01);

Table 6. Correlation between total adult emergents, days to emergence of *E*. *cautella* and some physicochemical composition of cocoa beans fermented at different days.

	Fermt.	TAE	DAE	Acid value	Peroxide value	Iodine value	Free fatty acid (FFA)	pH
Fermt.	1.00							
TAE	-0.52**	1.00						
DAE	-0.39	0.35	1.00					
Acid Val.	-0.29	0.61**	0.47	1.00				
Perox. Val.	0.30	-0.09	-0.45*	-0.28	1.00			
Iodine Val.	-0.40*	0.50**	0.14	0.68**	0.30	1.00		
FFA	-0.21	0.61**	0.46**	0.96**	-0.27	0.73**	1.00	
pH	-0.66**	0.28	-0.32	0.24	0.29	0.29	0.17	1.00

**Correlation is significant at (p > 0.01), (2 tailed). * Correlation is significant at (p > 0.05), (2 tailed). TAE, Total adult emergents; DAE, days to adult emergence; Fermt., fermentation.

and Pb and K (P>0.01). This study shows that Fe in combination with other mineral elements supported the larval development of *E. cautella* to adult stage at the required proportion in other variedly fermented cocoa beans while Ca at the concentration found in F_4 cocoa beans did not support larval development to adult stage, may be due to ionic imbalance in the larva system and consequent physiological disturbance as reported by Ahmed et al. (2010).

Similarly, the correlation matrix showing the interactions between fermented beans and the physico-chemical properties of fat extracts from the cocoa beans, total adult emergents and days to adult emergence is shown in Table 6. There was no significant differences (P = 0.01; P = 0.05) even as longer fermentation periods, lower the acid value, peroxide value, and free fatty acid values of the fat extract of the cocoa beans fermented for different days. Iodine value (P > 0.05) and pH value (P > 0.01) significantly reduced the longer the fermentation period. Acid value and free fatty acid value significantly (P > 0.01) influenced the total *E. cautella* adult emergents and also causes the days to adult emergence to increase. Similarly, iodine value significantly (P > 0.01) influenced positively the total number of adult emergent.

In conclusion, four-day fermentation of cocoa beans has been found to contain some nutritional and bio-chemical compounds responsible for inhibiting develop-ment of larvae and eventual adult emergence of *E. cautella* on such cocoa beans, but this study need be furthered to assess whether such cocoa beans fit the usage in the chocolate industry or any other medicinal usage, be evaluated for its biocidal and medicinal value at different durations in store.

Conflict of interests

The authors did not declare any conflict of interest.

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