

**THE IMPACT OF STORAGE ON THE PURPLENESS OF COCOA BEANS AND  
LEVEL OF INSECT DAMAGE IN STORED COCOA BEANS**

**BY**

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## DECLARATION

I do hereby declare that all the experimental work described in this thesis was carried out by me and that except for references to other people's work that have been duly acknowledged, this thesis either in part or whole has not been presented for any other degree elsewhere.



Dr. Rosina Kyerematen

ARPPIS Co-ordinator

## **DEDICATION**

**To the glory of God Almighty**

*And to my mother*

**Late. Laraba Buba Mbahi**



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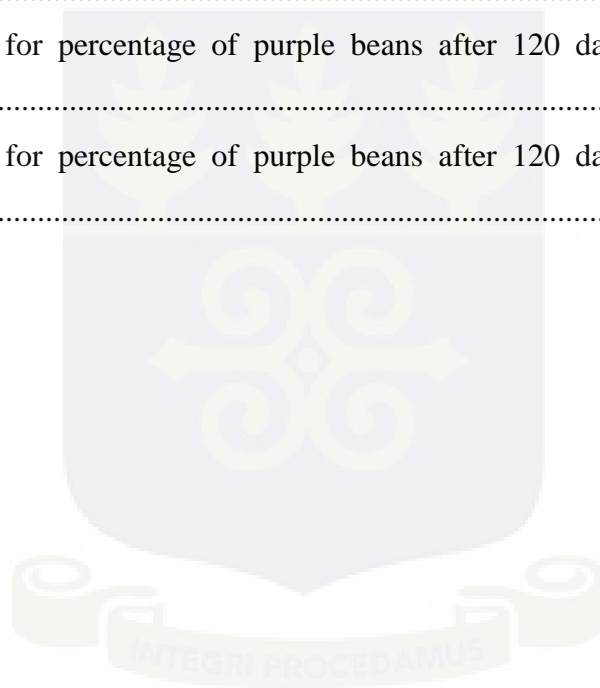
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## ABSTRACT

Cocoa is one of the most important cash crops in West Africa especially Ghana. It contributes 23% of the foreign export earnings from the agricultural sector in Ghana. Cocoa beans are fermented and dried before exporting. The fermentation period in Ghana is 6 to 7 days but some cocoa farmers under-ferment their cocoa beans leading to the development of purple cocoa beans. This study was carried out at CRIG and African Regional Postgraduate Programme in Insect Science (ARPPIS) at the University of Ghana to determine the impact of storage period and level of insect infestation on the level of purple cocoa beans. Cocoa beans taken from ripened pods were fermented for 1, 2, 3, 4 and 5 days to get the purple beans for the experiment and were sundried. *Ephestia cautella* and *Tribolium castaneum*, both singly and in combination, were introduced into the cocoa beans at the beginning of the storage and stored for 120 days. Cut test was used to determine the level of purpleness of the dry cocoa beans. Insect numbers were counted and the contaminants produced by these insects were observed. Percentage weight loss of the cocoa beans was determined using Count and Weigh method. Cocoa beans infested with *E. cautella* alone had the highest population of  $297 \pm 22.7$ . The highest weight loss was recorded in cocoa beans fermented for one and four days ( $10.1 \pm 1.87\%$  and  $10.1 \pm 8.74\%$ ). The population of *E. cautella* and weight loss increased with storage period. There was variation in the percentage of purple beans during the storage period. Cocoa beans fermented for 3 and 4 days had the highest percentage of purple beans. Cocoa beans fermented for one and two days were considered as slaty beans. Although there was variation in the percentage of purple beans, the grade of the beans did not vary. *Tribolium. castaneum* which is also a major pest of stored cocoa beans did not cause much damage to the whole cocoa beans but *E. cautella* alone caused significant damage to stored cocoa beans. The study was conducted using grade II cocoa beans

but the cocoa beans deteriorated over the storage period to sub-standard due to high levels of other defects such as germinated, flat and insect infested cocoa beans. High insect infestation contributed a great deal to the reduction in quality. From this study, poor fermentation and insect infestation are a great threat to the quality of cocoa beans. There was no significant reduction in the percentage purple beans over the storage period. Storage therefore had no impact on the percentage of purple cocoa beans. More attention should be given to *E. cautella* in terms of control.



## CHAPTER ONE

### 1.0 INTRODUCTION

Cocoa beans are the seeds from cocoa, *Theobroma cacao*. It is member of the Sterculiaceae family, which is a native to the Amazon region of South America and cultivated in the tropical regions of the world (Ardhana and Fleet, 2003). Each pod contains 30 to 40 beans embedded in a mucilaginous pulp. Raw cocoa beans have an astringent, unpleasant taste and flavour, and have to be fermented, dried and roasted in order to obtain the characteristic cocoa flavour and taste (Thompson *et al.* 2001).

World production of cocoa rose from 3,593m tonnes in the 2008/2009 crop season to 4,250 m tonnes in the 2010/2011 crop season and Africa contributes over 70% of the total production (ICCO, 2011). La Côte d'Ivoire and Ghana produce more than half of the world's cocoa beans. La Côte d'Ivoire produces 1.2 million tonnes (35%) and Ghana produces 0.73 million tonnes (21%) (World Cocoa Foundation, 2010). The commercial production of cocoa in Ghana began when the cocoa pod was brought from Fernando Po by Tetteh Quarshie in 1897 (Manu and Tetteh, 1987). Cocoa contributes 23% of Ghana's foreign export earnings from the agricultural sector, compared to 1.3% and 2.3% by timber and the non-traditional agricultural export, respectively (ISSER, 2012). Cocoa serves as the major source of revenue for the provision of socio-economic infrastructure in Ghana (ISSER, 2009). In 2010/2011 crop season, Ghana produced 1,024,150 tonnes which was the highest since Ghana registered its name in the international market as a producer of cocoa beans (Ghana Cocoa Board, 2011). Cocoa beans from Ghana are recognized because of their quality and therefore Ghana is regarded as the world leader in premium quality cocoa bean (Ntaimoah and Afrane, 2008).

Cocoa beans and products are food sources rich in phenolic compounds. Dreosti (2000) reported that 60% of the total phenolics in raw cocoa beans are flavonol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer). These compounds were reported to be potential candidates to combat free radicals, which are harmful to our body and food systems (Adamson *et al.*, 1999). Studies had demonstrated that the consumption of cocoa or chocolate reduced the risk of cardiovascular disease (Keen *et al.*, 2001). It helps to protect tissues against stress, and certain polyphenols work as preventive medicines for problems such as cardiovascular diseases, cancer, arthritis and autoimmune disorders (Mursu *et al.*, 2004).

The processing of cocoa beans consists of two major steps namely; fermentation and drying (Hii *et al.*, 2009). Fermentation begins immediately after the beans embedded in the mucilaginous pulp are removed from the pods. Fermentation methods vary considerably from country to country (Baker *et al.*, 1994). However, the two widely used methods are the heap and box fermentation (Nielson *et al.*, 2007; Hii *et al.*, 2009). Fermentation takes 6-7 days during which time the cocoa beans are mixed twice to ensure even fermentation. In order to moderate the initially bitter flavour of cocoa beans and to develop the typical flavour, the cocoa beans must be subjected to a fermentation process during which the highly bitter tannins present in them are oxidized, resulting in the formation of aromatic substances and the development of the typical brown to deep red-brown colour (Thompson *et al.*, 2001). The reduction in bitterness and astringency is as a result of diffusion of alkaloids (30% fall) and polyphenols (20% fall) out of the cocoa beans (Camu *et al.*, 2008a). The heat associated with fermentation causes the cocoa beans to lose their viability.

Fully ripened, properly fermented and thoroughly dried cocoa beans are thus of a brown to dark red in colour and have a very fragile shell with a pure, bitter flavour (Camu *et al.*, 2008). Poorly

fermented cocoa beans are purple in colour when under-fermented or very dark in colour when over-fermented (Misnawi *et al.*, 2003). The traditional fermentation period (6-7days) in Ghana ensures the decrease of the polyphenol (purple) content in the cocoa beans to as low as between 9% and 15% but the 3-4 days fermentation can keep the polyphenol compound to as high as between 40% and 80% in the bean (Misnawi *et al.*, 2003). Purple beans occur when the fermentation has been terminated prematurely. Polyphenols in cocoa products are mostly responsible for the astringent sensation and they also contribute to the bitter taste and colour along with alkaloids, some amino acids, peptides and pyrazines (Bonvehi & Coll, 1997; Luna *et al.* 2002; Misnawi *et al.*, 2005). Research has shown that during fermentation, the polyphenol content of the cocoa beans decreases. If fermentation is terminated at three, four, five and six days, the polyphenol values will be 10.7%, 8.2%, 7.6% and 6.01% (wt/wt) respectively (Aikpokpodion and Dongo, 2010). This shows that beans which are fermentated for three days have high percentage of purple cocoa beans compared to cocoa beans fermentated for six days.

Immediately after fermentation the cocoa beans are dried, either traditionally by being spread in the sun on mats or using special drying equipment. In Ghana cocoa beans are mostly dried by spreading the beans and constantly raking them. Drying is done to reduce the moisture content to 6.0-7.5% and to retain the chocolate flavour developed during fermentation (Sukha, 2003). Sun drying is the most common and widely used method of drying cocoa beans in other West Africa and Latin America. The cocoa beans are placed on wooden platforms with mats(Ghana), or polypropylene sheets (Côte d'Ivoire) or concrete floors in layers ranging from 5-7 cm thick (Thompson *et al.*, 2001). Fermented, dried cocoa beans are stored for a period of 3 to 12 months in warehouses. The efficiency of the drying process and the degree of dryness will determine the shelf life of the product. Uniformly dried cocoa beans with a moisture content of 6.0-7.5%, when

stored at a relative humidity of 65-70%, will generally maintain that moisture, resist mould growth and insect infestation (Thompson *et al.*, 2001).

All cocoa beans are susceptible to both beetle and moth infestation. Some of the common beetles are *Lasioderma serricorne* (F.) (Cigarette beetle) and *Araecerus fasciculatus* (Degeer) (coffee bean weevil), which can pierce the shell of the bean thereby providing an entrance for moths such as tropical warehouse moth (*Ephestia cautella*) (Walker) and for moulds (Jonfia-Essien, 2001; 2004). In Ghana, dry cocoa beans in storage were monitored for insect pests from 1995 to 2000 and *L. serricorne*, *Tribolium castaneum* (Herbst), *E. cautella* and *Corcyra cephalonica* (Stainton) were among the eleven species identified as most important (Jonfia-Essien, 2004). Insect pests inflict damage on stored products mainly by direct feeding, but their mere presence in foodstuffs is a nuisance. In many species both larvae and adults cause damage, which may sometimes be devastating. Several factors may be responsible for insect infestation in storage and of special importance is the number of flying insects searching for food (Hodges *et al.*, 2002).

## 1.1 Justification

Good quality cocoa bean is not necessarily about the variety, or where it is grown, or whether it is processed by a small-holder or on an industrial scale. What matters is the quality of fermentation and drying, because this is the only way to unlock the unique flavours of cocoa beans (Samantha, 2005). Quality of dry cocoa beans in international trade is assessed on the percentage level of total mould, slaty, purple, insect infestation, flat, and germinated beans (COCOBOD, 2011). It has been observed through personal interaction with cocoa farmers that,

some farmers being under pressure from the buyers of cocoa beans do not allow their beans to undergo adequate fermentation. Some of the cocoa farmers just allow a few days fermentation while a few do not allow fermentation at all which results in purple cocoa beans (Adzaho, 2007). Thus purple cocoa bean is caused mainly by improper fermentation (Adzaho, 2007). However there is no scientific data that shows what happens to the already produced purple cocoa beans in storage and the effect of insects infestation on the purple cocoa beans. This study therefore aims at determining the impact of storage period and the level of insect infestation on the purpleness of cocoa beans.

## 1.2 Objectives

The main objective of this study was to determine the impact of storage and the level of damage caused by *E. cautella* and *T. castaneum* on the stored purple cocoa beans. The specific objectives were:

1. Determine the population of both insect pests in the stored purple cocoa beans and their contaminant.
2. Assess the damage to stored purple cocoa beans caused by *E. cautella* and *T. castaneum* singly and in combination.
3. Determine the impact of storage period on the purpleness of cocoa beans.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Origin and botany of cocoa

Cocoa (*Theobroma cacao*) is one of 22 species of the genus *Theobroma* and the family Sterculiaceae. It is a native of South America from where it has been introduced to various parts of the tropical forest belt since the 16<sup>th</sup> century (Motamayor *et al.*, 2002). The species *Theobroma cacao* consist of a large number of highly morphological variable populations (Osei-Bonsu, 2005). Based on morphological traits and geographical origin, two main genetic groups have been defined within the species; Criollo and Forastero (Motamayor *et al.*, 2002). The Criollo produces the most flavoursome cocoa but are difficult to establish and are also prone to attack by diseases and pests (Osei-Bonsu, 2005). The Forastero, which is produced widely in West Africa and constitutes the West Africa “Amelonado” is less flavoursome but can withstand disease and pest attack compared to Criollo (Mossu, 1992). A third group, Trinitario is a hybrid of Criollo and Forastero and their botanical features have all the intermediate features of Criollo and Forastero group (Mossu, 1992).

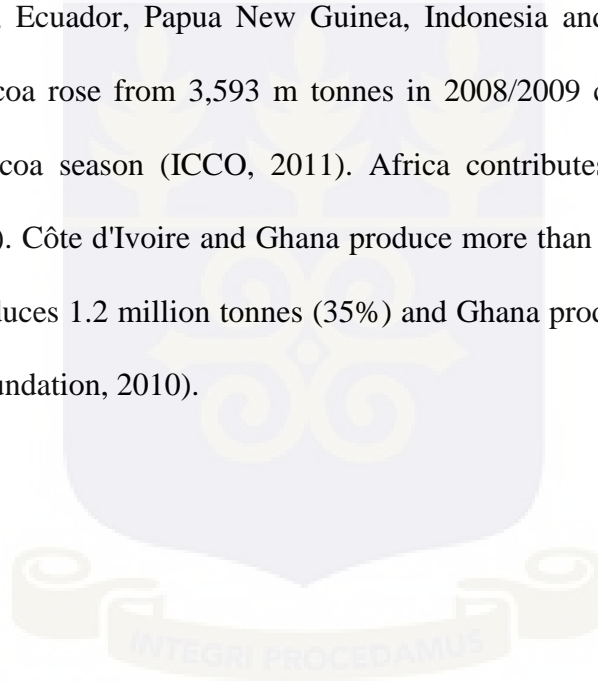
Cocoa is cauliflorous or truncate, which means that the flowers develop on the older parts of the trunk and branches, developing into fruits around the trunk (Opeke, 1992). The tree produces greenish white flowers at 1-5 years after planting in the field (Thompson *et al.*, 2001). These flowers are produced seasonally from cushions that emerge on the bark of the trunk and stem (Thompson *et al.*, 2001). A successfully pollinated flower produces pods. The development of the pod takes 5-6 month from pollination to ripening (Sanusi and Oluyole, 2005). The matured height of the tree is about 4-9 m with a round canopy of 6-8 m diameter if grown in isolation. These fruits are called pods and contain 20–40 seeds (Mossu, 1992; Dand, 1993). The economic

rotation for cocoa is estimated at 25– 30 years of age, after which yields may decline and replanting becomes necessary (Mossu, 1992; Dand, 1993).

## **2.2 Cocoa Production**

### **2.2.1 World production of cocoa**

There are many cocoa producing countries in the world but the major producers are from the African countries (Cote d'Ivoire, Ghana, Nigeria, Cameroon) (Table 2.1). Other major producing countries include Brazil, Ecuador, Papua New Guinea, Indonesia and Mexico (ICCO, 2011). World production of cocoa rose from 3,593 m tonnes in 2008/2009 cocoa season to 4,250 m tonnes in 2010/2011 cocoa season (ICCO, 2011). Africa contributes over 70% of the total production (IOCC, 2011). Côte d'Ivoire and Ghana produce more than half of the world's cocoa beans. Côte d'Ivoire produces 1.2 million tonnes (35%) and Ghana produces 0.73 million tonnes (21%) (World Cocoa Foundation, 2010).



**Table 2.1: World cocoa production estimates from 2008 to 2011**

<b>Countries</b>	<b>2008/2009</b>	<b>2009/2010</b>	<b>2010/2011</b>
Africa (thousand tonnes)			
Cote d'Ivoire	1223	1242	1511
Ghana	662	632	1025
Nigeria	250	235	240
Cameroon	224	205	230
Others	157	168	175
America (thousand tonnes)			
Brazil	157	161	200
Ecuador	135	150	145
Others	186	206	195
Asia and Oceania (thousand tonnes)			
Indonesia	490	550	440
Papua New Guinea	59	39	45
Others	48	44	45
<b>Total</b>	<b>3593</b>	<b>3631</b>	<b>4250</b>

Source: ICCO (2011)

### **2.2.2 Ghana cocoa production**

The commercial production of cocoa in Ghana began when the cocoa pod was brought from Fernando Po by Tetteh Quarshie in 1897 (Manu and Tetteh, 1987). In 1965/1966 production in Ghana reached an all-time peak of 560,000 tonnes but this fell to a very low level of 150 tonnes in 1983/1984 season resulting in the country losing her position to Cote d'Ivoire as the world largest producer of cocoa (Amanor, 2005). The ravages caused by insect pests and diseases, low producer price, unavailability of production inputs such as insecticides, fungicides, fertilizers, pesticide application equipment such as sprayers and labour, contributed to the dwindling of cocoa industry (Amanor, 2005). Since then Ghana became the second largest producers of cocoa in the world (Ntiamoah and Afrane, 2008) (Table 2.2).

In 2010/2011 cocoa season, Ghana produced 1,024,150 tonnes which was the highest production since Ghana registered its name in the international market as a producer of cocoa (COCOBOD, 2011). This was attributed to the efforts made in the disease and pest control programmes, the use of fertilizer to encourage the increased production of cocoa, and an increase in the producer price of cocoa (ISSER, 2012). Cocoa bean from Ghana is recognized for of its quality and therefore Ghana is regarded as the world leader in premium quality cocoa bean (Ntiamoah and Afrane, 2008).

**Table 2.2: Annual cocoa production in Ghana from 2000 to 2012**

<b>Crop year</b>	<b>Production (tonnes)</b>
2000/2001	389,772.00
2001/2002	340,562.00
2002/2003	496,846.00
2003/2004	736,975.00
2004/2005	599,317.69
2005/2006	740,457.63
2006/2007	614,531.63
2007/2008	680,781.06
2008/2009	710,642.19
2008/2010	632,037.25
2010/2011	1,024,554.00
2011/2012	879,347.90

Source: COCOBOD (2012).

### **2.3 Economic important of cocoa beans**

Cocoa is a main cash crop and a major source of income for many farmers in West Africa (Motamayor *et al.*, 2008). In Ghana, the importance of the cocoa industry to the economy cannot be over emphasized. Since the introduction of cocoa into the country, the industry which started with rudimentary production technology has made significant strides in major socio-economic contributions to date in such areas as employment, foreign exchange earnings, government revenue and gross domestic product (GDP). The cocoa industry in Ghana employs over 800,000 small holder farm families (Asamoah and Baah, 2003). Cocoa constituted 23% of the foreign

export earnings from the agricultural sector, compared to 1.3% and 2.3% contribute by timber and the non-traditional agricultural export (ISSER, 2012). In 2011, cocoa export increased by 25.6% in total value from US\$ 2285.2 million in 2010 to US\$ 2870.8 million (ISSER, 2012). It serves as the major source of revenue for the provision of socio-economic infrastructure in Ghana (ISSER, 2009).

The health benefit of cocoa cannot also be over emphasized. It helps to protect tissues against stress, and certain polyphenols work as preventive medicines for problems such as cardiovascular diseases, cancer, arthritis and autoimmune disorders (Mursu *et al.*, 2004). They act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals and their ability to stabilize membrane by decreasing membrane fluidity. (Aror *et al.*, 2000; Kromhout *et al.*, 2002; Lee *et al.*, 2003). Some polyphenols (such as proanthocyanidins) exert beneficial cardiovascular effects through inhibition of platelet aggregation (Murphy *et al.*, 2003). Other antioxidants found in cocoa beans include, catechins, epicatechin and procyanidins, polyphenols similar to those found in wine and tea (Carnesecchia *et al.*, 2002; Hatano *et al.*, 2002; Hermann *et al.*, 2006).

#### **2.4 Cultivation of cocoa**

Cocoa is cultivated in the humid tropical belt (Motamayor *et al.*, 2008). It requires a soil which can easily be penetrated by its roots, retain moisture during the dry season and permits the circulation of air and moisture (Motamayor *et al.*, 2002). Good cocoa soil are deep well drained, non gravelly top soil over a sandy clay loam layer with pH 8.5 and temperature maximum of 30-32<sup>0</sup>C and minimum of 18-21<sup>0</sup>C (Wood and Lass, 1985). Cocoa plant is highly susceptible to

drought. It is cultivated in areas with average rainfall of 1150 mm and 2500 mm; such areas lie along the equator in West Africa, Central and South America, and in Asia/Oceania (ICCO, 2008). The cocoa growing areas in Ghana are Ashanti, Brong Ahafo, Central, Eastern, Western and Volta Regions (Adzaho, 2007). The main cocoa-producing region is presently the Western Region, which accounts for more than 50% of total annual production (Obeng, 2010).

Just as other widely grown perennials such as coffee (*Coffea* spp.), black pepper (*Piper nigrum*) and tea (*Camellia sinensis*) cocoa is grown under shade trees (Willson, 1999). In West Africa, cocoa farmers retain desirable trees to provide shade for young cocoa during clearing and land preparation (Oke and Odebiyi, 2008). Planting of cocoa is usually carried out from April to July during the rainy season (Obeng, 2010). There are two main cocoa harvesting season in Ghana, the main and light crop season. The main crop season falls between September and February and the light crop season between March and August (Adzaho, 2007).

## **2.5 Harvesting and Post harvest handling of cocoa**

### **2.5.1 Harvesting**

Harvesting of cocoa is spread over several months and the peak harvest in Ghana is from October to December (Vos *et al*, 2003; Mikkelsen, 2010). Cocoa is also harvested at all times of the year once the tree is in its production stage (Wood and Lass, 1985). Harvesting must be carried out using specific techniques and tools. Sharp cocoa hook on a stick are used for pods higher up the trees. Secateurs can be used to harvest pods within easy reach. These tools should be kept clean (ICCO, 2008). According to Mossu (1992), harvesting of pods should be carried out at regular intervals of 10 to 15 days and should not exceed three weeks. A delay in

harvesting ripe pods lead to germination of the beans inside the pods and harvested unripe pods have smaller beans which when fermented produce poor quality of beans with possible lower fat content which are low in aromatic compounds (Wood and Lass, 1985).

### **2.5.2 Pod opening**

Cocoa pods are opened by means of cutlass or machete, club or by hitting them against a stone or stick. In Ghana the usual practice is by gathering the pods at convenient places in the farm (Plate 2.1) and breaking them with sticks (Asare, 2012). Opening of the pods can be delayed up to 14 days after plucking as this increase percentage of brown beans (Afoakwa *et al.*, 2012). Beans from delayed pod opening initiate rapid rise in temperature during fermentation through the activities of invertase (Tomlins *et al.*, 1993), a very important factor that leads to the death of the beans.

A delay between harvesting and pods opening also results in reduction in the concentration of pulp sugars and reduced pulp/cotyledon ratio of fresh beans (Nazaruddin *et al.*, 2006). During the period of delayed pod opening there is decrease in pulp volume per seed due to loss of moisture and inversion sucrose which reduces the amount of sugar leading to reduced acid production during fermentation (Biehl *et al.*, 1990). According to Biehl *et al.* (1990), improve cocoa flavour could be attributed to reduction in pulp volume which was linked to postharvest storage of pods or pre-conditioning the pulp prior to fermentation.

The normal practice at Cocoa Research Institute of Ghana (CRIG), Tafo is to break the pods three days after harvesting because this interval results in fermentation having a sharper rise in temperature and a higher maximum temperature than when the pods are broken shortly after harvesting (Afoakwa *et al.*, 2011). After opening of the pods the wet beans are separated from

the placenta before fermentation to avoid the incidence of double and multiple beans in the dry product (Asare, 2012).



**Plate 2.1: A heap of harvested cocoa pods awaiting opening**

### **2.5.3 Fermentation Processes**

Fermentation is the initial step needed to develop various flavour precursors in the beans (Hii *et al.*, 2009). Fresh cocoa beans have an astringent, unpleasant taste and have to be fermented, dried and roasted in order to obtain raw cocoa beans with desired characteristic flavour and taste (Thompson *et al.*, 2001). According to Thompson *et al.* (2001) and Jespersen *et al.* (2004), fermentation of cocoa is a complex interaction between filamentous fungi, yeasts, lactic acid bacteria, acetic acid bacteria, *Bacillus* spp and presumably their viruses. The main objective of fermentation is to solubilize and remove the pulp surrounding the beans. It is to create condition

that generate the precursors of chocolate flavour. These include breaking the beans and inducing their autolytic breakdown by endogenous enzymes as well as producing other flavour metabolites (Thompson *et al.*, 2001; Jespersen *et al.*, 2004).

During fermentation, yeast from the family *Enterobacteriaceae* convert the sugar from the pulp surrounding the beans to ethanol (Schwan and Wheals, 2004; Nielsen *et al.*, 2007) under anaerobic condition and cause depectinization of the pulp thus enabling the pulp to flow away and ingress of air (Schwan and Wheals, 2004). Lactic Acid Bacteria (LAB) mainly from the genus *Lactobacilli* convert sugars from the pulp and organic acids mainly into lactic acid (Camu *et al.*, 2007) and under aerobic condition, Acetic Acid Bacteria (AAB) mainly from the genus *Acetobacter* (Camu *et al.*, 2008) initially produced by the yeasts, oxidize the ethanol to acetic acid and then to carbon dioxide and water producing more heat and raising the temperature (Dand, 1993; Beckett, 1994). Turning of the beans makes condition become more aerobic and halts the activity of lactic acid (Dand, 1993; Beckett, 1994; Camu *et al.*, 2008).

Ethanol and acetic acid diffuse into the beans, and in combination with the heat produced by the exothermic bioconversion, causes the death of the seed embryo and disruption of the internal cellular integrity of the beans (Voigt *et al.*, 1994; Thompson *et al.*, 2001; Schwan and Wheals, 2004; Nielsen *et al.*, 2007). This allows complex biochemical processes to take place in the cotyledon leading to the breakdown of protein to peptides and amino acid (Lerceteau *et al.*, 1999; Buyukpamukeu *et al.*, 2001) and breakdown of anthocyanins to anthocyanidins and sugar (Wollgast and Anklam, 2000). Therefore, these biochemical changes lead to the formation of precursor molecules that are necessary for the development of the characteristic flavour, aroma,

and colour of the beans (Camu *et al.*, 2007). At the end of fermentation of cocoa beans, changes in colour from purple to brown are widely used to predict flavour potential of the beans (Ardhana and Fleet, 2003). Therefore to maintain a good quality of cocoa beans, adequate fermentation that last between 6-7 days should be under taken (Ardhana and Fleet, 2003).

### **2.5.3.1 Fermentation methods**

Several fermentation methods are used around the world which includes heaps, boxes, trays and baskets. The heap and box fermentation are the most widely used (Nielson *et al.*, 2007; Hii *et al.*, 2009). After pod harvest, cocoa beans are piled in either heaps, boxes, trays or baskets, covered with plantain leaves (Fowler, 1999) and left to ferment for 6 days (Afoakwa *et al.*, 2008). On the first day, the adhering pulp liquefies and drains off, with steady rises in temperature. By the third day, the bean mass will have heated typically around 45<sup>0</sup>C, remaining at 45–50<sup>0</sup>C until the fermentation is complete (Fowler, 1999; Kealey *et al.*, 2001).

In Ghana, the heap fermentation system dominates (Baker *et al.*, 1994). The beans are piled on banana or plantain leaves placed on the ground and covered with other banana or plantain leaves with sticks on top (Mounjouenpou, *et al.*, 2008). Heap fermentation size ranges from 20 to 1000 kg (Baker *et al.*, 1994) (Plate 2.2). Aeration of the heaps by turning the beans after every 2 days (during the fermentation) is considered beneficial for the quality of the end product, but this scheme is not followed by all farmers (Baker *et al.*, 1994). To circumvent the laborious process of turning the heaps, tray fermentation has been developed at the (CRIG). In tray fermentation the raw cocoa beans are placed in 120 x 90 x 10 cm wooden tray and a tray can hold about 90 kg wet beans. The trays are stacked on top of each other, 4 – 12 trays high and placed on a wooden platform (Plate 2.3) to promote air circulation, avoid the ground absorbing the produced heat and

allow the sweating to drain away. The beans are covered with banana or plantain leaves to prevent air pockets. Air pockets will cool down the heat and promote the growth of moulds (Mikkelsen, 2010).



**Plate 2.2: Heap fermentation**



**Plate 2.3: Tray fermentation**

In box fermentation, the beans are placed in a set of three boxes, each measuring 45 cm × 45 cm × 45 cm, and with drainage holes at the bottom raised from the ground. The boxes are arranged one in front of the other at three levels with sliding tops and removable panels in front (Plate 2.4). These boxes are lined with plantain or banana leaves. The top box are filled with the beans and covered with plantain or banana leaves to retain the heat. After 2 days the beans are turned over and transferred into the next box. The beans are turned over again after another 2 days and transferred in to the third box to complete fermentation (Mounjouenpou, *et al*, 2008).

The baskets used in basket fermentation are of different sizes, but generally can hold 30 to 40kg of beans and sometimes up to 90kg. The baskets are weaved from plant materials and are therefore not airtight. The inside of the basket is lined with banana or plantain leaves and the bottom remains uncovered to allow the sweating drain away. Then the beans in the basket are covered with banana or plantain leaves and left to ferment (Plate 2.5). Turning of the beans are done on the second and fourth day (Mossu, 1992)



**Plate 2.4: Box fermentation**



**Plate 2.5: Basket fermentation**

### **2.5.3.2 Duration of fermentation**

The duration of cocoa beans fermentation depends on the variety of the beans, season, the condition of the harvested pods, climate, volume of the mass of cocoa undergoing fermentation and the method of fermentation (Mossu, 1992; Aneani and Asamoah, 2004). On the first day, the adhering pulp liquefies and drains off, with steady rises in temperature. Under anaerobic conditions micro-organisms produce acetic acid and ethanol that inhibit germination and contribute to structural changes such as the removal of the compartmentalization of enzymes and substrates. The structural changes cause movements of cytoplasmic components through the cocoa cotyledon and this generally take place between 24–48 hours of bean fermentation. By the

third day, the beans mass will have heated typically around 45<sup>0</sup>C, remaining at 45-50<sup>0</sup>C until the fermentation is complete (Fowler, 1999; Kealey *et al.*, 2001).

In Costa Rica, fermentation period is not more than 3 days and drying start after only one day fermentation in order to take advantage of the sun (Wood, 1957). In Nigeria, fermentation period ranges from 5 to 6 days (Dongo *et al.*, 2009). In Ghana, fermentation duration is 6 days on average with a range of 4-7days depending on the weather and condition of harvested pods. Inadequate sunshine during the rainy season prompts 4 days fermentation whilst some farmers extend their fermentation period to even 7 days during the dry season (Aneani and Asamoah, 2004). Beans from freshly harvested pods are fermented for 6-7 days whereas those from exclusively ripe pods are fermented for 4 days (Aneani and Asamoah, 2004). Forastero and Trinitario type of cocoa are usually fermented for 4-6 days while 2-3 days for the Criollo type (Mossu, 1992).

Clapperton *et al.*, (1994), reported that 3 days fermented beans have marginal increase in beans flavour an indication of under fermentation but 5 and 7 days fermentation showed no appreciable difference in flavour. Fermentation is terminated when the beans swell, certain odour are developed, the cotyledon turn brown and the temperature falls. All these occur on average after 5-6 days (Mossu, 1992).

#### **2.5.4 Drying of cocoa beans**

Drying is done to reduce the moisture content to 6.5 to 7.5% (Sukha, 2003) which is safe for storage and transportation. Drying also promotes continuation of the oxidation process of fermentation. During drying there is reduction in bitterness and astringency and development of the chocolate brown colour of well fermented beans (Fagunwa *et al.*, 2009). Also drying retains the chocolate flavour developed during fermentation (Sukha, 2003).

Cocoa beans are usually dried as a thin layer of about one or two beans thick under typical tropical weather using sun drying. Sun drying is the most common and widely used method of drying cocoa beans in West Africa and Latin America. The beans are placed on wooden platforms, mats, polypropylene sheets, or concrete floors in layers ranging from 5 to 7cm thick (Thompson *et al.*, 2001). Sun drying may last between 7 and 21 days depending on the weather (Takrama & Adomako, 1996). Under favourable and sunny conditions, cocoa beans at two to three beans thick can still be dried without any significant loss in quality, but quality loss will be expected under adverse weather conditions. In Ghana, farmers sundry the beans on mats raised off the ground (Plate 2.6), the drying mats are erected in the villages where the beans could be spread out in the morning turned and cleaned during the day and heaped at night or in the event of rain to prevent wetting of the beans by dew during the night and rain water during rainfall (Adzaho, 2007).



**Plate 2.6: Drying of cocoa beans**

Sun drying can also be carried out in a drying ‘autobus’, which is a shelter and ramps at different heights allowing trays to slide from the sun under the roof. This is used for drying cocoa beans at CRIG, New Tafo (Plate 2.7).



**Plate 2.7: Drying ‘Autobus’ at CRIG**

Hii *et al.*, (2003) reported that lower loading of 20kg on drying mats have lowest percentage of purple and highest in percentage of brown beans. The lower loading on drying mats ensures better aeration. Beans loading at 20kg did not suffer from putrefactive activities, produced beans with good appearance and are extremely light in surface mould, high in fermentation index and acceptable in terms of odour and liquor sensory evaluation.

The rate of drying also affect the quality of beans, if drying is slow, there is a danger that moulds will develop and penetrate the testa. There is a further danger that off-flavour may arise. On the other hand rapid drying may prevent the oxidation changes being completed thus resulting in excessive acidity in cocoa beans which is unsuitable for chocolate manufacture (Barel, 1998), Another method of cocoa bean drying used in Malaysia is the direct solar dryer; it has been

developed for smallholders. It uses direct sunlight to dry the beans placed inside a transparent enclosure which has the advantage of protecting the cocoa beans from unfavourable weather condition (Hill *et al.*, 2006). The solar dryer prototype consists of two sections, the drying platform and the transparent enclosure. The drying platform is constructed with plywood and the transparent material used for the enclosure was ultraviolet-stabilized polyethylene film (Hill *et al.*, 2006).

Ovens are also used for drying cocoa beans; they are simple dryers that use heat to dry the beans. They can be heated either by hot air from an external fire or by the base of the oven. The beans are spread on trays, allowing the air to permeate through a ladder system (Mossu, 1992). Mechanical dryers are also used where big volumes of cocoa beans are to be dried. The cocoa beans are placed on rotating platforms that circulate in a tunnel through which hot air is blown. Drying cocoa beans with mechanical dryers takes 10–20 hours, depending on the initial moisture-content of the cocoa bean (Mossu, 1992). This rapid drying prevents the continuation of the oxidative stage of fermentation of the beans hence preventing the reduction of constringency and bitterness in the cocoa product.

### **2.5.5 Storage**

The efficiency of the drying process determines the shelf life of the product to be stored (Thompson *et al.*, 2001). Uniformly dried cocoa beans with a moisture content of 6.5 to 7.5%, when stored at a relative humidity of 65%-70%, will generally maintain that moisture; resist mould growth and insect infestation (Thompson *et al.*, 2001). However, since cocoa beans are hygroscopic, their sudden removal from cool to warm areas without sufficient temperature equilibration would cause moisture migration (Jonfia-Essien and Navarro, 2010).

In West Africa, some companies are trying to introduce bulk handling in containers, but most of the trade is still in jute bags of about 65 kg (Navarro, *et al.*, 2007). Cocoa beans are normally stored in form of whole beans in jute bags for relatively short periods. During storage and transportation they are subjected to problems of insect infestation, mould contamination and moisture exchange between atmosphere and the beans (Villers *et al.*, 2007). Cocoa beans are hygroscopic and will absorb moisture under very humid conditions until they reach equilibrium. The ambient humidity must not exceed 70% (Mossu, 1992). Warehouses are practical and appropriate structure for storing and protecting cocoa beans (Plate 2.8). A well managed cocoa bean warehouse should be free from insect infestation which causes devastating effect on the beans (Jonfia-Essien and Navarro, 2010). In Nigeria some farmers store their dried beans on bare floor while others stored on raised platforms. The former practice will contribute to mould development due to the humidification of the beans. Equally, the beans are prone to rodent and insect pest attack (Dongo *et al.*, 2009).



**Plate 2.8: Cocoa beans stored in jute sack in a typical warehouse**

From Villers *et al.* (2007), hermetic storage (HS) technology has emerged as a significant alternative to other methods of storage that protect commodities from insects and moulds. This technology, also termed sealed storage, airtight storage, or assisted hermetic storage, is a form of bio-generated modified atmosphere (MA) (Plate 2.9).

Hermetic storage is based on the principle of generation of an oxygen-depleted, carbon dioxide-enriched interstitial atmosphere caused by the respiration of the living organisms in the ecological system of a sealed storage (Navarro *et al.*, 2007). Jonfia-Essien *et al.* (2008) reported that cocoa beans that are stored using Hermetic storage (Cocoon) maintain their quality. They also reported that high populations of the insects were out of the bags of cocoa beans and were found dead inside the Cocoon. Based on their results, use of hermetic structures made of gastight plastic liners can be a safe and viable alternative to permanent structures for organic protection of cocoa beans for extended periods and it seems to be the most promising method for storing cocoa beans. In Trinidad, cocoa is never stored for a significant period of time before shipment, thus reducing the chance of picking up strong off odour, increased moisture levels and mould growth (Sukha, 2003).



**Plate 2.9: Hermetic storage of cocoa beans.**

Stored cocoa beans can be easily maintained and treated, transported to and from the warehouse and regularly checked for insect or fungal infestation. In Ghana, cocoa beans are stored in up-country warehouses before they are finally evacuated by Licensed Buying Companies (LBC's) to the main take-over centers (mainly Tema port, Takoradi port and the Kaase inland port) from where they are shipped to other countries (Obeng, 2010).

## **2.6 Impact of storage on purple cocoa beans**

Fermented, dried beans can be stored for periods of 3 to 12 months in warehouses found in exporting and importing countries and factories before the beans are processed into chocolate. Storage management plays a vital role in maintaining the quality of cocoa beans in storage. Particularly dry and cool conditions are preferred. Lower temperatures would result in maintaining better quality cocoa beans by inhibiting Free Fatty Acid (FFA) and insect development. Prolonged storage of cocoa beans especially in damp conditions results in a noticeable staling (Lopez and Dimick, 1995). Hence, it is advisable to store the beans for not more than 6 month (Woods and Lass, 1985). If cocoa beans need to be stored for a longer period in the tropics, special precautions must be taken to ensure that quality does not deteriorate (Mossu, 1992).

In 2008, Jonfia-Essisen *et al.* reported that there were some reduction in the total purple colour of some of the cocoa beans stored for 9 weeks and reported that the difference were not significant enough to cause any change in the grade. Cocoa with high percentage of purple beans gives a bitter and astringent chocolate.

## 2.7 Insects infestation and beans purpleness

Insect infestation can start from the farm but mostly from the drying mats. At the end of a season, the mats are usually rolled up and stored but they often carry the eggs and pupae of insects which may emerge to infest the next crop. Similarly the area around artificial drying can provide a breeding site for pests (Wood and Lass, 1985; Jonfia-Essien, 2004). Infestation can also occur during transportation to the warehouse for storage or shipment. The majority of insects associated with stored cocoa beans are those which also infest other dried food stuffs, such as grain, seeds and dried fruit.

Cocoa beans are susceptible to attack by several species of insect such as beetles and moths. Some of the common beetles are *Tribolium castaneum* (Herbst) (Rust-red flour beetle) *Lasioderma serricorne* (Fabricius) (Cigarette beetle) and *Araecerus fasciculatus* (Degeer) (Coffee bean weevil), which can pierce the shell of the bean thereby providing an entrance for moths such as tropical warehouse moth (*Ephestia cautella*) (Walker), the rice moth, *Corcyra cephalonica* (Stainton) and for moulds (Jonfia-Essien, 2001; 2004). Storage beetles are attracted to cocoa beans and cause damage by boring holes in the beans or feeding on the nib while the moths, in addition to causing damage by larval feeding, produce silken threads, which builds up into dense webbing that binds the cocoa beans (Jonfia-Essien *et al.*, 2007). Their feeding action causes loss of weight of the commodities thus reducing its commercial and nutritional value (Dharmaputra *et al.*, 2000). In Ghana, dry cocoa beans in storage were monitored for insect pests from 1995 to 2000 and *Lasioderma serricorne*, *Tribolium castaneum*, *Araecerus fasciculatus*, *Ephestia cautella* and *Corcyra cephalonica* were among the eleven species identified as most important (Table 2.3) (Jonfia-Essien, 2004).

**Table 2.3: Insect pests of cocoa beans found in Ghana**

Order	Family	Genus and species
Coleoptera	Tenebrionidae	<i>Tribolium castaneum</i> (Herbst)
Coleoptera	Silvanidae	<i>Oryzaephilus mercator</i> (Fauvel)
Coleoptera	Anabiidae	<i>Lasioderma serricorne</i> (Fabricius)
		<i>Araecerus fasciculatus</i> (Degeer)
Coleoptera	Bostrichidae	<i>Rhyzopertha dominica</i> (Fabricius)
Coleoptera	Cucujidae	<i>Cryptolestes ferrugineus</i> (Stephens)
		<i>Cryptolestes pusillus</i> (Schonherr)
Coloptera	Nitidulidae	<i>Carpophilus hemipterus</i> (Linnaeus)
		<i>Carpophilus dimidiatus</i> (Fabricius)
Lepidoptera	Pyralidae	<i>Corcyra cephalonica</i> (Stainton)
		<i>Ephestia cautella</i> (Walker)

Source: Jonfia-Essien, 2004

### 2.7.1 *Tribolium castaneum*

The rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae) is a polyphagous, cosmopolitan pest in stores or wherever durable products and other dried foods are processed. It is often the most common secondary pest species in the pest complex attacking stored products. It is also a facultative predator and scavenger. Both adults and larvae are known

to prey on immature stages of *Corcyra cephalonica* (rice moth) which is a potential competitor within stored product environments (Alabi *et al.*, 2008).

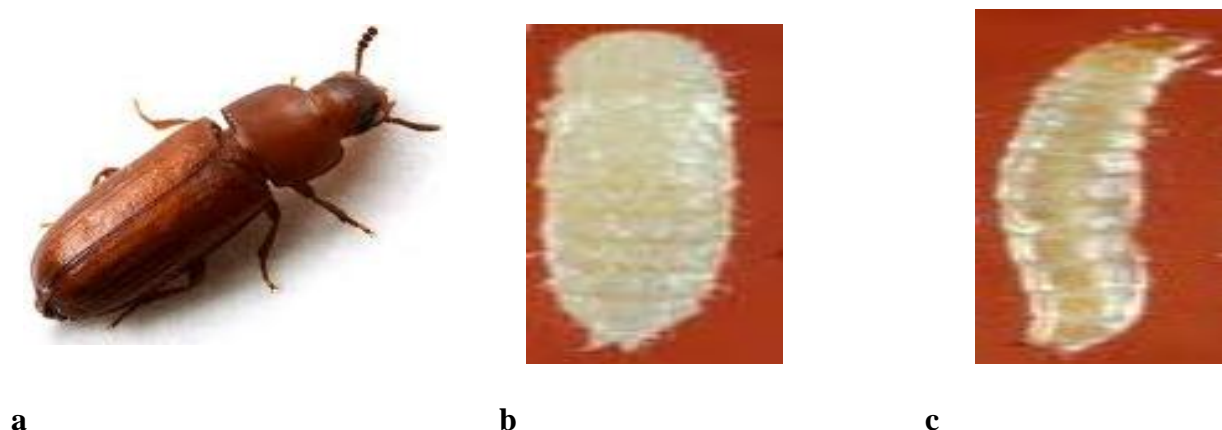
The original habitat of *Tribolium* spp. is thought to be under the bark of trees or rotting logs where they originally fed as fungivores or saprovores, occasionally scavenging insect eggs and pupae (Suresh *et al.*, 2001; Alabi *et al.*, 2008). *T. castaneum* is thought to have originated from India from where it has spread throughout the warm temperate, tropical and subtropical regions of the world (Obeng-Ofori, 2008). It has a wide range of host and an unspecialized feeder on durable products, including cereal grains, and grain products, cocoa, coffee, nuts, spices, dried fruits, peas and animal products as well as dry museum specimens (Obeng-Ofori, 2008). This beetle has been found to be one of the most common insects infesting cocoa beans in Ghana (Finkelman *et al.*, 2003; Jonfia-Essian, 2004).

The rust red flour beetle is 2.3-4.4mm in length and it is shiny, flattened, oval and reddish-brown (Bennett, 2003). Adult females lay about 300-400 sticky eggs directly on the stored product throughout their life (about 2-3 eggs per day) (Bennett, 2003). Under optimum conditions of 35°C and 75% relative humidity, the eggs hatch into larvae within 5-12 days and the larval period varies from 22 to more than 100 days (Bennett, 2003). The number of larval instars varies widely ranging from 5-11 depending on the individual, environmental conditions, and availability of food resources. The larvae are active, but generally hide within the food, away from light (Mason, 2003).

The last instar larva is brown-headed (Plate 2.10), with a slender body and length of about 6mm (Bennett, 2003). It transform into a naked pupa without any form of protection. Initially it is white but gradually it changes to yellow then to brown and finally into the reddish-brown adult

(Plate 2.10) (Calvin, 1990). The pupal stage last for five days (Boateng and Obeng-Ofori, 2008). The development period from egg to adult is about 26 days at 32 – 35<sup>0</sup>C and above 70% relative humidity (Mason, 2003), but it is greatly prolonged by cold weather (Calvin, 1990). This short phase of the life cycle may contribute to its high intrinsic rate of increase (Boateng and Obeng-Ofori, 2008). The adult are long-lived and may live for more than 3 years (Mason, 2003; Baldwin and Fasulo, 2007). Longevity of adults has been shown to increase with the degree of damage to grains (Baldwin and Fasulo, 2007). Adults of rust red flour beetle are very active, quickly running for cover whenever they are disturbed, and can be found either on the surface or deep within the stored produces.

Infestation by *T. castaneum* leads to persistent and pungent odour in the produce due to secretion of benzoquinones from a pair of abdominal defence glands (Obeng-Ofori, 2008) when agitated or are crowded (Mason, 2003). Both the larvae and the adult cause damage to cocoa beans by feeding (chewing through the outer coating of stored produce when the moisture content is above 12%) and contaminating the produce with their cast skin, frass and fecal pellets (Calvin, 1990; Bennett, 2003). Therefore the presence of red flour beetle in a storage environment indicates increase in moisture content and presence of moulds (Calvin, 1990).



**Plate 2.10: Life stages of *T. castaneum* (a) Adult (b) Pupa (c) Larvae**

### 2.7.2 *Ephestia cautella*

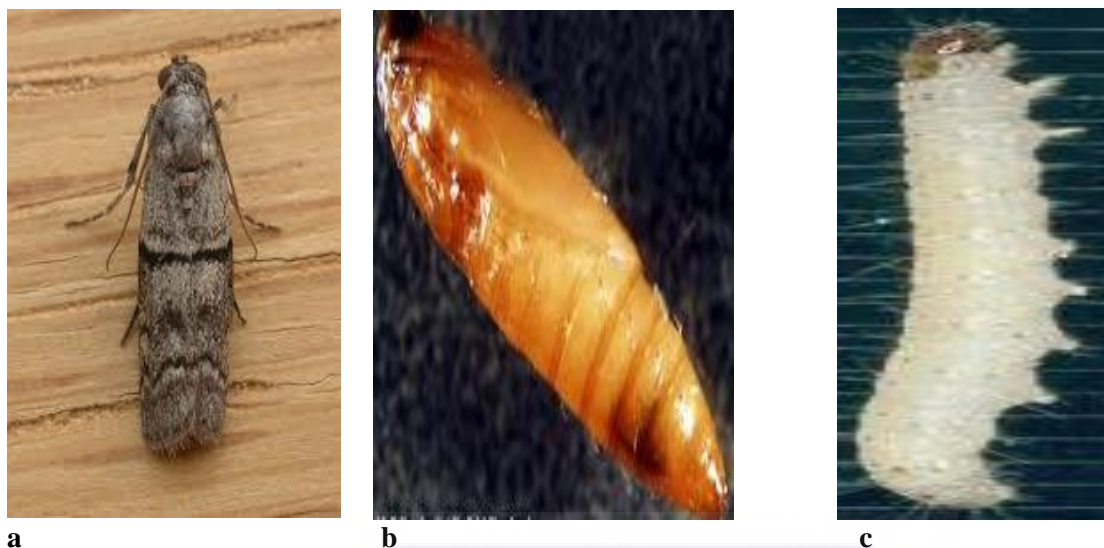
Tropical warehouse moth, *Ephestia cautella* (Walker) (Pyralidae: Lepidoptera) is one of the most wide spread and abundant major pest of stored products (Jonfia-Essian, 2004). Known also as almond moth or cocoa moth *E. cautella* is found in the tropics and warmer regions of the world. It can be a serious pest in heated storage facilities in temperate regions (Obeng-Ofori, 2008). It has an extensive host-food range and attacks a variety of stored products including cereals and cereals products, cotton seeds and cotton cake, dates, ground nuts, confectionary (biscuits and chocolates), spices and dried onions causing damage during harvest, storage, processing and exportation (Dharmaputra *et al.*, 1999; Chandra 2006; Obeng-Ofori, 2008). This moth is one of the most common insect pests infesting cocoa beans in Ghana (Finkelman *et al.*, 2003; Jonfia-Essian, 2004).

The female adults produce sex pheromones which attract potential mates for mating and lay up to 300 eggs directly in cracks or crevices on grain after which they die (Obeng-Ofori, 2008). The eggs are translucent yellow with a sculptured surface and at 30<sup>0</sup>C they hatch in approximately 3-6 days (Obeng-Ofori, 2008). The larvae (Plate 2.11) range from 1.5-20 mm in length and are

light brown with dark brown spots with sparse hair-like setae on the body. There are five larval instars and the larval period, under optimum conditions of 30°C and 70% relative humidity, is about 22 days (Obeng-Ofori, 2008).

Larvae web the grains together and also wander about leaving silken tunnels behind. In heavy infestation, the matured larvae migrate away from the produce in search of crevices to pupate. The dispersal behaviour is mediated by a pheromones secreted from the salivary glands of the larvae (Obeng-Ofori, 2008). The larva pupates for 7 days in a whitish cocoon and the Pupa is pale brown in colour (Plate 2.11). Adult emergence from the cocoon usually occurs during the late afternoon. Under optimum conditions, development from egg to adult takes 29-31 days depending on moisture content, temperature and type of food (Chandra, 2006). Adults avoid strong light and rest in dark places with wings folded over the abdomen. The adult have major peak of egg laying and flight activity at dusk and minor peak before dawn (Chandra, 2006; Obeng-Ofori, 2008).

In cocoa beans infestation, the larvae pupate in the outer layers of the jute bags, but a few do so in the inner layers (Jonfia-Essian, 2004). Damage of cocoa beans is usually caused by the feeding habit of the larvae. They preferentially feed on seed germs, therefore reducing seed variability. The larvae produce a lot of webbed galleries and join the beans together. Frass, faecal matter and silk-filled galleries contaminate the beans resulting in both qualitative and quantitative loss. In heavy infestation the surface of the entire stock can be covered by silken webs left behind by the wandering larvae (Chandra 2006; Obeng-Ofori, 2008).



**Plate 2.11: Life stages of *E. cautella* (a) Adult (b) Pupa (c) Larvae**

### **2.7.3 Impact of insect infestation on purple cocoa beans**

Insect and other storage pests cause serious damage to stored commodities. Insect infestation of stored cocoa beans, results in the breakdown of cocoa butter and this causes increase in the free fatty acid level in the beans (Jonfia-Essien, 2006). Fungal contaminants are also responsible for substantial increase in FFA content (Basilico *et al.*, 2001; Magan *et al.*, 2003). Cocoa butter is the main vegetable fat used in the manufacture of chocolate because of its rheological, textural and chemical characteristics (Lipp and Anklam, 1998). Its hardness depends on the saturated and unsaturated fatty acid content bound in triglycerides, and on FFA content (Guehi *et al.*, 2008). Although purpleness of cocoa beans is not much of defect, countries like Malaysia prefer to use such beans only for cocoa liquor, powder and cocoa butter production instead of chocolate (Misnawi *et al.*, 2003).

Obeng, (2010) reported that cocoa beans infested with *E. cautella*, *T. castaneum* and *L. serricornis* led to appreciable damage which reduced the quality of the cocoa beans. Dharmaputra

*et al.*, (2000) and Kresnowati (1999) reported that unfermented cocoa beans infested by insect had high damage than fermented cocoa beans. The populations of insect pests of cocoa were more on unfermented cocoa beans than fermented cocoa beans (Dharmaputra *et al.*, 2000). This is to say that insect cause more damage on poorly fermented cocoa beans.

## **2.8 Purpleness of cocoa beans**

In fresh cocoa beans, the cotyledon contains a small proportion of intensely coloured cells dispersed among colourless cells (Bartley, 2005). The coloured cells contain most if not all the polyphenolic compounds which play a significant part in the internal changes. The colour of the cotyledon of cocoa varies from white (lack of anthocyanin pigment) to varying shade of pink, violet to purple (Bartley, 2005). In some cotyledons, various degree of mottle is encountered, resulting from mixing of pigment in the cells of the cotyledon. Niemenak *et al.* (2006) have shown that total phenols, catechin, epicatechin and anthocyanins in fresh and fermented-like cocoa beans were genotype dependent. The quality and/or quantity of polyphenolic compounds depends on genetic diversity as well as many environmental factors such as light intensity, humidity, temperature, use of fertilizer and other stress factors such as wounding and infection (Cabrita *et al.*, 2000; Wang and Zheng, 2001; Vallejo *et al.*, 2003; Stintzing and Carle 2004). Cellular disruption and seed death initiate various enzymatic and non-enzymatic reactions between seed components. These reactions develop a range of flavour precursors (peptides, amino acids, reducing sugars and polyphenols), and also affect the colour of the beans (Adeyeye *et al.*, 2010). Therefore, the polyphenol content in the cocoa beans is among the factors responsible for the purple colour of the cotyledon. Nazaruddin *et al.* (2001) reported that the total

polyphenols ranged from 45–52 mg/gin cocoa liquor, 34–60mg/gin in cocoa beans, and 20–62mg/gin in cocoa powder.

During fermentation, the polyphenol content of the beans decreases, so also is the purple colour of the cotyledon. Research has shown that if fermentation is terminated at day 3, the polyphenol content will be 10.7% (wt/wt) and if fermentation is terminated at day 4, 5 and 6, the polyphenol values will be 8.2, 7.6 and 6.01% (wt/wt) respectively (Aikpokpodion and Dongo, 2010). This shows that beans that are fermented for 3 days have high polyphenol content hence high number of purple colour beans compared to beans fermented for 6 days (Aikpokpodion and Dongo, 2010) The reduction of bitterness and astringency and browning of the cotyledon is due to the oxidation of polyphenols to insoluble tannins through the agency of the enzyme polyphenoloxidase (Wood and Lass, 1985). Therefore the degree of fermentation determines the change in the cotyledon colour of cocoa beans from purple to brown.

## **2.9 Grading standard for purple beans**

In Ghana the revised grading standard for purple beans are grade 1, grade II, grade II\* and sub standard (Table 2.4) (QCD, 2005). Commercially, the degrees of fermentation of cocoa beans are assessed by the cut test in which 300 beans are cut lengthwise and the colour of the cotyledon recorded (Misnawi *et al.*, 2003). According to Dand (1993), the cut test has an inherent limitation of totally depending on the sight of the analyst, which cannot be used as a standard for such measurements.

**Table 2.4: Grading standard in Ghana for purple beans count**

<b>Grade</b>	<b>Purple</b>
Grade I	20% of purple beans count
Grade II	20.3-35% of purple beans count
Grade II*	35.5-45% of purple beans count
Sub-standard	>45% of purple beans count

### **2.10 Quality of cocoa beans**

In farm maintenance, regular weeding of farm, spraying of cocoa trees against pests and diseases, removal of epiphytes and climbers, harvesting at the appropriate time when the pods are fully matured and well ripened as well as proper fermentation and drying improve the quality of cocoa beans (Magan *et al.*, 2003). Exposure of cocoa beans to high humidity is most likely to occur at stages between harvest and final consumption. Then, poor post-harvest management can lead to rapid deterioration in the quality of cocoa, severely decreasing of commercial and nutritional values (Magan *et al.*, 2003).

Cocoa of merchantable quality should be well fermented, thoroughly dry and free from smoky beans, flat beans, abnormal odour and from any evidence of adulteration. It must also be free from living insects, frass, contaminants from insects and reasonably free from broken beans. Thus virtually it should be free from foreign matter and pesticides residues are not allowed. Beans sizes must be uniform in the sense that not more than 12% of the beans should fall below the range of  $\pm 0.33$  of the average weight (BCCCA, 1996).

According to Misnawi *et al.* (2003) high percentage of fully purple cocoa beans is not considered as a severe default of quality because some countries such as Malaysia are currently making use of the unfermented and partly fermented cocoa beans especially for cocoa liquor, powder and cocoa butter production.

The colour of a normal sample of cut beans covers a range from the chocolate brown of fully fermented beans to the fully purple beans that have been poorly fermented. Beans are grouped as fully fermented partly brown/ partly purple, fully purple and slaty in cut test for easy assessment of the degree of fermentation. The fully fermented beans are brown in colour with the convolution of the cotyledon tending to separate when the bean is properly dry. The fully purple beans on the other hand are without any brown patches and the cotyledons are pressed tightly together (Plate 12) (BCCCA, 1996). Beans described as partly brown/ partly purple are not defective and should be present at least to the extent of 20%. A proportion of 30-40% is acceptable but samples with more than 50% have probably been poorly fermented and may give rise to bitter and astringent flavour (BCCCA, 1996). Poor post-harvest management can lead to rapid deterioration in the quality of cocoa beans.



**Plate 2.12: Purple cocoa beans**

Generally cocoa beans are grouped into; Grade 1 if the bulk contain 3% mouldy (Table 2.5) (cocoa beans on the internal part of which mould is visible to the naked eye), 3% slaty (cocoa bean which shows a slaty colour on half or more of the surface exposed by a cut made lengthwise through the center) and 3% other defects (insects infested, thus a cocoa bean the internal parts of which are found to contain insects at any stage of their development or to show sign of damage caused which are visible to the naked eye and including germinated or flat beans) (Table 6) 7.5% moisture and 0 foreign matter. Grade II must contain not more than 4% mouldy, 8% slaty, 6% other defect, 7.5 moisture and 0 foreign matters (Dand, 1993; BCCCA, 1996).

**Table 2.5: Grading standard for quality cocoa beans**

<b>Grade</b>	<b>Mould (M)</b>	<b>Slaty (S)</b>	<b>All Other Defects (AOD)</b>	<b>Purple (P)</b>
Grade I	3	3	3	20
Grade II	4	8	6	20.3-35
Grade II*	4	8	6	35.5-45
Sub-standard	>4	>8	>6	>45

**Table 2,6: Defective beans and their causes**

Defective beans	Cause
Mouldy	Develops when moisture content has not been reduced to less than 7.5%. Cocoa beans on the internal part of which mould is visible to the naked eye
Slaty	Develops from unfermented beans and have not developed the characteristic chocolate aroma and brown colour. Cocoa bean which shows a slaty colour on half or more of the surface of the cotyledon exposed by a cut made lengthwise through the center.
Purple	Develops from under fermentation. Glycosides have not yet broken down. Cocoa beans which shows a purple colour on half or more of the surface of the cotyledon.
All other defects (flat, germinated, insect infestation and dark beans)	<ol style="list-style-type: none"> <li>1. Flat beans are beans collected from immature pods.</li> <li>2. Germinated beans are beans from unharvested, ripe pods on the tree for several weeks and not turning the beans during fermentation.</li> <li>3. Insect infestation are cocoa bean with internal parts of which are found insects at any stage of their development or to show sign of damage caused which are visible to the naked eye.</li> <li>4. Dark beans are beans from black pod diseased pods, too slow drying of the beans or drying of the beans on metals.</li> </ol>

## CHAPTER THREE

### 3.0 MATERIALS AND METHOD

#### 3.1 Study Area

The harvesting, fermentation and drying of cocoa beans was carried out at CRIG while as the storage of the cocoa beans, culturing of the insect and cut test was carried out at the research laboratory of African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Legon.

#### 3.2 Culturing of insects

The adults of *Ephestia cautella* and adults of *Tribolium castaneum* were obtain from the Research Laboratory, Quality Control Company Ltd (COCOBOD). They were reared separately in an incubator at University of Ghana Entomology Laboratory at a temperature of  $30\pm 2^{\circ}\text{C}$  and relative humidity of  $70\pm 5\%$  (Jonfia-Essien, 2004). These insects were selected because they are among the major insect pests attacking cocoa beans, are abundant and easy to handle. *E. cautella* was reared on a mixture of ground maize, wheat bran and glycerol in a ratio of 8:8:1 (W/W) (Jonfia-Essien, 2001) and *T. castaneum* on crushed dry cocoa beans, (Jonfia-Essien, 2001) (Plate 3.1). Males of *E. cautella* were distinguished from the females by a dark patch (testes) on the back, which is visible at the fifth instar larva (Sasaki *et al*, 2002). The males of *T. castaneum* were distinguished from the female by their aggregation behavior and have a setiferous patch on the posterior side of the fore femur (Bousquet, 1990).



**Plate 3.1: Culturing of insect pests in an incubator**

### **3.3 Fermentation and drying of cocoa beans**

Matured and fully ripe cocoa pods were harvested from cocoa plantation at Cocoa Research Institute of Ghana (CRIG), Tafo. The harvested pods were opened using a wooden stick. The beans were separated from the placenta and any black or diseased beans, germinated beans, placenta fragments or pieces of shell were removed. The wet cocoa beans were grouped into five samples. Plantain leaves were neatly laid on the ground covering an area big enough to avoid the beans touching the ground and 50 kg of the wet cocoa beans were piled up in a heap on the leaves. The beans were covered tightly with banana leaves. This was done for each of the samples. Each sample was fermented for either 1,2,3,4 or 5 days with consecutive openings and turning after every 2 days except for beans fermented for one and two days. After drying the grade of the beans was determine using cut test. The grade of the cocoa beans used for this present study was grade II.

After each fermentation period, the cocoa beans were sun-dried by spreading them on mats placed on raised platforms. The mats were rolled up at night to cover the cocoa beans from dew

and rain. The beans were hand stirred and mixed constantly to ensure uniform drying. Each sample of the fermented cocoa beans was dried to 6.9% moisture content; this was done at CRIG, while the packing of the cocoa beans into jute bags and stored for the various experimental periods was done at ARPPIS.

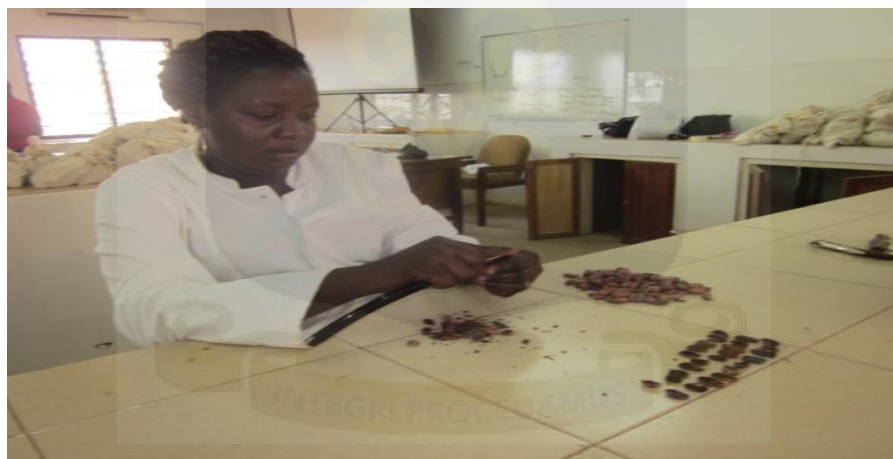
### 3.4 Storage of dry cocoa beans and determination of purpleness

Five 2 kg samples representing 1, 2, 3, 4, and 5 days fermentation period were stored separately in miniature prototype 30 cm × 30 cm jute sacks (Plate 3.2) for four months at  $30 \pm 2^\circ\text{C}$  and relative humidity of  $70 \pm 2\%$ , the prevailing conditions at the cocoa warehouses in Ghana (Jonfia-Essien, 2004). Completely randomized design (CRD) was used and each treatment (fermentation period) was replicated four times. Jute sacks were used in order to conform to the approved standard of storing cocoa beans in Ghana.



**Plate 3.2: Miniature jute filled with dry cocoa beans**

Cut test was used to determine the purpleness of the dry cocoa beans. The cut test is one of the cocoa grading schemes in Ghana; it is based on visual assessment of quality of cocoa, which relies on changes in colour of the beans (Misnawi *et al.*, 2003). Three hundred beans were sampled from each jute sack and cut length-wise through the middle to expose the internal surface of the two cotyledons (Plate 3.3). The cut cocoa beans were examined visually in good daylight, the number of purple (deep, pale, partly brown/partly purple) beans recorded and percentages worked out. Percentages of other defective categories (mouldy, slaty, insect infested, flat and germinated beans) were also determined (Plate 3.4). These were done before storage and repeated monthly during storage for each treatment. Each treatment had four replication.



**Plate 3.3: Researcher cutting cocoa beans for analysis**



**Plate 3.4: Cut beans**

### **3.5 Insect population estimation**

A mixture of 10 fifth instar larvae of *E. cautella* and 10 adult *T. castaneum* were introduced using putter into the jute sacks containing 2 kg of cocoa beans that had been fermented at various fermentation days (treatment). Ten fifth instar larvae of *E. cautella* and 10 adult *T. castaneum* were separately introduced using aspirator into another batch of jute sacks containing 2 kg each of the dry beans. Each of the jute sacks was inserted into gray baft (Plate 3.5) before storage to prevent migration of insects from one bag of the dry beans to another. The fifth instar larvae of *E. cautella* was used because it is very easy to distinguish the males and the females at this stage (Sasaki *et al*, 2002) and also because the fifth instar (last larval instar) feed vigorously before pupating (Chandra 2006). The temperature of the store room was  $30 \pm 2^{\circ}\text{C}$  and relative humidity of  $70 \pm 2\%$  and also free from insect. On monthly basis, each sample was sieved using a wooden sieve (Plate 3.6) and the numbers of both live and dead insects (larva, pupa and adult) in each sack was counted to determine the changes in insect populations. The dead ones were counted because they were assumed to have completed their life cycles and therefore contributed to the percentage weight loss of the beans. The live ones were returned into their respective sack.



**Plate 3.5: Gray baft bags containing jute sacks with dry cocoa beans.**



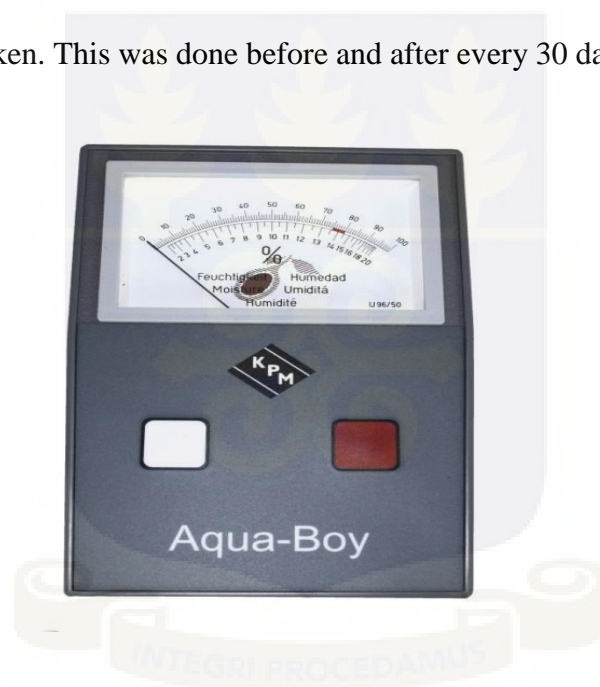
**Plate 3.6: Researcher sieving cocoa beans to obtain insect and contaminants for analysis**

### **3.6 Determination of Contaminants produced by the two insects.**

On monthly basis, each sample was sieved using a wooden sieve (Plate 3.6), the live insect found in the residue were picked using putter. The contaminants which were obtain after sieving and removing the live insects were observed, weighed and recorded monthly for a period of four months using completely randomized design (CRD) and each treatment was replicated four times.

### 3.7 Determination of moisture content of cocoa beans

The moisture content of the beans was determined using Aqua-Boy moisture meter (Plate 3.7). The voltage of the moisture meter battery was assessed by depressing the red button to ensure that the needle of the indicator was deflected within the red area. The cup electrode of the moisture meter was filled with 6 to 10 cocoa beans and tightly closed. The cup electrode was inserted into the measuring jack. The white measuring button was depressed and the moisture content read directly on the centesimal scale of the instrument. This was repeated three times and the average value was taken. This was done before and after every 30 days for 120 days.



**Plate 3.7 An Aqua-Boy (Source Jonfia-Essien, 2004)**

### 3.8 Damage and Loss assessment of dry cocoa beans in the various treatment (fermentation days)

Count and weigh method (Boxall, 1986) was used to determine the weight loss in the various dry cocoa beans. From each sack 100g of cocoa beans was sampled, and sorted into damaged and undamaged. The damaged beans were those with holes or any physical defects. Both the

damaged and undamaged beans were counted and weighed. Each treatment was replicated four times. The percentage weight loss was calculated as follows:

$$\% \text{ Weight loss} = ((UNd) - (DNu) / U(Nd + Nu)) * 100$$

U = Weight of Undamaged beans

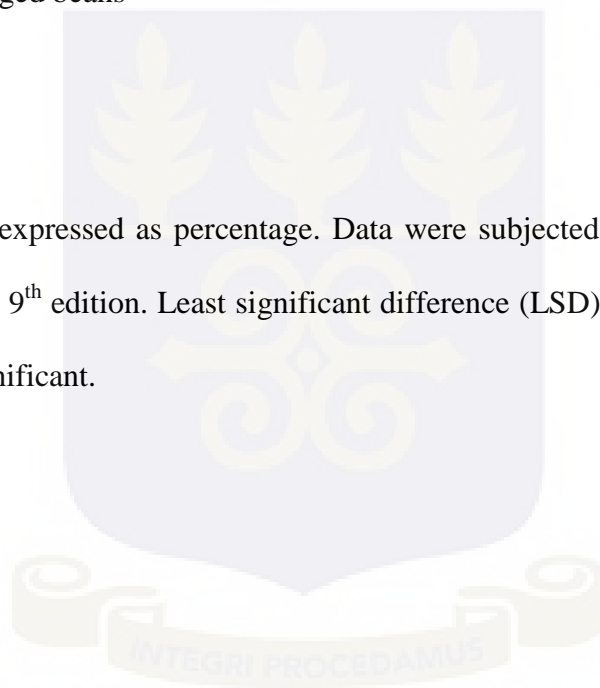
Nd = Number of damaged beans

D = Weight of Damaged beans

Nu = Number of undamaged beans

### 3.9 Data analysis

The cut test score were expressed as percentage. Data were subjected to Analysis of Variance (ANOVA) using Genstat 9<sup>th</sup> edition. Least significant difference (LSD) was used to separate the means at 5% level of significant.

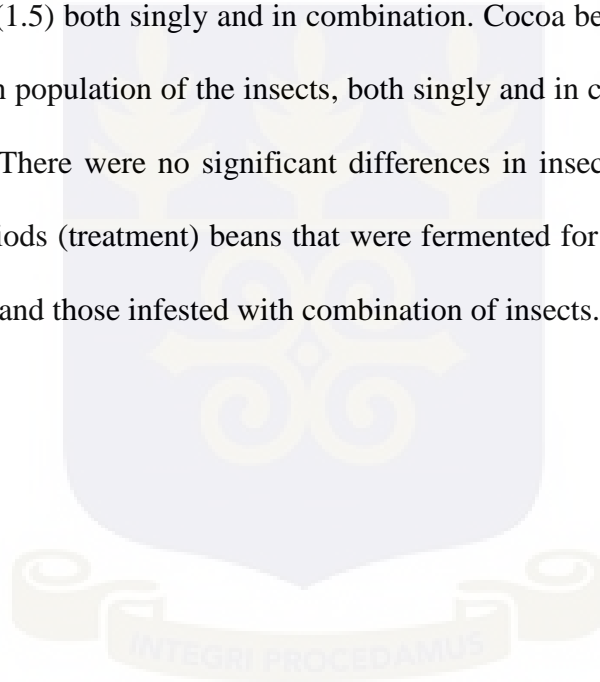


## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Population estimate of *E. cautella* and *T. castaneum* in stored purple cocoa beans

Generally the population of *E. cautella* in the cocoa beans increased with increasing storage period that of *T. castaneum* decreased with increasing storage period but only increased at the end of storage for 2 and 5 days (Table 4.1). *E. cautella* alone recorded the highest mean population of 297.0 at the end of the storage period of 120 days whilst *T. castaneum* recorded the lowest mean population (1.5) both singly and in combination. Cocoa beans fermented for 3 days recorded the lowest mean population of the insects, both singly and in combination, at the end of the storage (Table 4.1). There were no significant differences in insect populations among the various fermentation periods (treatment) beans that were fermented for different days and beans that were singly infested and those infested with combination of insects.



**Table4.1: Population trends of *E. cautella* and *T. castaneum* in dry cocoa beans stored for 120 days**

Days of fermentation	Treatment	Mean number of insect after days of storage			
		30	60	90	120
1	<i>E. cautella</i>	8.5±1.2	48.3±3.1	158.0±12.0	297.0±22.7
	<i>T. castaneum</i>	8.3±1.6	3.8±1.8	2.8±1.8	1.5±0.3
	<i>E. cautella, T.castaneum</i>	2.8±0.8, 4.5±2.0	2.0±1.0, 1.5±0.3	21.3±20.6, 2.0±1.2	29.5±17.1, 1.5±0.6
	Control	0.0	0.0	0.0	0.0
<b>LSD (p&lt;0.05)</b>		<b>4.7</b>	<b>5.8</b>	<b>36.9</b>	<b>43.9</b>
2	<i>E. cautella</i>	5.5±1.6	30.5±14.1	78.5±35.1	181.5±52.4
	<i>T. castaneum</i>	11.8±2.8	4.3±2.5	1.0±0.4	1.5±0.9
	<i>E. cautella, T.castaneum</i>	13.5±12.5, 8.3±2.9	23.0±11.9, 8.5±5.5	59.5±42.8, 15.8±10.6	71.0±41.4, 14.3±8.4
	Control	0.0	0.0	0.0	0.0
<b>LSD (p&lt;0.05)</b>		<b>20.4</b>	<b>29.9</b>	<b>86.8</b>	<b>103.7</b>
3	<i>E. cautella</i>	5.8±1.5	13.5±5.0	27.3±13.2	75.5±41.9
	<i>T. castaneum</i>	16.0±2.8	4.3±0.8	4.5±1.6	3.5±1.4
	<i>E. cautella, T.castaneum</i>	9.3±5.7, 3.3±0.9	16.5±8.7, 4.8±1.5	60.0±48.1, 1.0±0.7	46.8±21.6, 6.5±3.9
	Control	0.0	0.0	0.0	0.0
<b>LSD (p&lt;0.05)</b>		<b>10.2</b>	<b>15.8</b>	<b>76.9</b>	<b>72.9</b>
4	<i>E. cautella</i>	11.0±2.7	29.3±11.6	72.8±8.3	137.0±42.0
	<i>T. castaneum</i>	17.3±3.0	4.8±1.5	3.5±1.5	2.8±2.8
	<i>E. cautella, T.castaneum</i>	14.8±4.2, 3.3±0.6	48.5±12.8, 5.8±2.3	96.3±19.6, 11.8±4.0	206.5±39.2, 11.3±3.9
	Control	0.0	0.0	0.0	0.0
<b>LSD (p&lt;0.05)</b>		<b>9.0</b>	<b>27.0</b>	<b>33.4</b>	<b>88.9</b>
5	<i>E. cautella</i>	23.3±7.4	58.0±11.0	111.0±9.0	143.3±48.6
	<i>T. castaneum</i>	14.8±3.3	6.0±1.6	1.5±1.0	2.8±1.6
	<i>E. cautella, T.castaneum</i>	31.8±18.4, 40.0±11.2	23.3±10.9, 6.3±0.6	32.5±19.6, 5.0±2.8	45.3±17.8, 7.0±4.0
	Control	0.0	0.0	0.0	0.0
<b>LSD (p&lt;0.05)</b>		<b>35.4</b>	<b>24.0</b>	<b>33.5</b>	<b>80.0</b>

#### 4.2 Contaminants in stored cocoa beans produced by *E. cautella* and *T. castaneums*

Generally, the contaminants consisted of pupal cases, frass, fragment of cocoa, silken threads and dead insects. All the treatments with *E. cautella* had dead insects, faeces and silken thread as major components of the contaminants (Plate 4.1). They also had the highest mean weight of contaminants. The control had mainly fragments of cocoa beans. The weight of contaminants in the treated cocoa beans were significantly higher ( $p < 0.05$ ) than that produced in the control throughout the storage period except for beans that were fermented for 3 days (Table 4.2). Cocoa beans infested with *E. cautella* had the highest mean weight of contaminants ( $21.34 \pm 8.12\text{g}$ ) whilst the control recorded the lowest weight of contaminants ( $0.07 \pm 0.01\text{g}$ ).



**Plate 4.1: Contaminants produced by *E. cautella***

**Table 4.2: Weight of contaminants produced by *E. caudella* and *T. castaneum* on dry cocoa beans stored for 120 days**

Days of fermentation	Treatment	Mean weight of contaminant after days of storage			
		30	60	90	120
1	<i>E. caudella</i>	0.51±0.1	3.6±0.7	8.6±2.6	21.0±4.0
	<i>T. castaneum</i>	0.2±0.07	0.3±0.1	0.4±0.1	0.8±0.2
	<i>E. caudella, T.castaneum</i>	0.2±0.1	0.3±0.1	0.6±0.2	2.4±1.9
	Control	0.1±0.0	0.1±0.0	0.2±0.1	0.2±0.1
<b>LSD (p&lt;0.05)</b>		<b>0.2</b>	<b>1.1</b>	<b>4.1</b>	<b>6.8</b>
2	<i>E. caudella</i>	0.3±0.0	2.0±1.0	2.7±1.1	21.3±8.1
	<i>T. castaneum</i>	0.1±0.0	0.2±0.0	0.3±0.0	0.8±0.1
	<i>E. caudella, T.castaneum</i>	1.3±0.6	4.0±2.4	5.3±3.4	8.9±3.1
	Control	0.1±0.0	0.2±0.0	0.2±0.1	0.3±0.1
<b>LSD (p&lt;0.05)</b>		<b>1.0</b>	<b>4.0</b>	<b>5.5</b>	<b>13.4</b>
3	<i>E. caudella</i>	0.5±0.4	1.4±0.7	2.2±1.1	2.3±1.3
	<i>T. castaneum</i>	0.1±0.0	0.3±0.1	0.5±0.1	0.5±0.1
	<i>E. caudella, T.castaneum</i>	0.3±0.1	2.6±2.2	4.2±3.0	3.1±1.9
	Control	0.2±0.0	0.2±0.0	1.1±0.8	1.2±1.0
<b>LSD (p&lt;0.05)</b>		<b>0.6</b>	<b>3.5</b>	<b>5.0</b>	<b>3.8</b>
4	<i>E. caudella</i>	0.5±0.2	0.9±0.4	1.3±0.4	7.8±2.4
	<i>T. castaneum</i>	0.2±0.1	0.4±0.1	0.4±0.1	1.0±0.5
	<i>E. caudella, T.castaneum</i>	1.1±0.1	1.7±0.4	2.3±0.3	15.6±4.9
	Control	0.1±0.0	0.1±0.0	0.2±0.0	0.3±0.0
<b>LSD (p&lt;0.05)</b>		<b>0.4</b>	<b>0.8</b>	<b>0.8</b>	<b>8.5</b>
5	<i>E. caudella</i>	1.4±0.2	1.8±0.3	2.1±0.2	9.7±0.3
	<i>T. castaneum</i>	0.1±0.0	0.3±0.1	0.3±0.1	0.6±0.1
	<i>E. caudella, T.castaneum</i>	0.2±0.1	0.5±0.3	1.6±0.8	2.3±0.9
	Control	0.1±0.0	0.2±0.0	0.2±0.0	0.3±0.1
<b>LSD (p&lt;0.05)</b>		<b>0.3</b>	<b>0.6</b>	<b>1.3</b>	<b>1.5</b>

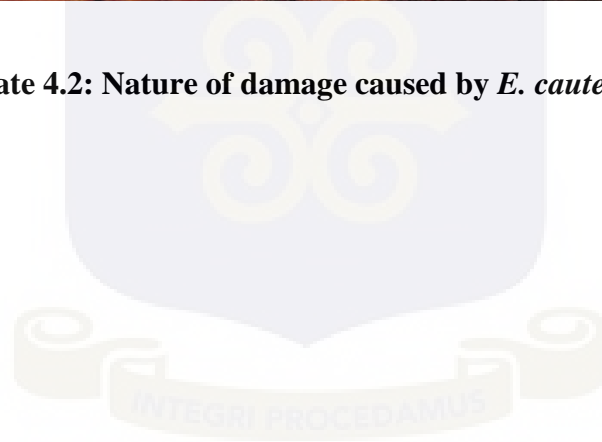
### 4.3 Damage caused by *E. cautella* and *T. castaneum* on stored dry cocoa beans

The percentage weight losses caused by the two insect pests on the stored cocoa beans varied significantly irrespective of whether they occurred separately or in combinations in cocoa beans which were stored under the temperature of  $31.5 \pm 1.0^{\circ}\text{C}$  and relative humidity of  $83 \pm 4\%$  (Appendix 1). On the other hand, the moisture content of the stored cocoa beans was not significantly different among the various treatments (Appendix 1).

Generally, the percentage weight loss increased with storage period. Cocoa beans infested with *E. cautella* produced the highest weight losses ranging from  $1.56 \pm 0.6\%$  at the start of storage to  $10.1 \pm 1.87\%$  at the end of storage. The highest weight losses of  $10.1 \pm 1.87\%$  and  $10.14 \pm 8.74\%$  were recorded in cocoa beans fermented for one day and four days respectively and there were caused by *E. cautella*. The percentage weight loss that resulted from infestation by *E. cautella* alone for 120 days of storage was significantly higher than that caused by *T. castaneum* alone, and also the two insects in combination, except for beans fermented for four days (Table 4.3). Infestation by *T. castaneum* alone caused the lowest damage to the stored beans throughout the storage period. There was no significant difference between the beans fermented for three days but stored for 90 days and beans fermented for four days but stored for 30 and 120 days. The level of damage was higher in cocoa beans infested with *E. cautella* (Plate 4.2).



**Plate 4.2: Nature of damage caused by *E. cautella***



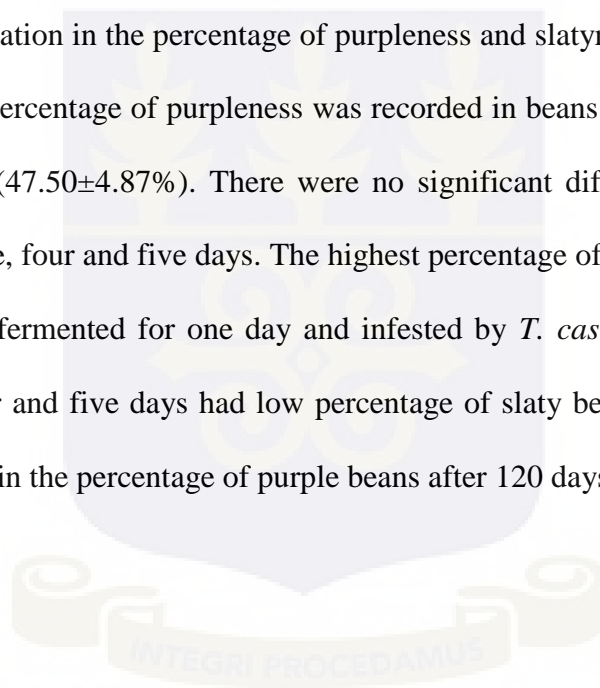
**Table 4.3: Mean percentage weight loss of dry cocoa beans infested with *E. cautella* and *T. castaneum* after varying periods of storage.**

Days of fermentation	Treatment	Mean weight loss after days storage (%)			
		30	60	90	120
1	<i>E. cautella</i>	1.6±0.6	3.2±0.6	7.2±0.6	10.1±1.9
	<i>T. castaneum</i>	0.0±0.0	0.1±0.0	0.5±0.1	1.2±0.4
	<i>E. cautella, T.castaneum</i>	0.6±0.2	0.6±0.3	0.8±0.4	0.9±0.3
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>0.9</b>	<b>1.0</b>	<b>1.1</b>	<b>3.0</b>
2	<i>E. cautella</i>	1.8±0.3	2.1±0.5	4.6±0.4	8.7±3.1
	<i>T. castaneum</i>	0.0±0.0	0.3±0.2	0.3±0.1	0.9±0.3
	<i>E. cautella, T.castaneum</i>	0.8±0.3	2.0±0.3	2.5±1.3	3.6±1.4
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>0.6</b>	<b>0.9</b>	<b>2.1</b>	<b>5.3</b>
3	<i>E. cautella</i>	0.6±0.2	1.2±0.4	3.7±1.4	4.6±0.7
	<i>T. castaneum</i>	0.0±0.0	0.1±0.1	0.3±0.1	0.7±0.2
	<i>E. cautella, T.castaneum</i>	0.5±0.3	1.0±0.3	2.5±1.1	3.1±1.7
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>0.5</b>	<b>0.7</b>	<b>2.8</b>	<b>2.8</b>
4	<i>E. cautella</i>	0.7±0.4	2.2±0.5	4.8±0.8	10.1±8.7
	<i>T. castaneum</i>	0.0±0.0	0.1±0.1	0.3±0.1	0.5±0.2
	<i>E. cautella, T.castaneum</i>	0.7±0.5	0.9±0.1	4.8±1.4	7.3±1.4
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>0.9</b>	<b>0.7</b>	<b>2.5</b>	<b>13.6</b>
5	<i>E. cautella</i>	1.4±0.2	2.5±0.6	3.8±1.2	4.9±0.5
	<i>T. castaneum</i>	0.0±0.0	0.1±0.0	0.6±0.6	1.1±0.9
	<i>E. cautella, T.castaneum</i>	0.4±0.1	0.6±0.2	2.2±0.7	3.3±1.0
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>0.4</b>	<b>1.0</b>	<b>2.2</b>	<b>2.2</b>

#### **4.4 Determination of purpleness and slatyness of dry cocoa beans after 120 days of storage.**

The results of the cut test of cocoa beans for purpleness and slatyness are summarized in Table 10. Cocoa beans fermented for one day had higher percentage of slatyness while beans fermented for three and four days had high percentage of purpleness (Table 4.4). The differences between each treatment for beans fermented for five days were not significant except at the beginning of the storage.

Generally, there was variation in the percentage of purpleness and slatyness as the storage period prolonged. The highest percentage of purpleness was recorded in beans fermented for three days and stored for 90 days ( $47.50 \pm 4.87\%$ ). There were no significant differences in slatyness for beans fermented for three, four and five days. The highest percentage of slatyness of  $99.0 \pm 0.41\%$  was recorded for beans fermented for one day and infested by *T. castaneum*. Generally beans fermented for three, four and five days had low percentage of slaty beans. There was neither a decrease nor an increase in the percentage of purple beans after 120 days of storage.



**Table 4.4: Cut test score (%) for purpleness and slatyness of dry cocoa beans infested with two insects and stored for 120 days.**

Days of fermentation	Treatment	% purpleness after days of storage				% slatyness after days of storage			
		30	60	90	120	30	60	90	120
<b>1</b>	<i>E. cautella</i>	0.0±0.0	11.5±2.0	0.0±0.0	0.0±0.0	97.3±1.3	87.8±3.5	80.3±2.7	46.8±4.5
	<i>T. castaneum</i>	0.0±0.0	5.8±5.4	0.3±0.3	2.0±1.4	99.0±0.4	93.8±5.3	95.8±1.0	84.8±3.3
	<i>E. cautella,T.castaneum</i>	1.3±1.0	9.0±1.4	0.5±0.5	2.8±2.1	97.8±0.6	90.8±1.4	96.8±2.0	85.5±3.1
	<b>Control</b>	0.1±0.3	10.8±1.3	0.0±0.0	0.3±0.3	98.5±0.5	89.0±1.2	96.8±1.0	86.5±1.2
<b>LSD (p&lt;0.05)</b>		<b>NS</b>	<b>9.4</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>5.6</b>	<b>10.0</b>
<b>2</b>	<i>E. cautella</i>	9.3±2.4	15.8±4.0	3.0±0.9	7.8±2.1	55.0±4.2	82.0±5.1	23.3±2.1	27.5±4.7
	<i>T. castaneum</i>	12.0±1.1	13.0±1.1	23.3±3.2	13.3±3.5	47.0±1.6	69.8±16.6	86.8±1.8	39.3±3.9
	<i>E. cautella,T. castaneum</i>	9.8±2.9	21.5±2.8	6.8±2.0	15.0±4.5	55.0±2.7	26.3±3.3	69.8±3.0	27.0±5.0
	<b>Control</b>	7.5±0.9	27.0±2.9	8.5±0.7	18.5±3.0	56.0±1.4	27.3±3.6	81.8±1.1	39.0±2.2
<b>LSD (p&lt;0.05)</b>		<b>NS</b>	<b>NS</b>	<b>6.1</b>	<b>NS</b>	<b>NS</b>	<b>27.8</b>	<b>6.5</b>	<b>NS</b>
<b>3</b>	<i>E. cautella</i>	19.5±2.8	29.0±3.4	40.8±7.6	28.0±3.0	1.3±0.5	0.0±0.0	0.8±0.5	0.3±0.3
	<i>T. castaneum</i>	23.5±1.6	39.8±1.4	47.5±4.9	39.0±2.0	0.0±0.0	0.0±0.0	0.5±0.3	0.3±0.3
	<i>E. cautella,T. castaneum</i>	20.5±5.0	24.3±6.4	33.3±2.3	33.8±2.3	0.5±0.5	1.0±0.7	0.3±0.3	0.0±0.0
	<b>Control</b>	25.3±0.5	21.5±1.2	39.5±6.7	32.5±6.5	0.5±0.3	1.0±1.0	0.8±0.5	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>NS</b>	<b>11.6</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>4</b>	<i>E. cautella</i>	21.3±0.5	21.3±1.3	30.3±1.9	30.0±2.3	0.8±0.5	0.0±0.0	1.3±0.6	0.5±0.3
	<i>T. castaneum</i>	26.5±1.6	22.8±2.0	40.3±2.9	24.3±1.1	0.5±0.3	0.8±0.5	0.5±0.3	0.5±0.3
	<i>E. cautella,T. castaneum</i>	25.5±2.5	24.3±1.1	38.3±3.1	26.3±3.8	0.5±0.3	0.0±0.0	1.5±0.5	0.5±0.3
	<b>Control</b>	26.5±1.0	26.8±3.2	26.8±1.8	32.0±2.4	0.8±0.5	0.3±0.3	0.3±0.0	0.0±0.0
<b>LSD (p&lt;0.05)</b>		<b>NS</b>	<b>NS</b>	<b>7.6</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>5</b>	<i>E. cautella</i>	29.5±0.7	25.3±1.9	25.5±5.8	25.0±3.5	0.5±0.3	1.0±0.4	0.8±0.3	0.5±0.3
	<i>T. castaneum</i>	25.5±1.9	23.8±2.0	26.5±1.3	25.5±2.6	0.3±0.3	1.0±0.6	0.3±0.3	0.5±0.5
	<i>E. cautella,T. castaneum</i>	21.8±1.0	24.0±1.8	24.5±1.9	23.0±2.1	1.3±0.3	1.0±0.6	0.5±0.3	0.0±0.0
	<b>Control</b>	26.3±1.7	21.5±1.0	28.5±3.9	24.8±1.1	1.0±0.4	0.5±0.5	0.8±0.3	0.3±0.3
<b>LSD (p&lt;0.05)</b>		<b>4.3</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>

#### 4.5 Percentage of other defects (OD) of dry cocoa beans after 120 days of storage.

Other defects (OD) include Insect infestations, flat and germinated beans. These were recorded during the cut test analysis. Generally, the number of insects found inside the cut cocoa beans (Plate 4.3) during the cut test increased with prolonged in storage period. Cocoa beans stored for 120 days had high percentage of insect infestation. Cocoa beans fermented for one day and infested with *E. cautella* had the highest percentage of insect infestation at the end of storage (45.5±4.3%). Higher percentage of the infestation was found in cocoa beans infested with *E. cautella* than those infested with *T. castaneum*. At the end of storage period, beans fermented for five days and infested with *T. castaneum* had the lowest percentage of the insect. The percentage of flat and germinated beans was generally low. Cocoa beans fermented for three days had low percentage of other defect (Table 4.5) whereas beans fermented for one day and infested with *E. cautella* had the highest percentage of other defect. There was considerable variation in the percentage of other defects during the storage period.



**Plate 4.3: Insect found inside cocoa beans**

**Table 4.5: Cut test score (%) for other defects of dry cocoa beans infested with two insects after 120 days of storage.**

Days of fermentation	Treatments	Other Defects after days of storage			
		30	60	90	120
1	<i>E. cautella</i>	2.0±1.1	1.8±1.1	17.0±3.1	45.5±4.4
	<i>T. castaneum</i>	0.3±0.3	0.3±0.3	0.5±0.3	2.0±1.1
	<i>E. cautella, T. castaneum</i>	0.0±0.0	0.0±0.0	3.0±2.4	4.3±2.8
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LSD (p<0.05)		NS	NS	6.0	8.2
2	<i>E. cautella</i>	1.3±0.3	1.5±0.9	7.8±3.8	26.5±7.8
	<i>T. castaneum</i>	0.5±0.3	0.8±0.8	1.8±0.9	2.0±1.7
	<i>E. cautella, T. castaneum</i>	2.3±1.3	0.5±0.3	9.8±5.4	18.5±10.3
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LSD (p<0.05)		NS	NS	NS	20.0
3	<i>E. cautella</i>	0.0±0.0	0.0±0.0	3.3±3.3	7.8±4.3
	<i>T. castaneum</i>	0.0±0.0	0.0±0.0	0.3±0.5	2.0±0.9
	<i>E. cautella, T. castaneum</i>	0.5±0.5	0.0±0.0	2.3±1.4	8.3±6.5
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LSD (p<0.05)		NS	NS	NS	NS
4	<i>E. cautella</i>	0.3±0.5	2.0±0.9	2.0±1.1	17.0±6.4
	<i>T. castaneum</i>	0.3±0.3	1.3±0.5	0.3±0.3	2.0±1.1
	<i>E. cautella, T. castaneum</i>	0.3±0.5	5.0±1.5	4.8±1.9	28.0±8.1
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LSD (p<0.05)		NS	2.8	3.4	16.0
5	<i>E. cautella</i>	0.5±0.5	5.0±0.0	7.5±0.9	15.8±2.4
	<i>T. castaneum</i>	0.3±0.3	0.3±0.3	5.0±2.6	0.5±0.5
	<i>E. cautella, T. castaneum</i>	0.8±0.8	2.8±1.1	0.5±0.3	10.3±3.0
	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
LSD (p<0.05)		NS	1.8	4.3	6.0

## CHAPTER FIVE

### 5.0 DISCUSSION

#### 5.1 Population estimate of *E. cautella* and *T. castaneum* in stored purple cocoa beans.

The population of insect pests are said to increase in the absence of predators, availability of food and favourable environmental conditions (Fischer, 2002). Jonfia-Essien (2008) reported that the populations of *E. cautella* and *T. castaneum* increase significantly with duration of storage in conventional storage. The increase in insect numbers may be due to favourable environmental conditions, availability of food, and absence of competitors and predators which favour the growth and development of insects. In this study, the availability of the cocoa beans served as a food reservoir for the insects which contributed to their multiplication. The insects were exposed to temperature of  $31.5 \pm 1.0^{\circ}\text{C}$  and relative humidity of  $83 \pm 4\%$  which are optimum for the insect development (Mason, 2003). Moreover, there were no predators and parasitoids to affect the pest population negatively.

The population of *E. cautella* increased with storage period and had the highest population both in single and mixed infestation probably because the larvae of *E. cautella* are internal feeder and they can move and feed inside cocoa beans (Dharmaputra *et al*, 2000). Their short life-span, availability of cocoa beans and favourable environmental conditions also contributed to their multiplication, compared to *T. castaneum* which is a secondary insect (Kurunakaran and Jayas, 2004) and newly hatched larvae of *T. castaneum* survive only when suitable food is readily available. Consequently, the population of *T. castaneum* in single infestation decreased during the storage because the dry cocoa beans were whole grains. However, populations of *T. castaneum* in combination with *E. cautella* were relatively higher, this might be due to the presence of latter's larvae which were able to feed on the whole beans thus making them suitable

for *T. castaneum* to also feed and increase in number. In contrast, Obeng (2010) reported that the population *T. castaneum* in single infestation increases after 180 days of storage probably because she used brown cocoa beans (fully fermented) while purple cocoa beans was used in this study.

The populations of both insects in single and in combination were low in cocoa beans fermented for three days and high in beans fermented for one day and four days. This was because all the cocoa beans were under-fermented and according to Dharmaputra *et al*, (2000) properly fermented beans had lower population of *E. cautella* than unfermented and under-fermented beans.

#### **5.1.1 Contaminants produced by *E. cautella* and *T. castaneum* on dry stored cocoa beans.**

Insects inflict damage on stored cocoa beans mainly by direct feeding and contamination with pupal cases, frass, silken threads and dead insects. Species in genus *Tribolium* are known to produce toxic quinines which also contaminate the cocoa beans (Jonfia-Essien, 2006). Other contaminants found during the present study were fragments from cocoa beans. The weights of the contaminants were significantly higher ( $p < 0.05$ ) than those produced in the control for one and five days. As the storage period increased weights of the contaminants also increased probably due to increase in the insect biological activities. Therefore cocoa beans stored for more than 30 days contained more contaminants as a result of insect infestation.

The larvae of *E. cautella* mainly produced a lot of webbed galleries which bind the beans together and they also wander about leaving silken tunnels behind (Chandra, 2006) thus contaminating the cocoa beans. From the present study beans infested with *E. cautella* produced the highest levels of contaminants probably due to high population. Dead insects (especially adult) were the major component of the contaminants probably because the adults of *E. cautella*

do not feed but lay eggs and die (Obeng-Ofori, 2008). For this reason, good quality cocoa meant for export must be free from live or dead insects especially *E. cautella*. In cocoa beans fermented for three days, the weight of the contaminants was low because of low population of insects found in the beans.

The major contaminants found in cocoa beans infested with *T. castaneum* were frass, pupal cases and fragment of cocoa beans. The weights of the contaminants were generally low, this is as a result of low numbers of the insects found in the cocoa beans and due to the fact that *T. castaneum* is a secondary pest and will only feed on grains that have been fed by other insects (Bailey, 2007). The observed low contaminant in cocoa beans infested with *T. castaneum* was in support of the work by Obeng (2010).

## **5.2 Damage caused by *E. cautella* and *T. castaneum* to dry stored cocoa beans.**

Insect infestation is one of the most important causes of deterioration of stored grains ecosystem (Dharmaputra *et al*, 2000). Insect pests inflicted damage mainly by feeding and their feeding action caused loss of weight to the cocoa beans thus reducing their nutritional and commercial value.

Cocoa beans infested with *E. cautella* gave the highest percentage weight loss after 120 days of storage. This is probably as a result of their higher numbers in the cocoa beans. The larvae of *E. cautella* caused more damage than *T. castaneum* and the increase in weight loss correlated with the number of insects that infested the cocoa beans. Therefore special attention should be given to *E. cautella* in terms of control as it can cause Ghana great loss in terms of exportable cocoa. Cocoa beans infested with *T. castaneum* alone caused the least weight loss. Both the highest and least weight loss was found in beans fermented for four days. This is in agreement with Obeng

(2010), who also reported that infestation by *E. cautella* on cocoa beans stored for 180 days caused more damage than *T. castaneum*.

Cocoa beans fermented for four days gave relatively higher percentage weight loss than those fermented for five days. Cocoa beans fermented for four days are considered as under-fermented cocoa. However, previous study showed that damage caused on fermented cocoa beans stored for 6 month (180 days) was lower than the damage on under-fermented beans (Dharmaputra *et al*, 2000). Kresnowati (1999) also reported that the percentage of damage beans on fermented cocoa beans was lower than that of unfermented beans. This shows that properly fermented cocoa beans could be stored for a longer period.

### **5.3 Determination of purpleness of stored dry cocoa beans.**

A purple cocoa bean shows purple colour on half or more of the surface of the cotyledon. Generally, there was a variation in percentage of purple beans during storage. Jonfia-Essisen *et al*. (2008) reported that there was reduction in the total purple colour of cocoa beans but difference was not significant enough to cause any change in the grade. However, in this study, there was neither decrease nor an increase in the percentage of purple beans after days of storage.

The polyphenol content in cocoa beans is among the factors responsible for the purple colour, thus if the polyphenol content is high, the percentage of purple beans will be high. Aikopkpodion and Dongo (2010) determined the polyphenol content of cocoa beans during fermentation and recorded a decrease in the polyphenol content as fermentation period prolonged, a corresponding decrease in percentage of purple colour beans was also recorded. His finding suggests that fermentation leads to gradual reduction of polyphenol in cocoa beans. Polyphenols are mostly responsible for the astringent sensation as well as bitter taste and colour (Misnawi *et al*, 2002).

Adzaho *et al.*, (2011) also reported a decrease in anthocynins (polyphenolic compound) content with increase in fermentation period. Therefore determination of polyphenol content during storage should be studied.

At the beginning of the storage period there was high percentage of purple beans in cocoa beans fermented for 3, 4 and 5 days. Guehi *et al.* (2010) reported a higher percentage of purple beans in cocoa fermented for 4 days than in cocoa fermented for 5 days. Under-fermented beans usually produce high percentage of purple beans and cocoa with high percentage of purple beans gives a bitter and astringent chocolate. Although there was variation in the percentage of purple beans, the grade of the beans was not affected. Cocoa beans fermented for 5 days were found to be grade II after the storage period but beans fermented for 3 and 4 days reduced in quality from grade II to grade II\* based on purple cocoa beans. This is due to the fact that beans fermented for 5 days contained less than 35% purple beans after 120 days of storage while those fermented for 3 and 4 days had more than 35% purple beans. However, according to Misnawi *et al.* (2003) countries, such as Malaysia, are currently making use of the unfermented and under-fermented cocoa beans for cocoa liquor, powder and cocoa butter production, but due to its excessive astringency and bitterness, purple beans are undesirable in West Africa.

### **5.3.1 Determination of slatyness of stored dry cocoa beans.**

Slaty cocoa beans show a slaty texture on half or more of the surface of the cotyledon. Slaty cocoa beans were found in beans fermented for one and two days, in this study and the percentage of slaty beans decreased with increase in storage period. Jonfia-Essien (2008) reported that there was a decrease in percentage of slaty cocoa beans stored for 9 weeks. Slaty beans are a major defect because they cannot be used for chocolate, though they can be used for cocoa liquor, powder and cocoa butter production (Misenawi *et al.*, 2003). The highest percentage

of slaty beans was found at the beginning of the storage period. Cocoa beans fermented for one day had higher percentage of slaty beans compared to those fermented for 2 days. Cocoa fermented for 4 and 5 days had little or no slaty beans. The grade of the cocoa from this study based on slatyness were substandard for beans fermented for one and two days because they contained more than 8% slaty beans, and cocoa fermented for 3, 4 and 5 days were grade I because they contained less than 3% slaty beans. Guehi, *et al*, (2008) also reported low percentage of slaty beans in three Ivorian main cocoa producing regions probably due to the fact that the cocoa farmer fermented or under fermented their cocoa beans.

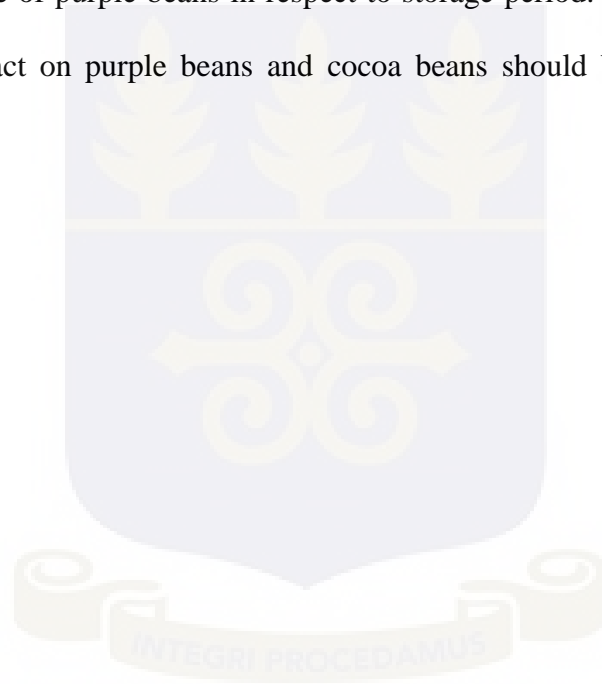
### **5.3.2 Determination of other defects (OD) of stored dry cocoa beans.**

The percentage of cocoa beans infested by the two insects increased with storage period. Larvae of *E. cautella* were found inside the beans during the cut test. The percentages of germinated and flat beans were lower than insect infested beans. Guehi *et al*, (2008) reported low percentage of insect infestation after cut test analysis in Brown, Black and Clustered cocoa beans. That *E. cautella* recorded highest percentage of beans infestation ( $45.5 \pm 4.37\%$ ) in the cut test analysis was probably due to the ability of the larvae to bore into the beans and feed. Cocoa beans fermented for one day had the highest percentage of other defects while the control had the lowest percentage; this was because the beans were not infested with insect.

In general, infestation of cocoa beans by *E. cautella* is a threat to quality of cocoa beans in Ghana. From this study, it has been established that population of *E. cautella* increased significantly in under-fermented infested stored cocoa beans in conducive environmental conditions. Damage and percentage weight loss caused by *E. cautella* were quite extensive, resulting in high weight loss. The contaminants produced by the insect in the cocoa beans stored for 120 days also affected the quality of cocoa beans. *T. castaneum* which is also a major pest of

stored cocoa beans did not cause much damage on the whole cocoa beans. This implies that the moth *E. cautella* is more important economically, than the beetle *T. castaneum*.

It is a well known fact that under-fermentation of cocoa beans leads to the production of purple beans and cocoa beans fermented for one day produce slaty beans which are normally considered as unfermented cocoa beans (BCCCA, 1996). In this study *E. cautella* fed more on unfermented beans than under-fermented beans. Therefore, unfermented and under-fermented cocoa beans should not be stored for more than 30 days. Also from this study there was neither a decrease nor an increase in percentage of purple beans in respect to storage period. This implies that storage does not have any impact on purple beans and cocoa beans should be fermented for 6 days before storage.



## CHAPTER SIX

### 6.0 CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

The study was conducted using grade II cocoa beans but the cocoa beans deteriorated over the storage period to sub-standard due to high levels of other defects such as germinated, flat and insect infested beans. High insect infestation contributed a great deal to the reduction in quality. The tropical warehouse moth (*E. cautella*) alone caused significant damage to stored cocoa beans. Cocoa beans fermented for one and two days were all slaty. It has been shown in this study that poor fermentation and insect infestation pose a great threat to the quality of cocoa beans. These factors deserve attention in effect to maintain quality of cocoa beans during storage. There was no significant reduction in the percentage purple beans over the storage period. Storage duration therefore had no impact on the percentage of purple cocoa beans.

#### 6.2 Recommendations

Cocoa beans are of great economic importance in Ghana and in order to maintain the quality, they should be properly fermented (6 days fermentation period) and stored in an environment which is free from stored product pests, with moisture content of 6.5-7% at a relative humidity of 65-70% to prevent deterioration by insect pests. If cocoa beans need to be stored for more than 30 days, adequate control measures should be applied especially against *E. cautella*. Further studies should be done to determine the level of damage on stored cocoa beans caused by other storage insect pests of cocoa beans such as *Cryptolestes* sp, *Lasioderma serricorne*, *Carpophilus* sp and *Araecerus fasciculatus*.

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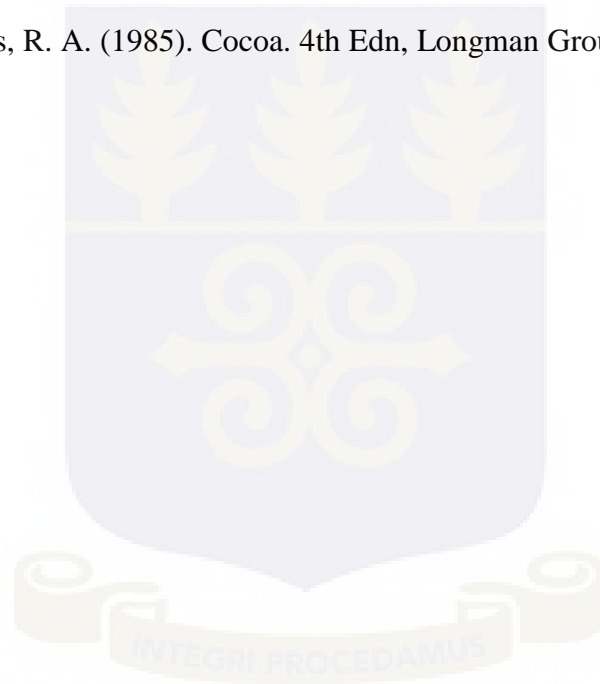
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## LIST OF APPENDICES

**Appendix 1: Mean moisture content of stored dry cocoa beans infested with insects after days of storage.**

Days of fermentation	Treatment	Mean moisture content after days of storage			
		30	60	90	120
1	C	6.7±0.1	6.9±0.1	6.6±0.0	6.3±0.0
	E	6.9±0.1	6.6±0.1	6.5±0.1	5.3±0.1
	T	6.9±0.0	7.1±0.1	6.9±0.1	6.4±0.1
	ET	6.8±0.1	6.8±0.1	6.5±0.1	6.3±0.1
<b>LSD (P&lt;0.05)</b>		<b>0.3</b>	<b>0.2</b>	<b>0.2</b>	<b>0.2</b>
2	C	6.3±0.0	7.2±0.1	6.6±0.1	6.3±0.4
	E	6.4±0.2	6.5±0.1	6.2±0.2	6.1±0.2
	T	6.0±0.1	7.2±0.1	6.8±0.1	6.6±0.1
	ET	6.2±0.1	7.1±0.1	6.2±0.2	6.5±0.1
<b>LSD (P&lt;0.05)</b>		<b>0.3</b>	<b>0.35</b>	<b>0.4</b>	<b>0.7</b>
3	C	6.6±0.0	7.1±0.1	6.5±0.1	6.4±0.2
	E	6.7±0.1	7.1±0.1	6.5±0.1	6.6±0.1
	T	7.1±0.2	7.1±0.3	6.6±0.2	6.7±0.1
	ET	6.7±0.1	7.0±0.1	6.5±0.2	6.6±0.0
<b>LSD (P&lt;0.05)</b>		<b>0.4</b>	<b>0.5</b>	<b>0.3</b>	<b>0.4</b>
4	C	6.5±0.1	7.4±0.1	6.5±0.1	6.3±0.1
	E	6.5±0.1	6.9±0.1	6.5±0.1	6.3±0.1
	T	6.4±0.1	7.4±0.1	6.9±0.1	6.3±0.2
	ET	6.6±0.1	6.9±0.1	6.4±0.1	5.7±0.2
<b>LSD (P&lt;0.05)</b>		<b>0.3</b>	<b>0.30</b>	<b>0.2</b>	<b>0.5</b>
5	C	8.4±0.1	7.1±0.2	6.9±0.1	6.9±0.1
	E	7.6±0.5	6.9±0.1	6.8±0.2	6.2±0.1
	T	7.8±0.5	7.3±0.1	6.8±0.2	6.9±0.1
	ET	7.9±0.2	7.2±0.1	6.7±0.2	6.5±0.2

<b>LSD (P&lt;0.05)</b>	<b>1.1</b>	<b>0.4</b>	<b>0.5</b>	<b>0.4</b>
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**Population trend of two insects**

**Appendix 2A**

**Number of insects analysis**

**Appendix 2: ANOVA for number of insects after 120 days of storage for beans fermented for one day**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	96.500	32.167	3.53	0.049
Residual	12	109.500	9.125		
Total	15	206.000			

LSD = 4.654

Insect	E	T	ET.E	ET.T
Mean	8.50	8.25	2.75	4.50

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	6313.25	2104.42	149.87	<.001
Residual	12	168.50	14.04		
Total	15	6481.75			

LSD = 5.773

Insect	E	T	ET.E	ET.T
Mean	48.25	3.75	2.00	1.50

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	67852.5	22617.5	39.53	<.001
Residual	12	6865.5	572.1		
Total	15	74718.0			

LSD = 36.85

Insect	E	T	ET.E	ET.T
Mean	158.0	2.8	21.2	2.0

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	247870.7	82623.6	101.95	<.001
Residual	12	9724.8	810.4		
Total	15	257595.4			

LSD = 43.86

Insect	E	T	ET.E	ET.T
Mean	297.0	1.5	29.2	1.5

**Appendix 3: ANOVA for number of insects after 120 days of storage for beans fermented for two days.**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	153.5	51.2	0.29	0.831
Residual	12	2105.5	175.5		
Total	15	2259.0			

LSD = 20.41

Insect	E	T	ET.E	ET.T
Mean	5.5	11.8	13.5	8.2

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	1809.2	603.1	1.61	0.240
Residual	12	4506.8	375.6		
Total	15	6315.9			

LSD = 29.86

Insect	E	T	ET.E	ET.T
Mean	30.5	4.2	23.0	8.5

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	15859.1	5286.6	1.66	0.227
Residual	12	38107.3	3176.1		
Total	15	53965.4			

LSD = 86.8

Insect	E	T	ET.E	ET.T
Mean	78.5	1.0	59.5	15.75

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	80796.6	26932.7	5.94	0.010
Residual	12	54377.4	4531.7		
Total	15	135173.6			

LSD = 103.7

Insect	E	T	ET.E	ET.T
Mean	181.5	1.5	71.0	14.3

**Appendix 4: ANOVA for number of insects after 120 days of storage for beans fermented for three days.**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	367.69	122.56	2.79	0.086
Residual	12	526.25	43.85		
Total	15	893.94			

LSD = 10.20

Insect	E	T	ET.E	ET.T
Mean	5.8	16.0	9.2	3.2

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	459.5	153.2	1.46	0.274
Residual	12	1255.5	104.6		
Total	15	1715.0			

LSD = 15.76

Insect	E	T	ET.E	ET.T
Mean	13.5	4.2	16.5	4.8

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	8853.6	2951.2	1.19	0.356
Residual	12	29872.4	2489.3		
Total	15	38724.5			

LSD = 76.9

Insect	E	T	ET.E	ET.T
Mean	27.3	4.5	60.0	1.0

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	14271.5	4757.3	2.13	0.150
Residual	12	26834.6	2236.3		
Total	15	41105.8			

LSD = 72.9

Insect	E	T	ET.E	ET.T
Means	75.5	3.5	46.8	6.5

**Appendix 5: ANOVA for number of insects after 120 days of storage for beans fermented for four days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	447.69	149.23	4.41	0.026
Residual	12	406.25	33.85		
Total	15	853.94			

LSD = 4.11

Insect	E	T	ET.E	ET.T
Mean	11.0	17.2	14.8	3.2

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	5265.7	1755.2	5.72	0.011
Residual	12	3681.2	306.8		
Total	15	8946.9			

LSD = 26.98

Insect	E	T	ET.E	ET.T
Mean	29.2	4.8	48.5	5.8

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	24879.7	8293.2	17.64	<.001
Residual	12	5641.2	470.1		
Total	15	30520.9			

LSD = 33.40

Insect	E	T	ET.E	ET.T
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Mean                    72.8                    3.5                    96.2                    11.8

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	118375.0	39458.2	11.86	<.001
Residual	12	39922.7	3327.9		
Total	15	158298.5			

LSD = 88.9

Insect	E	T	ET.E	ET.T
Mean	137.0	2.8	206.5	11.3

**Appendix 6: ANOVA for number of insects after 120 days of storage for beans fermented for five day**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	1419.7	473.2	0.90	0.471
Residual	12	6330.2	527.5		
Total	15	7749.9			

LSD = 35.39

Insect	E	T	ET.E	ET.T
Mean	23.2	14.8	31.8	40.0

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	7176.2	2392.1	9.87	0.001
Residual	12	2907.5	242.3		
Total	15	10083.8			

LSD = 23.98

Insect	E	T	ET.E	ET.T
Mean	58.0	6.0	23.2	6.2

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	31118.0	10372.7	21.98	<.001
Residual	12	5664.0	472.0		
Total	15	36782.0			

LSD = 33.47

Insect	E	T	ET.E	ET.T
Mean	111.0	1.5	32.5	5.0

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	51196.0	17065.0	6.33	0.008
Residual	12	32360.0	2697.0		
Total	15	83556.0			

LSD = 80.0

Insect	E	T	ET.E	ET.T
Mean	143.3	2.8	45.3	7.0

## Appendix 2B

### Weight of contaminants Analysis

#### Appendix 7: ANOVA for weight of contaminant after 120 days of storage for beans fermented for one day

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	0.41615	0.13872	7.70	0.004
Residual	12	0.21605	0.01800		
Total	15	0.63220			

LSD = 0.2067

Insect	C	E	ET	T
Mean	0.067	0.507	0.200	0.205

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	33.4091	11.1364	20.95	<.001
Residual	12	6.3774	0.5315		
Total	15	39.7866			

LSD = 1.123

Insect	C	E	ET	T
Mean	0.11	3.57	0.33	0.29

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	200.626	66.875	9.55	0.002
Residual	12	83.989	6.999		
Total	15	284.615			

LSD = 4.076

Insect	C	E	ET	T
Means	0.19	8.56	0.62	0.35

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	1187.90	395.97	20.15	<.001
Residual	12	235.83	19.65		
Total	15	1423.73			

LSD = 3.13

Insect	C	E	ET	T
Mean	0.2	21.0	2.4	0.8

**Appendix 8: ANOVA for weight of contaminants after 120 days of storage for beans fermented for two days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	4.1459	1.3820	3.44	0.052
Residual	12	4.8261	0.4022		
Total	15	8.9720			

LSD = 0.977

Insect	C	E	ET	T
Mean	0.08	0.32	1.31	0.08

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	39.733	13.244	2.02	0.165
Residual	12	78.677	6.556		
Total	15	118.410			

LSD = 3.945

Insect	C	E	ET	T
Mean	0.16	1.97	4.02	0.24

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	69.61	23.20	1.82	0.197
Residual	12	152.75	12.73		
Total	15	222.36			

LSD = 5.497

Insect	C	E	ET	T
Mean	0.21	2.72	5.26	0.25

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	1160.76	386.92	5.12	0.016
Residual	12	906.62	75.55		
Total	15	2067.37			

LSD = 13.39

Insect	C	E	ET	T
Mean	0.3	21.3	8.9	0.8

**Appendix 9: ANOVA for weight of contaminant after 120 days of storage for beans fermented for three days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	0.3098	0.1033	0.69	0.577
Residual	12	1.8037	0.1503		
Total	15	2.1135			

LSD = 0.5973

Insect	C	E	ET	T
Mean	0.145	0.465	0.340	0.133

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	14.330	4.777	0.92	0.461
Residual	12	62.319	5.193		
Total	15	76.648			

LSD = 3.511

Insect	C	E	ET	T
Mean	0.24	1.40	2.55	0.29

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	31.47	10.49	0.98	0.435
Residual	12	128.55	10.71		
Total	15	160.02			

LSD = 5.043

Insect	C	E	ET	T

Mean 1.14 2.23 4.17 0.46

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	15.668	5.223	0.86	0.488
Residual	12	72.888	6.074		
Total	15	88.555			

LSD = 3.797

Insect	C	E	ET	T
Mean	1.24	2.27	3.09	0.49

**Appendix 10: ANOVA for weight of contaminant after 120 days of storage for beans fermented for four days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	2.68432	0.89477	12.58	<.001
Residual	12	0.85347	0.07112		
Total	15	3.53779			

LSD = 0.4109

Insect	C	E	ET	T
Mean	0.083	0.465	1.120	0.155

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	5.7886	1.9295	6.63	0.007
Residual	12	3.4916	0.2910		
Total	15	9.2802			

LSD = 0.831

Insect	C	E	ET	T
Mean	0.11	0.90	1.68	0.36

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	2.68432	0.89477	12.58	<.001
Residual	12	0.85347	0.07112		
Total	15	3.53779			

LSD = 0.4109

Insect	C	E	ET	T
Mean	0.11	0.90	1.68	0.36

Mean            0.083        0.465        1.120        0.155

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	606.95	202.32	6.72	0.007
Residual	12	361.36	30.11		
Total	15	968.32			

LSD = 8.45

Insect	C	E	ET	T
Mean	0.3	7.8	15.5	1.0

**Appendix 11: ANOVA for weight of contaminant after 120 days of storage for beans fermented for five days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	4.89762	1.63254	38.25	<.001
Residual	12	0.51223	0.04269		
Total	15	5.40984			

LSD = 0.3183

Insect	C	E	ET	T
Mean	0.128	1.427	0.112	0.220

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	6.6797	2.2266	13.56	<.001
Residual	12	1.9705	0.1642		
Total	15	8.6502			

LSD = 0.624

Insect	C	E	ET	T
Mean	0.22	1.80	0.52	0.27

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	10.5766	3.5255	5.07	0.017
Residual	12	8.3364	0.6947		
Total	15	18.9130			

LSD = 1.284

Insect	C	E	ET	T

Mean            0.23        2.14        1.56        0.32

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	233.9550	77.9850	80.06	<.001
Residual	12	11.6891	0.9741		
Total	15	245.6441			

LSD = 1.521

Insect	C	E	ET	T
Mean	0.29	9.71	2.19	0.62

### APPENDIX 3

#### PERCENTAGE WEIGHT LOSS ANALYSIS

##### Appendix 12: ANOVA for percentage weight loss after 120 days of storage for beans fermented for one day

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	6.4915	2.1638	6.07	0.009
Residual	12	4.2775	0.3565		
Total	15	10.7690			

LSD = 0.920

Insect	C	ET	T	E
Mean	0.00	0.54	0.00	1.56

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	27.5156	9.1719	20.84	<.001
Residual	12	5.2808	0.4401		
Total	15	32.7963			

LSD = 1.022

Insect	C	ET	T	E
Mean	0.00	0.55	0.07	3.19

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	137.1877	45.7292	88.38	<.001
Residual	12	6.2092	0.5174		
Total	15	143.3968			

LSD = 1.108

Insect	C	ET	T	E
Mean	0.00	0.79	0.46	7.15

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	269.300	89.767	23.80	<.001
Residual	12	45.267	3.772		
Total	15	314.567			

LSD = 2.992

Insect	C	ET	T	E
Mean	0.00	0.86	1.17	10.10

**Appendix 13: ANOVA for percentage weight loss after 120 days of storage for beans fermented for two days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	9.1141	3.0380	21.86	<.001
Residual	12	1.6675	0.1390		
Total	15	10.7816			

LSD = 0.5743

Insect	C	ET	T	E
Mean	0.000	0.782	0.000	1.840

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	10.7614	3.5871	10.80	0.001
Residual	12	3.9871	0.3323		
Total	15	14.7486			

LSD = 0.888

Insect	C	ET	T	E
Mean	0.00	0.97	0.31	2.14

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	55.167	18.389	10.32	0.001
Residual	12	21.380	1.782		
Total	15	76.548			

LSD = 2.056

Insect	C	ET	T	E
Mean	0.00	2.49	0.25	4.57

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	182.06	60.69	5.13	0.016
Residual	12	141.93	11.83		
Total	15	323.99			

LSD = 5.299

Insect	C	ET	T	E
Mean	0.00	3.62	0.92	8.67

**Appendix 14: ANOVA for percentage weight loss after 120 days of storage for beans fermented for three days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	1.1474	0.3825	3.10	0.067
Residual	12	1.4783	0.1232		
Total	15	2.6258			

LSD = 0.5408

Insect	C	ET	T	E
Mean	0.000	0.492	0.030	0.597

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	4.2231	1.4077	6.11	0.009
Residual	12	2.7643	0.2304		
Total	15	6.9874			

LSD = 0.739

Insect	C	ET	T	E
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Mean            0.00        1.00        0.13        1.16

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	37.319	12.440	3.90	0.037
Residual	12	38.271	3.189		
Total	15	75.590			

LSD = 2.751

Insect	C	ET	T	E
Mean	0.00	2.46	0.30	3.69

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	55.320	18.440	5.42	0.014
Residual	12	40.839	3.403		
Total	15	96.159			

LSD = 2.842

Insect	C	ET	T	E
Mean	0.00	3.07	0.66	4.63

**Appendix 15: ANOVA for percentage weight loss after 120 days of storage for beans fermented for four days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	2.0367	0.6789	1.87	0.189
Residual	12	4.3639	0.3637		
Total	15	6.4006			

LSD = 0.929

Insect	C	ET	T	E
Mean	0.00	0.74	0.00	0.69

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	13.0282	4.3427	20.17	<.001
Residual	12	2.5839	0.2153		
Total	15	15.6122			

LSD = 0.715

Insect	C	ET	T	E
Mean	0.00	0.93	0.06	2.23

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	86.398	28.799	11.25	<.001
Residual	12	30.708	2.559		
Total	15	117.106			

LSD = 2.465

Insect	C	ET	T	E
Mean	0.00	4.76	0.26	4.79

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	304.05	101.35	1.29	0.321
Residual	12	939.68	78.31		
Total	15	1243.73			

LSD = 13.63

Insect	C	ET	T	E
Mean	0.0	7.3	0.5	10.1

**Appendix 16: ANOVA for percentage weight loss after 120 days of storage for beans fermented for five days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	5.06317	1.68772	24.49	<.001
Residual	12	0.82707	0.06892		
Total	15	5.89024			

LSD = 0.4045

Insect	C	ET	T	E
Mean	0.000	0.405	0.018	1.385

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	16.5871	5.5290	12.96	<.001
Residual	12	5.1198	0.4266		

Total 15 21.7069  
LSD = 1.006

Insect	C	ET	T	E
Mean	0.00	0.63	0.09	2.52

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	35.481	11.827	5.63	0.012
Residual	12	25.201	2.100		
Total	15	60.681			

LSD = 2.233

Insect	C	ET	T	E
Mean	0.00	2.16	0.55	3.81

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	58.831	19.610	10.02	0.001
Residual	12	23.496	1.958		
Total	15	82.327			

LSD = 2.156

Insect	C	ET	T	E
Mean	0.00	3.27	1.06	4.94

#### APPENDIX 4

#### PERCENTAGE OF PURPLE BEANS IN STORED COCOA BEANS ANALYSIS

#### Appendix 17: ANOVA for percentage of purple beans after 120 days of storage for beans fermented for one day

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	4.1875	1.3958	1.43	0.284
Residual	12	11.7500	0.9792		
Total	15	15.9375			

LSD = 1.525

Insect	C	E	ET	T
Mean	0.50	0.00	1.25	0.00

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	78.50	26.17	0.71	0.565
Residual	12	442.50	36.88		
Total	15	521.00			

LSD = 9.36

Insect	C	E	ET	T
Mean	10.8	11.5	9.0	5.8

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	0.6875	0.2292	0.73	0.552
Residual	12	3.7500	0.3125		
Total	15	4.4375			

LSD = 0.861

Insect	C	E	ET	T
Mean	0.00	0.00	0.50	0.25

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	21.500	7.167	1.14	0.373
Residual	12	75.500	6.292		
Total	15	97.000			

LSD = 3.864

Insect	C	E	ET	T
Mean	0.25	0.00	2.75	2.00

**Appendix 18: ANOVA for percentage of purple beans after 120 days of storage for beans fermented for two days and infested with two insects**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	41.25	13.75	0.83	0.502
Residual	12	198.50	16.54		
Total	15	239.75			

LSD = 6.27

Insect	C	E	ET	T
Mean	7.5	9.2	9.8	12.0

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	465.69	155.23	4.71	0.021
Residual	12	395.75	32.98		
Total	15	861.44			

LSD = 8.85

Insect	C	E	ET	T
Mean	27.0	15.8	21.5	13.0

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	947.25	315.75	20.10	<.001
Residual	12	188.50	15.71		
Total	15	1135.75			

LSD = 6.106

Insect	C	E	ET	T
Mean	8.50	6.75	3.00	23.25

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	241.25	80.42	1.79	0.202
Residual	12	538.50	44.88		
Total	15	779.75			

LSD = 10.32

Insect	C	E	ET	T
Mean	18.5	7.8	15.0	13.2

**Appendix 19: ANOVA for percentage of purple beans after 120 days of storage for beans fermented for three days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	84.69	28.23	0.80	0.518
Residual	12	423.75	35.31		
Total	15	508.44			

LSD = 9.16

Insect	C	E	ET	T
Mean	25.2	19.5	20.5	23.5

Variate: 60 day

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	775.25	258.42	4.58	0.023
Residual	12	676.50	56.38		
Total	15	1451.75			

LSD = 11.57

Insect	C	E	ET	T
Mean	21.5	29.0	24.2	39.8

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	409.5	136.5	1.04	0.411
Residual	12	1579.5	131.6		
Total	15	1989.0			

LSD = 17.68

Insect	C	E	ET	T
Mean	39.5	40.8	33.2	47.5

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	245.69	81.90	1.36	0.302
Residual	12	723.75	60.31		
Total	15	969.44			

LSD = 11.96

Insect	C	E	ET	T
Mean	32.5	28.0	33.8	39.0

**Appendix 20: ANOVA for percentage of purple beans after 120 days of storage for beans fermented for four days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	75.188	25.062	2.51	0.108
Residual	12	119.750	9.979		
Total	15	194.938			

LSD = 4.867

Insect	C	E	ET	T
Mean	26.50	21.25	25.50	26.50

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	66.00	22.00	1.33	0.312
Residual	12	199.00	16.58		
Total	15	265.00			

LSD = 6.27

Insect	C	E	ET	T
Mean	26.8	21.2	24.2	22.8

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	524.00	174.67	7.11	0.005
Residual	12	295.00	24.58		
Total	15	819.00			

LSD = 7.64

Insect	C	E	ET	T
Mean	26.2	30.2	38.2	40.2

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	148.25	49.42	1.86	0.191
Residual	12	319.50	26.62		
Total	15	467.75			

LSD = 7.95

Insect	C	E	ET	T
Mean	32.0	30.0	26.2	24.2

**Appendix 21: ANOVA for percentage of purple beans after 120 days of storage for beans fermented for five days**

Variate: 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	121.500	40.500	5.31	0.015
Residual	12	91.500	7.625		
Total	15	213.000			

LSD = 4.254

Insect	C	E	ET	T
Mean	26.25	29.50	21.75	25.50

Variate: 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	29.25	9.75	0.81	0.513
Residual	12	144.50	12.04		
Total	15	173.75			

LSD = 5.346

Insect	C	E	ET	T
Mean	21.50	25.25	24.00	23.75

Variate: 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	35.00	11.67	0.22	0.882
Residual	12	642.00	53.50		
Total	15	677.00			

LSD = 11.27

Insect	C	E	ET	T
Mean	28.5	25.5	24.5	26.5

Variate: 120 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Insect	3	14.19	4.73	0.12	0.947
Residual	12	473.75	39.48		
Total	15	487.94			

LSD = 9.68

Insect	C	E	ET	T

Mean	24.8	25.0	23.0	25.5
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