UNIVERSITY OF CAPE COAST

STUDIES OF OCCURRENCE OF PURPLE BEANS IN COCOA PRODUCED IN GHANA

BY

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MARCH, 2007

DECLARATION

CANDIDATE'S DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

..... 08/08/07

DANIEL KWABLA ADZAHO DATE

SUPERVISOR'S DECLARATION

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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ABSTRACT

The study investigated the occurrence of high purple beans counts of cocoa produced in Ghana. The investigation consisted of a questionnaire survey of nineteen randomly selected districts in the six cocoa growing regions in Ghana and laboratory studies at the Cocoa Research Institute of Ghana. The studies were carried out from August 2005 to October 2006.

Fermentation of cocoa beans for six or seven days did not guarantee the complete removal of anthocyanins and purpleness from the cocoa beans.

It was observed that good quality dry cocoa beans had a Fermentation Index of one and above; 2.0mg/kg and below residual anthocyanins and pH of 5.4-5.5. Cocoa beans of partly brown/partly purple and pale purple colour were observed to have the same residual anthocyanins contents of the brown coloured cocoa beans considered to be of good quality. The residual anthocyanins contents of the fifteen cocoa cultivars after six days of fermentation varied considerably. Anthocyanins contents of the cultivars tested after six days fermentation, shows that only T85/799 x *Amelonado* and T60/887 x *Amelonado* contained 2.0mg/kg. However, cultivars with anthocyanins contents more than 2.0mg/kg reduced significantly below 2.0mg/kg after seven days fermentation except for T60/887 x IMC 60 and T60/887 x Be 8. The percentage purpleness of *Criollo*, *Amelonado*, *Amazonia* and all T85/799 crosses were found to be less than 20% while all the T60/887 crosses had purple beans of more than 20% except for T60/887 x IMC 60.

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DEDICATION

To the Glory of God and to the memory of Kotomensah Ladzaka and Rosa Afafavi Agbogah

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CHAPTER ONE

INTRODUCTION

Cocoa *(Theobroma cacao* L.*)* is described by many as the 'Golden Tree', an apt description derived from its ripe golden pods that hang on the brown stem against the green background of leaves (Plate 1). Tetteh Quarshie was reported to have brought the pods or seeds of cocoa to Ghana from Fernando Po in 1879 (Legg, 1972). Various Missionaries of the Basel mission, which had a station at Akropong had earlier introduced cocoa to Ghana for ornamental purposes (Wanner, 1962).

Theobroma cacao L. belongs to the family *Sterculiaceae* and has two main types, *Criollo* that contributes about 10% of the World cocoa production and commonest type and *Forastero*, which yields smaller, flatter and purple beans (Appiah, 2004). A third variety, the *Trinitario* (Leeds and Jackson, 1973) is a more disease- resistant hybrid of the *Criollo* and *Forastero* cross which is regarded as a flavour bean. In Ghana, three types of cocoa are grown. These are *Amelonado* (30.0%), *Amazon* (40.1%), *Hybrid* (26.4%) and a mixture of the three (3.5%) (CSSVDCU, 2005). According to Clapperton (1993), the type of planting material has a major influence on the flavour and colour of the cocoa beans.

Mossu (1992) reported that cocoa could be successfully grown in areas with rainfall between 1000mm and 3000mm and on lands, which support the heavy type of tropical rainforest. In these areas, the dry season does not exceed three months and the rainfall is not less than 100mm per month with temperatures ranging between a maximum of 30°C to 32°C and a minimum of 18°C to 21°C (Mossu, 1992).

Cocoa beans originate as seeds in fruit pods of the tree *Theobroma cacao.* Each pod contains 30-40 beans, embedded in a mucilaginous pulp (Nielsen *et. al.*, 2005).

The cocoa plant is generally seed propagated; however, vegetative methods could be used. Flowering and fruiting start at the age of 4 to 5 years and the trees continue bearing fruits for more than 50 years in areas with good soil and suitable climate, and at 30 to 40 years under marginal conditions (Krug and Quartey-Papafio, 1964). Improved varieties developed at CRIG start flowering and fruiting at lesser age of 2 to 3 years (Adu-Ampomah *et. al.*1996). After pollination, the pods grow slowly for about 40 days, after which growth becomes rapid and reaches a maximum in about 75 days. The development of the pods takes 5 to 6 months from fertilization to fullness (Mckelvie, 1956).

In Ghana, there are two cocoa harvesting seasons namely, the main and light crop seasons. The main crop-harvesting season falls between September and February with peak harvesting period usually in November. The light crop-harvesting season is between March and August. The bean sizes show distinct pattern between the main and the light crop seasons. About 68.68% of main crop

season beans are large in size, 28.41% are medium in size, 1.98% is a small sized bean and remnants form 0.93%. In contrast, the light crop beans are mostly medium in size (51%) and small sized (35%) with a small proportion of large sized beans (2%) and relatively large proportions of remnants (14%) (QCD, 2005). The harvesting pattern of cocoa for Tafo in Ghana showed that harvesting reaches its peak in the month of November. The less pronounced peak has an advantage in spreading the task of harvesting and the amount of work involved during the fermentation and drying of the cocoa beans. In Nigeria, Toxopeus (1964) reported that 75% of the annual crops from *Amelonado* are harvested between September and January whereas only 50% of the *Amazon* crop was harvested during the same period. However, the other half of the *Amazon* crop was harvested between February and July.

Cocoa is successfully grown in six out of the ten regions of Ghana namely, Ashanti, Brong Ahafo, Central, Eastern, Western (South and North) and Volta.

Cocoa is of great economic importance to both the farmer and the nation and over the years it has helped to raise the living standards of farmers cropping it. The cocoa sector in Ghana employs over 800,000 smallholder farm families for whom cocoa contributes about 70-100% of their annual household income (COCOBOD, 1998). The sector also employs about 60% of the national agricultural labour force (Appiah, 2004).

Export of cocoa beans has been one of the major foreign exchange earners to Ghana. In 1999, cocoa accounted for 26.2% of total foreign exchange earning of Ghana's export (ISSER, 2000) and 22.6% in 2000 (ISSER, 2002).

Government revenues from international trade transactions of cocoa are derived basically from two main sources: the import related taxes and export duties on cocoa. Cocoa export taxes alone formed 20.5% of the 1999 total revenue from international trade taxes and 3.8% of government revenue in 2000. Cocoa sub-sector contribution to Gross Domestic Product (GDP) was about 4% in 2000 (ISSER, 2002).

Cocoa is used as a raw material in many products and is highly appreciated for its characteristic brown chocolate colour, aroma and flavour. In Ghana, locally produced items from cocoa are cocoa butter, cooking fat, lighting oil, ointments, hard soap, soft soap from cocoa butter and cocoa oil, candle, cocoa as fuel, cocoa ash fertilizer, cocoa bread and biscuit (Are and Gwynne-Jones, 1973). Cocoa butter is remedy for burns, fever, dry lips, snakebites and wounds (Ghana Cocoa Board, 2007). Cocoa by-products like cocoa jam; cocoa vinegar; cocoa brandy; body pomade and animal feeds are also developed by Cocoa Research Institute of Ghana. In Western Nigeria, cocoa balls, rolls, flakes, biscuits, chi chin, cocoa popcorn (*'gugu'*) and cocoa butter are made from cocoa beans (Are and Gwynne-Jones, 1973).

Research has also shown that cocoa has the potency to reduce the stress women go through during menopause (Kettenberg, 2000). Cocoa contains more antioxidants, chemicals that have been shown to fight cancer, hypertension, stroke, heart attack, diabetes, asthma, aging and erectile dysfunction (Wollgast and Anklam, 2000; Kettenberg, 2000; Ghana Cococa Board, 2007). Chocolate has the ability to improve potency in men.

Fresh cocoa beans have an astringent, unpleasant taste and flavour and have to be fermented, dried and roasted to obtain the desired characteristic cocoa flavour and taste (Thompson *et. al.*, 2001). Fermentation takes place in the pulp surrounding the beans. The pulp is rich in carbohydrates and has a relatively low initial pH3.3-4.0, primarily due to high concentration of citric acid (Roelofsen and Giesberger, 1947; Pettipher, 1986; Thompson *et.al.*, 2001; Ardhana and Fleet, 2003).

The quality of Ghana cocoa is acclaimed the best in the World. The activities of the farmer contribute up to 60 to 70% of the quality with 30 to 40% from researchers, Licensed Buying Companies (L.B.Cs.), haulage or transport companies and COCOBOD as the manager of the cocoa industry (Buatsie, 2005).

In the 2003/2004 main crop cocoa seasons, however, there were reports from some chocolate manufacturers of unusually high levels of purple beans in cocoa from Ghana. The incidence of purple beans was high with a national average of 27.3% (Personal communication) compared to 26.1% observed by Morinaga and Co. Ltd of Japan using cocoa from Ghana (Morinaga and Co Ltd., 2004). Purple beans, according to chocolate manufacturers, produce cocoa liquor that has less flavour and high acidity. The incidence of deep purple beans increased from 5.1 to 11.8% from 2003 to 2004 in Ghana cocoa imported by Morinaga and Co. Ltd of Japan. In terms of total purple beans, the increase for Morinaga and Co. Ltd. was 13.6 to 26.1% from 2003 to 2004. These reports coming soon after the recently introduced cocoa diseases and pests' control (CODAPEC) and

Cocoa Hi- Tech. programmes raised a lot of concern in the cocoa industry. Some farmers in the Western and Brong Ahafo regions also put the blame on COCOBOD for the loss of about 2000 tonnes of their cocoa in the 2003 and 2004 main crop season for lack of education on the purple beans problem (Daily Graphic, 2006).

The incidence of purple beans in Ghana cocoa has thus lowered status of Ghana as the premier grade one quality producer of bulk cocoa beans for the international market. Ghana has supplied grade one cocoa beans to the world market over the years, supplying 98% grade I cocoa beans of her total cocoa production (Aquah, 1999), until 2003/2004 cocoa season when the purple cocoa beans problem started. Total cocoa exports from Ghana between 2000/2001 to 2002/2003 cocoa crop year, according to grades were 98% grade I and 2% grade II. In 2003/2004 cocoa crop year, grade I cocoa export declined to 61% while grade II increased to 39%. Grade I and II cocoa beans export, as at 29th week of the 2004/2005 main crop year, were 33% and 67% respectively (Buatsie, 2005). At the end of the 2004/2005 cocoa seasons, grade I cocoa exported dropped further to 12.33% whilst grade II increased sharply to 37.56% and 50.11% declared as complete purple beans (QCD, 2005). The change in grade status of cocoa beans from Ghana was attributed to high levels of purple colour in the cotyledon of the dry cocoa beans. However, when grading of cocoa in Ghana was reviewed according to purple beans counts for the 2005/2006 cocoa year, only 0.35% of dry cocoa beans graded and sealed for export could qualify for

grade I while grade II represent 61.40%. Grade II* and sub-standard cocoa beans graded and sealed represent 38.23% and 0.02% respectively (QCD, 2005).

The purple bean menace has affected the profit margin of most private licensed cocoa buying companies in the country. At the moment, the licensed buying companies are paid only 70% of total cost of cocoa delivered to COCOBOD at the take- over centers for cocoa beans of purple content above 30% per 100 dry bean counts. The remaining 30% is paid only when the importers expressed satisfaction about reduction in the purple levels in the beans. Again, cocoa beans of purple levels above 45% per 100 dry bean counts are marked sub- standard, which attract only 50% of the cost of cocoa delivered to COCOBOD without any refund. There is the general believe among major stakeholders in the cocoa industry that the cocoa farmer in Ghana has compromised some of his post harvest processes and techniques. Educational programmes were organized for farmers nationwide on proper methods of processing cocoa so as to reduce the high occurrence of purple beans to acceptable levels. Purple levels in the beans however, still remain very high in most cocoa districts and regions of Ghana, exceeding the national average of 27.3% (Personal communication).

Wood and Lass (1985) reported that methods of processing cocoa by farmers had not been surveyed. The extent to which cocoa types grown in Ghana affect cocoa anthocyanin content had also not been unequivocally established and studies in this are rather scarce. Previous studies on purple beans have not

given a comprehensive interpretation to the term 'purple' as it had been largely subjective.

This study therefore seeks to conduct both social survey and scientific investigations into the causes of high incidence of purple cocoa beans in Ghana. Specifically, the study seeks to determine:

1. causes of high purple beans occurrence: The perception of the Farmer, Purchasing Clerk and Grading Staff' (G.S.) of Quality Control Division(COCOBOD).
2. effects of fermentation duration on anthocyanins level and fermentation index of mixed cocoa cultivars.
3. effects of length of pod storage on occurrence of purple beans.
4. effects of cocoa cultivar on anthocyanin levels in dry unfermented and fermented cocoa beans.
5. effects of different cocoa cultivars on purpleness in dry fermented cocoa beans.
6. anthocyanins levels of different categories of purple colour (deep purple, pale purple and partly brown/partly purple) and brown cocoa beans.
7. effects of environmental factors on purpleness in dry fermented cocoa beans.

Plate 1. Cocoa tree (*Theobroma cacao* L.) showing ripe golden pods hanging on the brown stem

CHAPTER TWO

LITERATURE REVIEW

Origin of cocoa

Linneaus (1753) in his first edition of species plantarum named the cocoa tree *Theobroma cacao*. The genus *Theobroma*, together with the genera *Herrania*, *Guazuma* and *Cola* occur in Africa and belong to the family *Sterculiaceae*. *Croillo*, *Forastero*,*Trinitario* are among the common species of cocoa grown.

Cuatrecasas (1964) divided the genus *Theobroma* into six sections containing twenty-two species. *Theobroma cacao* is the only species, which is cultivated commercially. The other better known species in the genus being *Theobroma bicolor* and *Theobroma gradiflorum*. *Theobroma bicolor* is typical of the genus as its inflorescences appear in the axils of the new leaves so that its large heavy pods are borne on the ends of the branches, which bend down when matured. The beans have white cotyledons and have been used as adulterant of cocoa in Central America. *Theobroma gradiflorum*, known as 'Cupuacu' in Brazil, is liked locally for the delicate flavour of the mucilage around the beans. *Theobroma cacao* is a diploid species with chromosome number of 20, and has been sub-divided into two subspecies by Cuatrecasas (1964). These are *Theobroma cacao sp cacao* and *Theobroma cacao spp. Sphaerocapum*. The

Theobroma cacao sp cacao consists of the *Criollo* populations of Central and South American whilst *Theobroma cacao spp. Sphaerocapum* includes all the other populations.

Cocoa varieties released to farmers over the years

Wood and Lass (1955) has identified two principal types of cocoa of commercial value depending on the colour of the seeds. These are the *Criollo* group with white, ivory or very pale purple cotyledon and the characteristically pale to deep purple cotyledon *Forastero* group. The West African *Amelonado* cocoa belongs to the *Forastero* groups as do the Mananhao Comun and Para types from Brazil (Mossu, 1992). The cotyledon of *Amelonado* is dark purple.

The Ghanaian cocoa industry was based exclusively on limited 19th-century introductions of a type of cocoa originating from the lower Amazon-basin and known as 'West African Amelonado'. This still accounts for a large proportion of all planting materials despite being vulnerable to cocoa swollen shoot virus (Thresh *et al.*, 1988). The West African Amelonado was grown almost exclusively for many years and formed the basis of the cocoa industry in Ghana and also elsewhere in West Africa. The bulk of cocoa produced in Ghana until 1969 was made up of 80% *Amelonado* and 20% of local *Trinitarios* (Adu-Ampomah *et.al.*, 1996).

The cocoa planting material used in Ghana and the changes that have occurred were considered in relation to the problems caused by Swollen Shoot Disease (Thresh *et al.*, 1988). In an effort to obtain local Cocoa Swollen Shoot Disease (CSSVD) resistant genotype, Posnette (1943a) made several collections

mainly *Amelonados* and local *Trinitarios* from all cocoa-growing areas of the then Gold Coast. In order to obtain the necessary variation needed for genetic improvement of the crop, Posnette again introduced semi- wild types from the headwaters of the Amazon; the Upper Amazon selections. These were introduced to Ghana as pods and numbered in sequence, with the general letter 'T' for Trinidad and the resulting trees are still known by these numbers (Adu-Ampomah *et al* 1999). Mixed Amazons were later developed through multiplication of thirteen of the Upper Amazon selections that included T60, T85, T87, Sca12 and IMC24 to replace the *Amelonados* and *Trinitarios*, which were easily susceptible to CSSVD attack. According to Thresh *et. al.*, (1988), Series II hybrids were later developed to supersede the Mixed *Amazons*. Series II hybrids (T79/501 x T85/799 and T60/887 x T60/888) were products of selected hybridized Upper Amazon clones and *Amelonado* or local *Trinitario* clones.

In 1969-1981, crossed hybrids of female parents (T63/967, T63/971, T79/467, T85/799 and PA7) and some selected Upper Amazon pollen parents (T79/501, T65/288, T60/887, Pa7 and IMC60) and progenies that combined a measure of resistance with satisfactory agronomic characteristics, notably high yield, low losses from black pod disease and bean quality acceptable to the chocolate manufacturing industries were released and planted by farmers in Cocoa Swollen Shoot Virus endemic areas.

Progenies of IMC60, SCA12, NA31-34, IMC47 and PA35, PA7 which all originated from Peru were extensively planted in Ghana and elsewhere. They

other genotypes with purple cotyledons produced the same intensity of pigment in all of the seeds as was exhibited in crosses between Catongo and *Criollo* genotypes with white cotyledons.

Bartley (1964b) observed that plants carrying the gene with those producing dark purple cotyledons have seeds with cotyledons of the dark shade whilst the seeds produced in crosses between the mutant type and green-fruited *Criollo* genotypes with white cotyledons are light purple in colour. Bartley concluded that the absence of anthocyanins pigment in any plant organ or combination of organs would be the result of genes that block the action of the enzymes responsible for the synthesis of anthocyanins.

Niemenak *et al* (2006) had shown that total phenols, catechin, epicatechin and anthocyanins in fresh and fermented-like beans were genotype dependent. Again, Luna *et al* (2002) reported different ranges of concentration of polyphenols in cocoa beans from Ecuadorian selfed and heterozygous population of cocoa clone EET95.

According to Macheix *et al.* (1990); Chalker-Scott, (1999); Cabrita *et al.* (2000); Vallejo *et al.* (2003); Stintzing and Carle, (2004); Cacho *et al.* (1992), the quality and /or quantity of polyphenolic compounds in plants depends on genetic diversity as well as on many environmental factors such as light intensity, humidity, temperature, use of fertilizers and other stress factors such as wounding and infections. Roubelakis-Angelakis and Kliewer, (1986); Kliewer, (1977); Cobbina and Miller, (1987); Wang and Zheng, (2001) have

also reported that light and temperature control accumulation of anthocyanins in plants.

Effects of harvesting time on quality of dried cocoa beans

Rohan (1963) noted that it is necessary to have a controlled frequency of harvest so as to ensure uniformity of pod ripeness. According to Mossu (1992), harvesting of pods should be carried out at regular intervals of 10 to 15 days, which in any event should not exceed three weeks. In Ghana, the pods are picked at intervals greater than the recommended three weeks (Hammond, 1953). Pods should be harvested when they are ripe. If the pods are left on the tree for too long, the seeds germinate. It is even more serious to harvest the pods before they are ripe because fermentation of the seeds in this case always produces a poor quality cocoa, which is low in aromatic compounds (Knapp, 1926).

Biehl (1961) recommended that the ripe cocoa should be harvested, and fermentation time increased as much as possible in order to avoid purple beans. Unripe cocoa fails to ferment properly because it contains less sugar than ripe cocoa (MacLean and Wickens, 1952; Saposhnikova, 1952, Rohan, 1963).

Effects of pod storage on purple beans

Several studies have examined the effect of post-harvest storage of pods on subsequent processes in the processing line on bean and chocolate quality (Berbet, 1979; Lewis and Lee, 1986; Meyer *et al.*, 1989; Tomlins *et al.*, 1993).

Kenten and Powell (1960) reported that interval between pod harvesting and breaking or opening has an influence on fermentation. Beans from delayed pod breaking initiate rapid rise in temperature during fermentation through the activities of invertase (Meyer *et. al.*1989; Tomlins *et al.* (1993), a very important factor that leads to the death of the beans. A delay between harvesting and pod breaking also resulted in a reduction in the concentration of pulp sugars and reduced pulp/cotyledon ratio of fresh beans (Lewis and Lee, 1986; Meyer *et al.*; 1989). Meyer *et al.* (1989) also reported that post- harvest storage of cocoa pods led to improved aeration of the ferments resulting in a rapid increase in temperature to more than 45°C within 20 hours. This results in suppression of lactic acid bacteria and increased sugar respiration by yeasts over alcohol fermentation. The major effect on sugar content was the conversion of about 25% of the total sucrose by invertase in the testa (Berbet, 1979). MacLean and Wickens (1951) found that storage of pods for two days or more produced significant temperature increase during the first 48 hours of fermentation. Furthermore, beans from pods stored for four days showed significantly lower temperature after 96 hours of fermentation than pods stored for one day only. They also found that increased length of pod storage has significantly reduced purple and wrinkle bean percentages.

There are varying suggestions as to how long the pods should be stored before breaking or opening. While some advocate for longer periods between 6-15 days (BCCCA, 1996) depending on cocoa genotypes, others suggest a period not more than six days. In Ghana, the recommended pod storage period is 3-4

days (Hancock, 1949; Allison and Rohan, 1958). Tomlins *et al.* (1993) also reported that pods stored for seven days before box fermentation in Malaysia had the least initial pulp volumes. According to Biehl *et al.* (1990) and Said *et al.*, (1990) improved cocoa flavour could be attributed to reduction in pulp volume which was linked to post–harvest storage of pods or pre-conditioning the pulp prior to fermentation. Anon (1981) also reported that delay in pod opening was to speed up fermentation by about 24 hours. According to Anon (1981), during the period of delay in pod opening, there is loss of moisture, which reduced the amount of 'sweatings' by 50% and allowed better aeration at the beginning of fermentation.

In Ghana, most of the farmers harvest for several days before opening the pods in order to gather sufficient quantity, but the effect must be to speed up the fermentation. Farmers with smaller farm holdings, however, break the pods immediately after harvesting for fermentation. The normal practice at the Cocoa Research Institute of Ghana, Tafo is to break the pods three days after harvesting, because this interval results in fermentations having a sharper rise in temperature and a higher maximum temperature than when the pods are broken shortly after harvesting (Hancock, 1949; Allison and Rohan, 1958).

In Papua New Guinea, farmers have been recommended to delay opening pods for three or four days after harvest for the same reason (Bridgland, 1959). According to Montserin (1952) when pods are heaped in the field and left for several days before breaking, the less ripe pods ripen and the beans in the fully ripe pods undergo a slight change. Under these conditions, a shorter

fermentation is required than when freshly picked pods are broken daily. Berbet (1979) determined the sugar content of freshly harvested ripe pods and those stored for six days after harvest and have observed that more glucose and fructose were present in samples stored for six days after harvest than in fresh ones.

MacLean and Wickens (1951) also reported that when pods were left for three days or more before breaking, West African *Amelonado* cocoa have its purple colour reduced from 82.0 to 39.6% and increase in temperature of 2°C in subsequent fermentation. MacLean and Wickens (1951), studied the influence of ripeness on recovery of dry cocoa and the percentage purple beans in dried products and have reported that a recovery of dry cocoa beans is in a descending order of 85.2% (under ripe), 81.3% (ripe) and 65.6% (over ripe) whiles percent purple beans were 70.2% (under ripe), 53.7% (ripe) and 28.1% (overripe). Jinap *et al.* (2000) have also reported a 12% slaty, 78% purple and 10% brown beans in Sulawesian and 3% slaty, 4% purple and 61% brown in Malaysia cocoa beans fermented immediately after harvest.

Effects of primary processing of cocoa on purple beans occurrence

The characteristic flavour of cocoa is the result of post- harvest fermentation, drying and manufacturing processes such as roasting (Cros *et al.*, 1999). Powell (1981) reported that, fermentation and drying comprise one of the most important processes involved in the manufacture of chocolate.

Fermentation of cocoa

According to Roelofsen (1958); 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* species and presumably, their viruses.

Seeds within ripe pods are microbiologically sterile. When the pod is opened with a knife, the pulps become contaminated with a variety of microorganisms, many of which contribute to subsequent fermentation. Organisms come mainly from the hands of workers, knives and unwashed baskets used for transport of seeds and dried mucilage left on the walls of boxes from previous fermentations (Roelofsen, 1958; Thompson *et al.* 2001 and Jespersen *et al., 2004*).

The main objectives of fermentation are to solubilise and remove the pulp surrounding the beans. It is to create conditions that generate the precursors of chocolate flavour. This involves killing the beans and inducing their autolytic breakdown by endogenous enzymes, as well as producing other flavour metabolites (Roelofsen, 1958; Thompson *et al.* 2001; and Jespersen *et al.*, 2004).

Schwan *et al.* (1995) observed two major events during fermentation. These are microbial action on the mucilaginous pulp, which produces alcohol and acids. It is also to liberate complex biochemical reactions triggered by diffusion of metabolites from microorganisms that occur in the cotyledon. *Amazonia* has more pulp than *Amalonado* resulting in more acetic and lactic acids being produced in *Amazonia* than *Amelonado* (Anon, 1982).

The cocoa pulp is a rich medium for microbial growth. It consists of 82-87% water, 10-15% sugar, 2-3% pentosans, 1-3% citric acid and 1-1.5% pectin (Roelofsen, 1958). Proteins, amino acids, vitamins (mainly vitamin C) and minerals are also present. The concentration of glucose, sucrose and fructose is a function of fruit age (Saposhnikova, 1952).

According to Quesnel (1972), sometimes fermentation of the pulp is abnormal when the mass becomes excessively slimy and "waterlogged" and the temperature does not rise in the normal way. He further observed that beans from such fermentation are difficult to dry and have a high proportion of cheesy texture and purple cotyledons. Quesnel (1972), noted that slimy fermentation is caused by forced ripening and rain-soaked cocoa. Beans, which were judged as insufficiently fermented, gave deep purple colour to the cotyledon after drying (Rohan, 1957a) which are characteristically hard and tough textured with the cotyledon not separated from the texture (Urquhart, 1961). Urquhart has observed that unfermented beans could be avoided by harvesting only ripe and slightly over ripe pods, discarding disease pods, turning the heap at 48 hourly intervals.

Method of fermentation

In Ghana, farmers traditionally cover their fermenting mass with plantain and banana leaves (Anim-Kwapong *et al.*, 2006). Aneani and Asamoah (2004) also reported that cocoa farmers in Ghana generally practiced the traditional heap-on-the-ground method of fermentation.

The heap method is the most popular method of fermenting cocoa on peasant farms, as it requires the simplest equipment at practically no cost (Rohan, 1963). He however, reported that box, basket and tray methods of fermentation are carried out at Cocoa Research Institute of Ghana. Knapp (1937) reported earlier that heap and box fermentation are the most commonly used methods in Ghana. According to Wood and Lass (1985), heap method of fermentation is generally used throughout West Africa but almost exclusively in Ghana where farmers are recommended to ferment their beans in heaps for six days, turning after two and four days.

Microbial fermentation of cocoa

Rombouts, (1952); Ostovar and Keeney, (1973) and Schwan *et al.*, (1986) reported that fermentation of cocoa is accomplished by a succession of microorganisms in four phases involving over 50 species. Phase I is dominated by yeasts, phase II by lactic acid bacteria, phase III, by acetic acid bacteria and phase IV, by *Bacillus* species

The major role of microorganisms is to produce acids and alcohols that will penetrate the testa and start the chemical reactions that will form the precursors of chocolate flavour (Schwan and Wheal, 2004). There is no evidence that enzymes from the microorganisms penetrate the testa and create flavour compounds. Hydrolytic enzymes inside the beans are activated by microbial metabolites such as acetic acid (Biehl *et al.* 1996; Biehl *et al.* 1993 and Voigt *et al* 1994).

Yeasts are the most effective microorganisms in ferments (Chick, 1980) and to be effective, their population should be high. The yeasts conduct an alcoholic fermentation and this involves the growth of *Kloeckera* and its teleomorphic form *Hanseniaspora*, *Saccharomyces*, *Candida*, *Pichia* and *Kluyveromyces* species. Some of the yeasts, including Candida spp. and Pichia spp., metabolize citric acid causing the pH value to increase from 3.5 to 4.2 in the pulp (Schwan and wheal, 2004). This allows for the growth of bacteria, conversion of sucrose, glucose and fructose to ethanol and CO_2 under low-oxygen and high- sugar conditions, which is eventually consumed oxidatively (Schwan *et al.*, 1995). Yeasts also produce organic acids (acetic, oxalic, phosphoric, succinic and malic) and some volatile organic compounds (Schwan and wheal, 2004). This may either contribute to the development of chocolate flavour or precursors of chocolate flavour.

Lactic acid bacteria ferment pulp sugar and utilize citric acid (Ardhana and Fleet, 2003). These involve the growth of *Lactobacillus*, *Leuconostoc* and *Lactococcus* species. Acetic acid bacteria (*Acetobacter and Gluconobacter spps.)* eventually grow and then oxidize ethanol initially produced by yeasts to acetic acid. Bacillus (*Bacillus subtilis* and *Bacillus licheniformis)* finally develop when the pH of the bean mass becomes less acidic and its temperature increases to 40-50°C (Carr and Davies 1980) due to the heat generated by the total process.

Schwan and Rose (1994) also reported that *Kloeckera apiculata* and *Saccharomyces cerevisiae var chevalieri* were the major producers of volatiles

such as isopropyl acetate, ethyl acetate, methanol, 1-propanol, alcohol, 2,3-butanediol, diethyl succinate, and 2-phenylethanol. Among the yeasts with high fermentative power is *Saccharomyces cerevisiae var.chevalieri*, which produces large amounts of aroma compounds. This suggests that these strains might be collaborating in the elaboration of aroma and flavour characteristics in cocoa (Schwan and Rose, 1994). Yeasts also produce pectinolytic enzymes (Rombouts, 1952; Gauthier *et al.* 1977; Sanchez *et al*, 1984). These break down the cement between the walls of the pulp cells and the resultant juice (or "cacao honey") drain away as 'sweatings'. The collapse of the parenchyma cells in the pulp between beans results in the formation of void spaces into which air percolates. Schwan *et al.* (1996) also reported that only four out of twelve yeast species showed pectinolytic activity. These are: *Kluyveromyces marxianus*, *Saccharomyces cerevisiae var chevalieri*, *Candida regopelliculosa* and *Kluyveromyces thermotolerans*. *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* showed substantial activities and only *Kluyveromyces marxianus* produced large quantities of heat stable endo- polygalacturonase. These enzymes have strong maceration activity, which reduced cocoa pulp viscosity during the first 36 hours of fermentation (Schwan *et al* 1996, Buamah et al., 1997)

More than 30 different species of bacteria have been isolated from fermentations (Carr *et al*, 1979; Ostovar *et al*, 1973; Passos *et al*, 1984; Passos *et al*, 1985; Schwan *et al*, 1986). The great majority of lactic acid bacteria utilize glucose via the Embden-Meyerhof-Parnas pathway, yielding more than 85% lactic acid. However, some species utilize glucose via the hexose

monophosphate shunt, forming 50% lactic acid, as well as combinations of ethanol, acetic acid, glycerol, mannitol, and carbon dioxide. Acetic acid bacteria are responsible for oxidation of ethanol to acetic acid and the oxidation of acetic acid to carbon dioxide and water. The exothermic reactions of acetic acid bacteria raise the temperature of the fermenting mass (Forsyth and Quesnel 1963). The acidity of cocoa bean preparations, the high temperatures in the fermenting masses, and the diffusion and hydrolysis of protein in the cotyledons has been attributed to the metabolism of these microorganisms (Forsyth and Quesnel 1963). Aerobic spore-forming bacteria, such as *Bacillus* strains, produce a variety of chemical compounds, including 2,3-butanediol, pyrazines, acetic acid, and lactic acid, under fermentative conditions, which may contribute to the acidity and perhaps, at times, to off-flavours of fermented cocoa beans (Schwan *et al*, 1986).

Duration of cocoa fermentation and occurrence of purple beans

The duration of fermentation depends on the cocoa variety and can last from 1.5 to 10 days (Forsyth and Quesnel, 1957). There are wide variations in durations of fermentation throughout the cocoa-growing world, even for cocoa of similar types. According to Forsyth and Quesnel (1957) the periods for fermenting cocoa is related to the amount of purple pigment present in the fresh beans. They went on to state that the deeper the colour, the longer should be the fermentation. *Criollo* beans which have little or no pigment, are fermented for much shorter periods than the purple *Forastero* types. It is however not exactly

known the extent to which the colour alone has influenced the choice of fermentation period (Duthie, 1937). Bellefroid (1935) reported that the size of the beans influences fermentation duration and that thin Venezuelan cocoa beans ferment more quickly than the thicker *Forastero* types.

Palma (1951), also suggested that duration of fermentation is dependent on the size, variety and season. Forsyth and Quesnel (1956) established a bimodal frequency histogram with maxima at two to three days and six to eight days corresponding to the *Criollo* and *Forastero*-type fermentations. As a result of these differences, it was recommended that mixed fermentation should be avoided (Wood and Lass, 1985). Rohan (1957a, 1958a, 1958b) reported that West Africa *Amelonado* could be fermented in shorter periods than was normally recommended without any adverse effect on quality. Clapperton *et al.* (1994) reported that three days fermented beans have marginal increases in bean flavour, an indication of under fermentation but five and seven days fermentations showed no appreciable differences in flavour. In Costa Rica fermentation period is not more than three days and are generally put out to dry after only one day in order to take advantage of any sun (Wood 1957a). According to Wood, the necessary care should be taken to ensure that the interval between pod breaking and the beginning of fermentation is not more than 48 hours. Mossu (1992) reported that the duration of fermentation depends on genetic structure of the selection, climate, and volume of the mass of cocoa undergoing fermentation and the method of fermentation.

Fermentation is arrested when the beans swell, certain odour is developed, the cotyledons turn brown and the temperature falls. All these occur on average after four to six days for the *Forastero* and *Trinitario* type of cocoa and two to three days for the *Criollo* type (Mossu, 1992; Schwan, 1986).

In Brazil, fermentation period is between three and six days but beans are not usually covered. In Venezuela, duration of fermentation is two or three days in wooden boxes. According to Palma (1951) majority of farmers still ferment for only three days, and this is probably a legacy from the time Venezuela cocoa was *Criollo* variety. He reported that Venezuela farmers still keep to three-day fermentation when *Forastero* was introduced leading to under-fermentation.

Hammond (1953) reported that some farmers in Ghana held the view that in wet weather, the period of fermentation should be less, and it is also believed that the length of fermentation should be varied according to the time lag between harvesting and breaking the pods. According to Aneani and Asamoah (2004), fermentation duration in Ghana is five and half days on average with a range of four and seven days, which depend on weather and condition of harvested pods. Inadequate sunshine during the rainy season prompts four days fermentation whilst some farmers extend their fermentation period to even seven days during the dry season. Beans derived from fresh harvested pods are fermented for six to seven days whereas those from exclusively ripe pods go for four days (Aneani and Asamoah, 2004).

Drying of cocoa and occurrence of purple beans

The drying process is a continuation of oxidative stage of fermentation and plays an important role in reducing bitterness and astringency, and developing the chocolate brown colour of well-fermented beans (Wood and Lass, 1985).

According to FAO (1969), moisture content of dried beans should not exceed 7.5%. For subsequent storage and transport, moisture contents over 8% are undesirable as this could lead to mould development inside the beans.

In Ghana, farmers sun dry the beans on mats raised off the ground. 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. According to Wood and Lass (1985) how long it takes to dry in the sun depends obviously on the weather. It is unusual for drying to be completed in less than a week, but during dull weather, the period may extend to two weeks or more. Sun drying is also the most popular method used by Malaysian smallholders to dry cocoa beans (Mahmod, 1999).

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

Wood and Lass (1985) also reported that the rate of drying has an important bearing on the flavour and quality of the dried beans. If drying is too slow, there is the danger that moulds will develop and penetrate the testa. There is also the danger that off-flavours may arise (Wood and Lass, 1985). On the other hand, rapid drying may prevent the oxidative changes being completed and may result in excessive acidity. According to Howat *et al.* (1957a), West African Amelonado cocoa was dried in 14 hours and chocolate manufactured from it showed no consistent difference in flavour from sun-dried beans. Jacquet *et al.* (1980), reported that increase in drying temperature increased astringency and acidity and recommended that drying temperature should not exceed 65-70°C.

The browning of the cotyledons is the most important aspect of drying and is associated with the oxidation of polyphenols by a polyphenol- oxidase system known to be active only in the presence of oxygen (Rohan, 1963). Kim and Keeney (1984) and Bonvehi and Coll (2000) reported that the amount of polyphenols is substantially reduced by enzymatic browning during drying. Some of the changes, which occur during fermentation, continue into the drying phase. Rohan (1958b), for example, has demonstrated that, on early removal of beans from fermenting mass, anthocyanins destruction continues actively. According to Forsyth (1952a), fresh beans, which were minced in air, turn brown very rapidly and polyphenols were completely destroyed within one hour and therefore must be turned during drying for good aeration.

Pettipher (1985) have observed that drying could reduce an existing anthocyanin content of the beans by 13-44% depending on the time of fermentation. He also observed that drying unfermented beans (day zero) resulted in a 79% loss of anthocyanin whereas fermenting for four days followed by drying resulted in a 97% loss of anthocyanins. Subsequently, there is reduction in purpleness of the dry beans by polyphenoloxidase systems, which is active only in the presence of oxygen (Pettipher, 1985).

Roelofsen and Giesberger (1947), suggested that since sections of beans exposed to air brown rapidly, absence of browning and the presence of purple in dried beans could be attributed to lack of oxygen during the period prior to inactivation of the polyphenoloxidase by dehydration. Griffiths (1957) has also shown that (-) epicatechin is the major substrate of polyphenol-oxidase. This is primarily responsible for the browning observed during drying. Leucocyanidins contributed to a very limited extent and practically no contribution from anthocyanins pigments.

Roelofsen (1958) reported a relationship between browning and the duration of fermentation. The longer the beans have been fermented the more complete is the browning during drying. There is, however, no evidence that flavour improves with increased browning, but full flavour and brown nibs usually occur simultaneously. According to Forsyth and Quesnel (1963), astringency in raw cocoa is due to the tannin effect of mono- and oligomeric polyhydroxyphenols, which are mainly flavonoids, leucocyanidin, catechin and

anthocyanin. These compounds are modified to some extent during fermentation in absence of oxygen.

Nib pH of fermented cocoa beans

The degree of acidity of a solution is a property of every aqueous solution that is important in all biological systems. This property is measured as the pH (Potential of Hydrogen) of the solution (Nester, *et al.*, 1995).

The quality of cocoa depends largely on its final pH since the formation of cocoa specific aroma precursors is strongly dependent on the degree of acidification of the nib during fermentation (Biehl *et al.*, 1982, 1985).

The pH for fresh cocoa beans and shells were found to be 6.5 and 3.6 respectively and these reached 5.3 and 5.9 after fermentation and drying (Takrama and Aculey, 2001). Cocoa with high cocoa specific aroma is obtained from only fermented cocoa, which results in moderate nib acidification of pH5.0-5.5. Acidification giving pH of 4.0-4.5 results in raw cocoa with low cocoa specific aroma potential (Biehl *et al.*, 1985), and therefore low quality cocoa products.

Kuebutornye *et al.* (2003), reported that fermentation may be deemed successful if nib pH reaches 5.3 and total acidity is about 0.98mEq of acetic acid regardless of cultivar. Jinap and Dimick, (1991, 1994); Lopez, (1983) also reported that preferred pH of dried beans is 5.2-5.5, and that value of pH more than 6 indicates that the beans are not well fermented and produces purple beans.

Fermentation Index (FI) of cocoa beans

Fermentation index is a measure of the degree of fermentation of cocoa beans. In fresh unfermented cocoa bean, the peak absorbance by the pigments occurs at 460nm wavelength while in the fermented cocoa bean, it occurs at 530nm. A ratio of absorbance at these two wavelengths thus reflects the degree of fermentability of the cocoa bean, which is referred to as Fermentation Index. According to Kuebutornye *et al.* (2003), cocoa is considered under- fermented when it yields a Fermentation Index (F.I.) of less than 0.80 and values above this are considered fully fermented. Jinap *et al* (2000), also reported that fermentation index greater than one is an indication for a well fermented cocoa beans.

Cocoa polyphenols and purple beans

Cocoa is rich in polyphenols. The main polyphenols found in cocoa beans are catechin, procyanidin, anthocyanin and flavonol glycoside (Zumbe, 1998). In unfermented cocoa beans, pigment cells make up about 11-13% of the tissue (Mossu, 1992). The pigment cells contain approximately 65-70% polyphenols and 3% anthocynins by weight.

In cocoa, unchanged anthocyanin pigments (cyanidin-3-galactoside and cyanidin-3-arabinoside) are responsible for most of the purple colour of fresh Forastero cocoa beans (Tomlins, 1993; Forsyth and Quesnel 1957a). These pigments, according to Forsyth and Quesnel (1957a), constitute 0.5% of the fat-free bean and 1.7% of the total phenolic content. In general, cocoa with more

than 20% residual anthocyanin was too harsh to be acceptable; while samples with less than 10% were acceptable and intermediate samples of 10-20% were variable (Kenten, 1965). A change in flavour is associated directly or indirectly with this change in colour and beans with 30% or more unchanged anthocyanin have a deep purple colour and chocolate made from them would have a harsh and bitter taste (Rohan, 1963).

During cocoa fermentation, polyphenols are subjected to biochemical modifications. Thus, anthocyanins are hydrolysed during fermentation and changed to colourless leuco-anthocyanin (Rohan, 1963; Kim and Keeney, 1984; Bonvehi and Coll, 2000). According to Zumbe (1998), approximately 20% of the polyphenols (by weight) remains at the end of the fermentation process. Total polyphenol content by weight in dry cocoa beans is estimated to be six to eight percent (Zumbe, 1998). The level of polyphenols will vary with the variety of cocoa bean and with degree of fermentation. Among the polyphenols, catechins are colourless, while the anthocyanins give rise to the purple colour of unfermented beans. According to Keasrley and Rodriguez (1981) the amount of anthocyanins is temperature dependent. They also stated that the rate of absorbance is lowest at room temperature of 20°C and that at 80°C most anthocyanins are released from the vacuole where they are stored. The high temperature disrupts the phospholipids bilayers. Enzymes and cells then become denatured because the active site changes shape hence the substrate will no longer fit the enzyme. At room temperatures however, the cells maintain strong and stable bonds so the anthocyanins cannot escape from the cell. Bergqvist *et al*

(2001) also established a correlation in high temperatures with a fall in anthocyanins levels. They further stated that at temperatures above 37°C, sugar accumulation is inhibited and the formation of anthocyanins is limited either through degradation or the inhibition of synthesis or both (Spayd *et al* 2002). Some farmers in Ghana refer to the purple colour in the dried cotyledon as "cocoa red" or "-cocoa purple" or "kokoo bisi" (Personal communication).

Laycock (1930) concluded from a study of fermentation of cocoa produced in Nigeria that the *Forastero* type cannot be prepared free from purple unless the beans are much matured and the fermentation unusually prolonged. Since over ripe beans usually germinate and over fermented beans results in putrifaction and off-flavour, there is possibly no practicable method of fermentation which will prevent the incidence of some purple beans in the product. According to Anon (1968), it is not possible to prepare a sample with 100 per cent fully fermented beans, nor is it desirable to attempt to do so.

Schutt-im-Hofe (1913) made controlled organoleptic assessments of samples in cocoa in various stages of oxidation and concluded that if the oxidation process was prolonged until all the beans had turned brown, the taste was less full and there were fewer aromas than in samples containing some of the reddish-violet beans mixed with the brown. Brown (1957) reported that fruity component essential to the West Africa type flavour is associated with partly or under fermented beans. He further observed that the presence of pale purple beans is likely to affect quality of the cocoa product. According to Brown (1957), the presence of pale purple beans is a safeguard against over-

fermentation, which generally results in loss of cocoa flavour and development of off-flavours.

Quesnel (1958) noted that purple beans constitute a serious defect of cured cocoa. The elimination of purple beans is therefore important to the chocolate manufacturer. According to FAO reports (FAO, 1961), mouldy, slaty and fully purple beans, in order of seriousness are the most objectionable categories of defective beans because their effects on the quality of the finish product are more serious than those of other defects.

Grading standards for purple beans

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 inadequately 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 (Anon, 1968). The fully fermented beans are brown in colour with the convolutions 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. Beans described as 'partly brown/ partly purple' are not defective and should be present at least to the extent of 20 per cent. A proportion of 30-40 per cent is acceptable but samples with more than 50 per cent have probably been inadequately fermented for some reason and may give rise to bitter and astringent flavour (Anon, 1968).

Grading standards permitted in Brazil and Malaysia for purple beans are 25 per cent and 30 per cent respectively. Powell (1981) also recommended that 50% or more of beans with chocolate brown cotyledons together with 20% or more of partly brown and partly purple beans should dominate a cut test analysis in order to obtain good flavour. He opposed the presence of slaty and fully purple beans as they form the basis of poor flavour. In Ghana, the revised grading standards for purple beans are: grade I (for purple beans counts up to 20 per cent), grade II (for purple beans counts between 20.3-30.0%), and grade II* (for purple beans counts between 30.3-45.0%) and purple beans counts above 45% are sub standard (Q.C.D., 2005).

Commercially, the degree of fermentation of cocoa beans is assessed by the cut test in which 100 beans are cut and the colours of the cotyledons are recorded (Wood, 1975). International Cocoa Standard is based on the cut test, which facilitates the detection of certain gross quality defects. According to Dand (1993) the cut test has an inherent limitation of totally dependent on the sight of the analyst, which cannot be used as a standard for such measurements. Cocoa of merchantable quality should be well fermented, thoroughly dry, and free from smoky beans, abnormal or foreign odour and from any evidence of adulteration. It must also be free from living insects and reasonably from free broken beans, fragments and pieces of shell and thus virtually free from foreign matter. Its size must be uniform in the sense that not more than 12% of the beans should fall beyond the range of ± 0.33 of the average weight (BCCCA, 1996). The Cocoa Standard Authority, The Ministry of Food and Agriculture in Ghana

and the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA) grade cocoa beans as Grade I if the bulk contains, 3% mouldy (cocoa bean on the internal parts of which mould is visible to the naked eye), 3% slaty (a cocoa bean, which shows a slaty colour on half or more of the surface exposed by a cut made lengthwise through the center), 3% other defects (insect- infested, thus, a cocoa bean, the internal parts of which are found to contain insects at any stage of development or to show signs of damage caused which are visible to the naked eye, and including germinated and flat beans), 7.5% moisture and 0 foreign matter. Grade II must contain not more than 4% mouldy, 8% slaty, 6% insect-infested (including germinated and flat beans), 7.5% moisture and 0 foreign matters (Dand, 1993; BCCCA, 1996).

CHAPTER THREE

MATERIALS AND METHODS

The studies conducted on the occurrence of purple beans in cocoa produced in Ghana were in two parts namely, a questionnaire survey and laboratory investigations.

Part one: Field survey

Introduction

Part one of the studies was a questionnaire survey conducted in 19 randomly selected districts from the six cocoa growing regions of Ghana, namely: Ashanti, Brong Ahafo, Central, Eastern, Western (North and South) and Volta regions (Fig 1). For easy administration and monitoring, COCOBOB has divided Western region into two sections as North and South.

The main objective of the preliminary survey was to validate the occurrence of purple beans in cocoa produced in Ghana. It was also to find out the knowledge of the farmer in post harvest processing of cocoa beans over the years. The survey was also meant to find out the views of the Farmer, Grading Staff (GS) of Quality Control Division (COCOBOD) and Purchasing Clerks (PCs) of Licensed Buying Companies (LBCs) on the possible causes of the purple beans. The findings were to form the basis for the laboratory investigations.

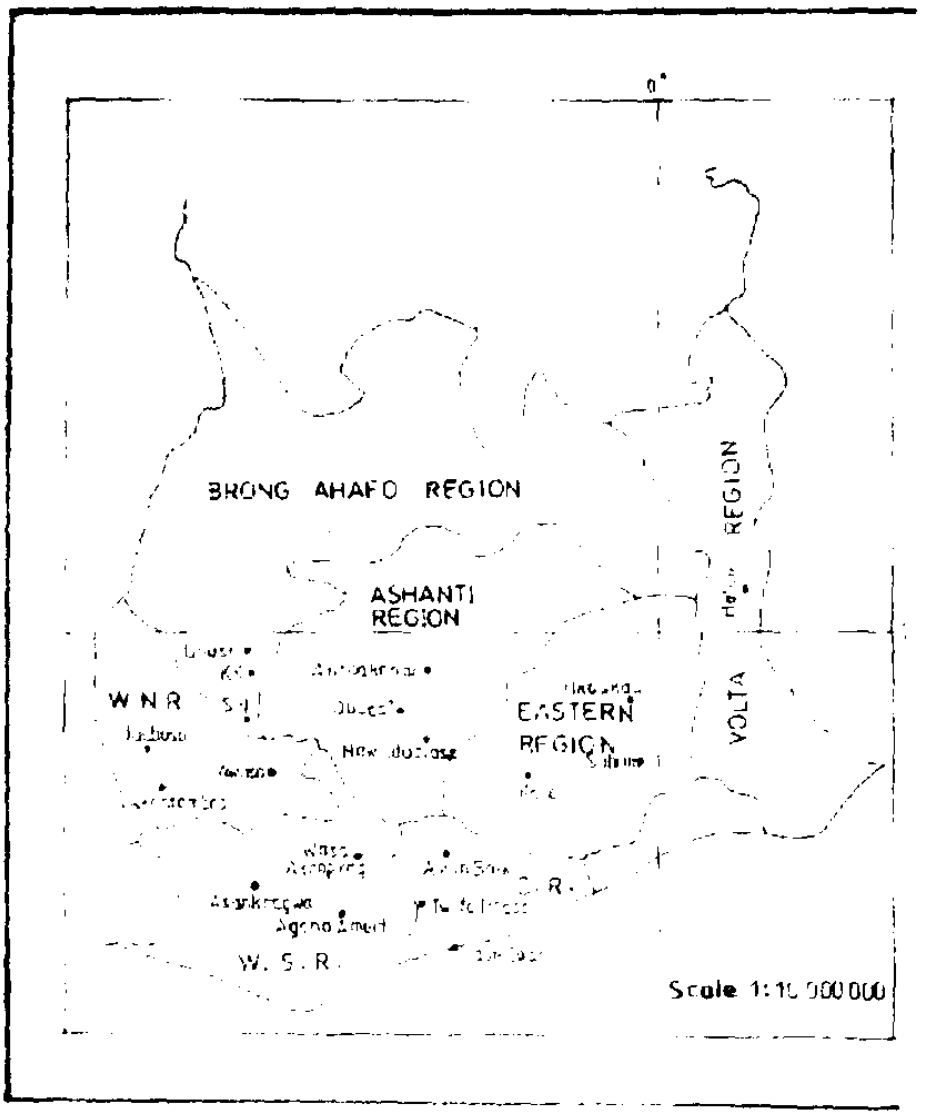

Fig. 1: Map of Ghana showing cocoa districts surveyed from six cocoa growing regions

Keys: SN = Sankore **KK** = Kukuom **WSR** = Western south region

WNR = Western north region **C R** = Central region

Questionnaire survey

A validated pre-tested questionnaire (Appendix 1) was administered to farmers, Grading Staff of the Quality Control Division (COCOBOD) and Purchasing Clerks of Licensed Buying Companies (LBCs) from all six cocoa growing regions of Ghana from August 9, 2005 to January 20, 2006. Three districts were randomly selected from each of the six cocoa growing regions except Volta where the only district was selected.

The questionnaire for farmers was divided into seven sections viz, biodata about the farmers, cocoa types grown, source of seedlings, observed differences in ripe cocoa pod content, harvesting, fermentation and drying, assessment of beans quality, purple beans and its possible causes. One hundred and seventy (170) farmers, 56 Grading Staff of the Quality Control Division (COCOBOD) and 91 Purchasing Clerks were selected and interviewed. Questionnaires for Grading Staff and Purchasing Clerks were divided into three sections viz, biodata about staff interviewed, beans quality assessment as well as purple beans and its possible causes. Farmers, Grading Staff and Purchasing Clerks were interviewed using guided questionnaire. Findings from the Grading staff and the Purchasing Clerks were meant to complement that of the Farmers.

Technique for sample selection

The snowball sampling technique was used since the exact number of farmers from each district could not be quantified. By this technique, a farmer was identified and used as an informant to identify other cocoa farmers and

those identified in turn identify yet others. Purchasing Clerks and some Chief Farmers from the selected districts also assisted in identifying the farmers. Grading Staff and Purchasing Clerks at post during the time of the survey were interviewed.

Sampling of dry cocoa beans from jute sacks in the cocoa growing regions of Ghana

Dry cocoa beans were removed from jute sacks packed in lots of 30 bags. The beans were removed from the sides, front and back of each bag by a stabbed metal horn. The beans horned were bulked and mixed thoroughly and quartered. One opposite quarter was rejected and the process repeated until a final sample of slightly more than 300 beans were obtained.

Moisture content determination of dry cocoa beans

A K.P. Mundinger GmbH KAM III 005677 cocoa moisture meter (AquaBoy) model, which is a high quality electronic moisture-measuring instrument, was used for moisture determination. The cup electrode of the moisture meter was two third filled with the bulked samples (10 beans) and the moisture value read from the meter scale.

The oven method was also used to standardize the AquaBoy readings. Thus, 10 beans of the dry commercial cocoa beans were crushed roughly in a mortar with the pestle within a minute to obtain the greatest dimension of particles not exceeding five millimeters, while avoiding the formation of a paste.

Ten grams of the crushed beans were weighed to the nearest 0.0002g and poured into dry petri dishes of known weights and quickly cooled in a desiccator for an hour. The dishes with lids containing the test samples were oven dried at 103°C in a Gallenkamp Hotbox oven (ov-160 model) without their lids for 72 hours. The dishes with the oven dried test samples were covered with their lids and quickly transferred into a desiccator until they were cooled to ambient temperature and then weighed again to the nearest 0.0002g. The moisture content of the samples was expressed as the percentage loss in mass.

Cut test

The cut test which is a cocoa grading scheme based on visual assessment of quality of cocoa and which relies on changes in colour of the beans is the standard test used to assess the suitability of cocoa beans for making chocolate. Points were given for bean dimension, colour, odour and the absence of imperfect beans. The procedure involved filling three equal sized white calico clothed sampling bags ($5.7dm^3$) with well-mixed beans. The mixed beans were quartered leaving a heap of slightly more than 300 beans, which were used to fill the sampling bags. Each sampling bag thus contained slightly hundred beans and was cut length-wise through the middle to expose the internal surface of the two cotyledons (Plate 2).

Plate 2: Cutting of dry cocoa beans to expose the internal surfaces of the two cotyledons

The cut beans were examined in good daylight and the percentage total purple (deep, pale and partly brown/partly purple) beans were determined and recorded. Percentages of other defective categories (mouldy, slaty, insect infested, flat and germinated beans) were also determined (Appendix 7).

Data analysis

The data gathered were coded and analyzed using descriptive statistics of the Statistical Package for Social Sciences now known as Statistical Product and Service Solutions (SPSS) software (**SPSS version 10.0).**

The descriptive statistics used were frequencies, percentages, means and standard deviations. MSTATC computer software was used for analysis of variance (ANOVA) and means separation of percentage purple beans occurrence of cocoa growing regions of Ghana.

Part two: Laboratory investigations

Studies in part two consisted of five experiments. Completely Randomized Design (CRD) was used with three replications.

Experiment one: Effect of fermentation periods on the degree of fermentation

Introduction

The objective was to determine the effect of different fermentation durations on the quality of cocoa. Data collected included: Fermentation Index (FI), nib pH, total anthocyanins and purple beans by count per 100 beans from cut test analysis.

Cocoa fermentation

Beans from mixed hybrids of cocoa from Cocoa Research Institute of Ghana (CRIG) plantation were used. The pods of the mixed hybrids were harvested within a period of four days and were split opened. The beans, which were separated from the placenta, were scooped from the pods and mixed thoroughly. A 100kg wet beans from the mixture were heaped on perforated banana leaves arranged on wooden poles in triplicates for free drainage and aeration. The heaps were then carefully covered with banana leaves, which were held in place with wooden poles. (Plate 3).

Fermentations were conducted under a raised shed of 2.5m high with a roof of tidal mesh, in the open. The wet beans were fermented for 168 hours with two turnings after 48 and 96 hours of fermentation. One kilogram wet beans were sampled at 30cm deep from each heap at 0, 24, 48, 72, 96, 120, 144, and 168 hour intervals. Samples of wet beans of weight 0.1kg were depulped by placing the beans in dry sawdust and robbed with the sawdust until all the pulps were removed. The beans were then cleaned of the sawdust and traces of moisture with clean white calico cloth and immediately oven dried for 168 hours at 50°C. The Gallenkamp Hotbox oven (ov-160 model) was used to dry the beans. After oven drying to a moisture content of 6.8%, the beans were kept in desiccators filled with sufficient desiccants for four hours before packing them in plastic rubber bags, sealed and stored in a refrigerator at -34.6°C. The heaps were later broken after 168 hours of fermentation and the beans sun dried.

Plate 3: Heap method of cocoa beans fermentation

Fermentation index determination

Fermentation index was determined using the method of Gur'eva and Tserevitinov (1979) detailed by Bakri *el al.*, (1994). Thus, 10g of the dry sample beans were collected after each of the fermentation periods and peeled and finely ground in a mortar with a pestle, and 0.1g weighed on an analytical balance (Stanton 462) into test tubes in triplicates. Ten millilitres of acidified methanol (i.e. 97:3 mixture of Methanol: HCl) were added and then homogenized on a vortex shaker for a thorough mixture. The homogenate was then allowed to stand in a cold room at 4°C overnight (20 hrs). Absorbance readings at 460nm and 530nm were taken using Cecil CE 7400 Spectrophotometer and 10mm curvette. The fermentation indices were calculated by the formula:

A460 nm/A530 nm (Gur'eva and Tserevitinov, 1979).

Where, **A** indicates Absorbance.

pH determination

The method of the 'Office International du Cacao et du Chocolat (1972)' was used to determine the nib pH. The testa and cotyledon of 10 beans from bulked samples were separated with a scalpel. The cotyledons were finely ground using mortar and pestle. Ten grammes each were weighed to the nearest 0.01g into 150ml conical flasks and 90ml of boiling water was added while being stirred and allowed to cool in an air-conditioned room. The stirring

continued to about 22°C. The pH of each sample was determined using Jenway Model glass electrode pH meter, Model 3310.

Total anthocyanin determination

A method developed by Misnawi *et al* (2002), was used to extract total anthocyanins. Ten beans each of the oven-dried samples of 0, 1, 2, 3, 4, 5, 6 and 7-day fermentations stored at -34.6°C in the freezer were selected at random and carefully peeled and ground using a pestle and a mortar into a powder. The powdered samples were defatted using Soxhlet apparatus. 10g of each powdered sample were weighed to the nearest 0.001g approximately into extraction thimbles. The thimbles were then sealed off with a solid cotton wool and placed into Soxhlet extractor. Six 250ml Erlenmeyer flask were filled with petroleum spirit (bp 60-80°C) and placed on a heating apparatus. The Soxhlet extractor and sealed thimbles were mounted on the Erlenmeyer flask (Plate 4). The petroleum spirit in the Erlenmeyer flask was heated to its boiling point to extract fat from the powdered cocoa for 8hr with at least ten siphoning per hour.

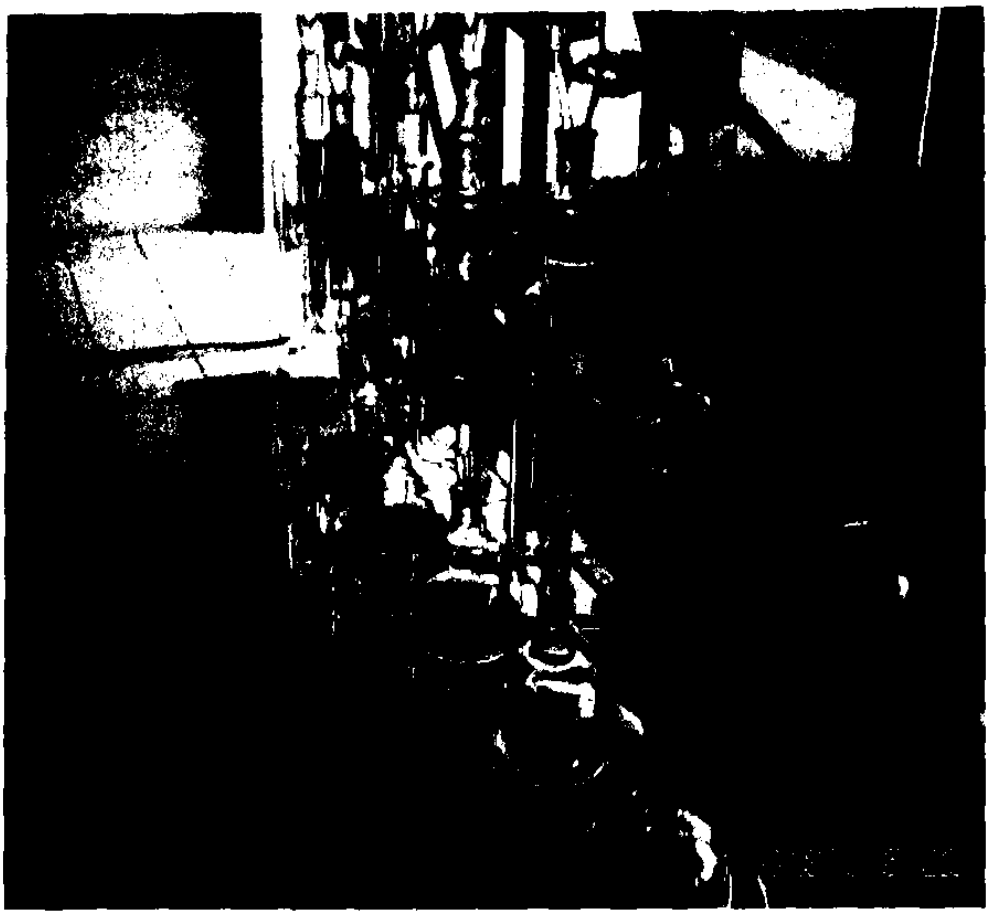

Plate 4: Soxhlet extractor used to defat the powder of cocoa cultivars

The defatted samples were dried in a Gallenkamp Hotbox oven, model ov 160 at 50°C for 16hr. The dried powders were ground again and anthocyanins extracted from 0.25g of each defatted sample. Thus, 0.25g of the defatted samples were weighed into test tubes in triplicate. The weighed samples were suspended in 12.5ml of 10 mM acidified (pH4.5) sodium acetate buffers for one hour. The suspensions were then incubated at 45°C in a shaking water bath at 160rpm.

After incubation, 12.5 ml of 0.2MHCl was added. Hydrochloric acid was used to extract the anthocyanins from cocoa beans as preliminary investigations had shown that it was more effective than water and these pigments were unstable in

neutral or alkaline solutions. The suspensions were then allowed to cool to room temperature. The mixtures obtained from incubations were filtered using Whatman #4 filter paper and the supernatants read spectrophotometrically for total absorbance (TOD) at 535nm on Cecil CE 7400 spectrophotometer

The content of total anthocyanins was calculated using Misnawi *et al.* (2002) formula as follows:

$$\textbf{Total anthocyanins (mg/kg)} = \frac{\textbf{TOD}}{(A_v \epsilon_{535})^{1\%}_{1cm/10}} \times \frac{1000}{1}$$

Where,

TOD = Total Optical Density (absorbance)

$(A_v \epsilon_{535})^{1\%}_{1cm}$ = Average extinction coefficient for total anthocyanins when 1 cm cuvette and 1% (10mg/ml) standards are used, the value is 982.

Experiment two: Effects of pod storage periods on occurrence of purple beans

Well-riped pods of mixed hybrid cocoa were harvested from cocoa plantations at Cocoa Research Institute of Ghana. The pods were harvested continuously for four days and were all broken on the fifth day. The pods harvested on the first day were stored for four days and those harvested on the

second, third, and fourth days being stored for three, two and one day respectively before breaking.

A 100kg heaps each of wet beans were made from pods stored for one, two, three and four days. A fifth heap of 100kg was made by mixing 25kg of wet beans sampled from pods stored for one, two, three and four days and labeled as pods stored for five days to simulate on-farm situation.

The wet beans for each of the pod storage durations and the mixed heap were fermented for 168 hours with two turnings after 48 and 96 hours of fermentation. 500g of beans were drawn 30cm from each heap after 120,144 and 168 hours and sun dried for two weeks to a moisture content of about 6.8% before carrying out cut test analysis on the dry samples fermented for the 120, 144 and 168 hours.

Experiment three: Effects of cocoa cultivar on the occurrence of purple colour in dry cocoa beans

Selection and harvesting of cocoa cultivars

Different cocoa cultivars namely, *Amelonado* (selfed), *Criollo* (selfed), *Amazonia* (selfed), as well as twelve different hybrid selections were identified by the Plant Breeding Division of Cocoa Research Institute of Ghana and used for the study.

The twelve hybrid selections were: T85/799 x Amelonado, T85/799 x T79/501, T60/887 x Amelonado. T85/799 x Sca9, T85/799 x Pa 150, T60/887 x Ma12, T60/887 x ICS 6, T60/ 887 x IMC 60, T60/887 x Be 8, T60/887 x

Catongo, T60/887 x Pound 7 and T60/887 x Pound 10. The letter 'T' stands for Trinidad where these cultivars were introduced from and the numbers in front were sequence of pod numbering during breeding.

All the twelve hybrid selections as well as the *Amazonia* and *Criollo* were harvested from the Plant Breeding Division plots at CRIG-Tafo, while the *Amelonado* (selfed) pods were collected from Apedwa cocoa seed garden (an experimental area of CRIG) in the Eastern region. Thirty pods were harvested from three different randomly selected plots for each hybrid but from one plot each for the *Amelonado*, *Criollo* and *Amazonia* cocoa type because the pods were insufficient.

The pods were broken four days after harvesting. Beans from each cultivar were put in separate fermentation nets and were lined in four wooden fermentation trays. The trays measured 1.2 x 0.9m and 10cm deep and had a wet bean capacity of about 90kg (Plate 5). Four trays were filled with mixed hybrid beans to cover the nets in the trays and stacked together so that the filled sections lie directly above each other and the whole stack was placed on an empty tray to allow for better drainage and aeration. The top tray was covered with banana leaves and held in place by pieces of wood. The beans were fermented for 144 and 168 hours.

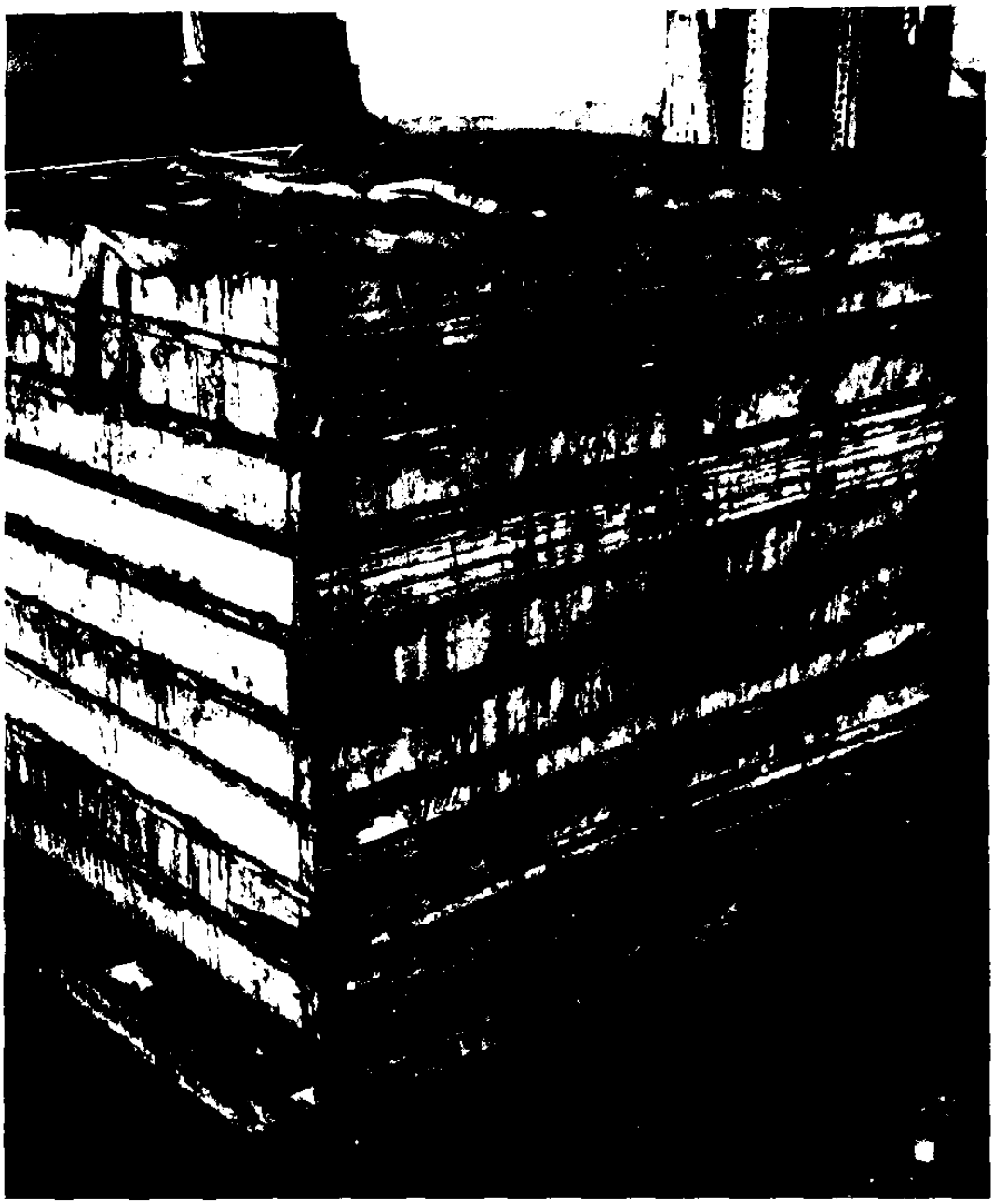

Plate 5: Tray method of cocoa fermentation

Hundred wet beans were drawn from each variety at day 0, 6 and 7. The beans were depulped and oven dried at 50°C and were allowed to cool before being stored in a freezer at -34.6°C and later used for total anthocyanins extraction.

Five hundred beans were also drawn from each variety fermented for six and seven days and sun-dried for two weeks to a moisture content of 6.8% and later used for purple beans count through cut test analysis (Table 16) and total anthocyanin determination. Means of purple beans counts and total anthocyanins in beans fermented for six and seven days were compared using t-test.

Total anthocyanin determination

The method developed by Misnawi *et al* (2002) as described earlier, was used to extract anthocyanins from all the selected cultivars. Total anthocyanins content was calculated for each cultivar and presented in (Table 15).

Percentage degradation and residual anthocyanins in the different cocoa cultivars (fig 4) after fermentation and drying were determined by the formula:

$$100 - \left[\frac{(ACY_u - ACY_f)}{ACY_f} \right] \times 100\%$$

where,

ACYu= Total anthocyanins in dry unfermented beans

ACYf= Total anthocyanins in dry fermented beans

Experiment four: Determination of total anthocyanin in different classes of purple colours and brown beans from different locations

Introduction

The objective was to determine total anthocyanins in different shades of purple colour (deep purple, pale purple and partly brown/ partly purple) in dry fermented beans with brown beans as control. This would serve as the basis for developing purple bean key as a standard for grading purple beans in Ghana.

Sample collection, defattening, total anthocyanin and pH determination

Dry cocoa samples were collected from different locations viz: CRIG, Suhum, Koforidua and Kade all in the Eastern region, Cape Coast (Central) and Sankore (Brong Ahafo) for cut test and purple colour shade determination.

The purple colours were classified as deep purple (Dp), pale purple (Pp), and partly brown/partly purple (Pb/Pp) and brown (B) (control) (Plates 6 a, b, c, d respectively). Beans which were brown in colour with convolutions of the cotyledons tending to separate when properly dry are classified as brown beans whereas partly brown/partly purple beans were those that show some evidence of blue or purple with brown patches and some fissures on the cut surface when properly dry. Pale purple beans refer to beans without any brown patches and fissures with the entire cut surface being blue or purple while deep purple beans were those in which the entire cut surface was blue or purple without any brown

patches and the cotyledons were pressed tightly together when the beans were properly dry. Each of these purple colour classes was defatted and total anthocyanin determined for each using the Misnawi *et al.* (2002) protocol as described in experiment 3. Total anthocyanin contents of the different classes of purple colour were compared (Table 17).

The pH for each purple colour class was determined using the method of the 'Office International du Cacao et du Chocolat (1972)' as described in experiment one and the results presented in Fig. 5.

Experiment five: Effects of climatic factors on purpleness of dry cocoa beans

A study of climatic effects on purple beans was carried out between the periods of February to August 2006.

A 100kg heap of wet cocoa bean was fermented for six days. The beans were carefully covered with sufficient plantain leaves to ensure good insulation and were thoroughly mixed after 48 and 96 hours. The fermented beans were sun dried for two weeks to a moisture content of about 6.8% using the K.P. Mundinger GmbH KAM III 005677 AquaBoy model.

Daily records on rainfall, relative humidity at 9am and 3pm, temperature maximum and minimum and sunshine hours were taken using rain guage, hygrometer, thermometer and sunshine meters respectively. Means of these climatic factors (rainfall, relative humidity, and temperature and sunshine hours) were determined at the end of each month and recorded.

Cut test analysis as described earlier was conducted on the dry sample beans for each month for purpleness, mouldy, slaty and other defects (viz: weevil, germinated and flat beans), (appendix 8). Purple bean counts (dependent variable) were correlated with climatic conditions (independent variables) within the period of February to August 2006, (Table 18).

Statistical analysis

MSTATC computer software was used for analysis of variance (ANOVA) and means separation. Microsoft Excel was also used to carry out regression and correlation analysis.

Experimental precautions

1. All glassware were thoroughly washed with liquid soap, rinsed in several changes of clean tap water and dried before use.
2. Separate Pasteur pipettes were used for each sample in reading absorbance at 460nm and 530nm.
3. Cuvette was thoroughly wiped of any liquid particle with soft tissue paper after loading with the supernatant before reading was done.

CHAPTER FOUR

RESULTS

PART 1

General observations

A survey of all the six cocoa growing regions in Ghana showed high occurrence of purple beans in processed cocoa with a national value of 32.3%. The survey also revealed that most farmers fermented their cocoa for periods of four to five days instead of six days recommended for the fermentation of cocoa in Ghana. Most farmers interviewed also "forced-ripe" the pods through cutlass wounds to induce early ripening. Some also mixed unripe pods harvested together with ripe ones before breaking them together for fermentation. In some cases, they harvested the matured but unripe pods together with the well ripe pods and fermented their beans together.

Farmers in all the cocoa growing regions generally used the heap method of fermentation. They rarely turned the beans in the heap during fermentation and those who did so turned it only once to remove placenta fermented together with the beans.

Large quantities of wet beans were found heaped on small sized drying mats in most cocoa growing areas especially in the heavy cocoa production regions in Ghana (Western North and South, Ashanti and Eastern).

Farmers stored pods for a shorter period after harvesting before pod breaking.

Cut test conducted on dry beans collected immediately from drying mats were purpler than those in beans stored for few weeks after drying.

Questionnaire Survey

Distribution of percentage of occurrence of purple beans in cocoa growing regions in Ghana for 2004/2005 crop season

Percentage of occurrence of purple beans in cocoa produced in Ghana in 2004/2005 cocoa crop season is presented in Table 1. The distribution of purple beans on regional basis indicated that the Volta region recorded the highest value of 38.7% followed by Western south (34.7%), Ashanti (33.0%), Western north (32.7%), Eastern (31.7%), Brong Ahafo (28.0%) and Central region being the least with 28.0%.

Purpleness of beans from Volta region was significantly higher than those from Brong Ahafo, Central and Eastern regions but not statistically significant from beans from Western south and north and Ashanti. From the table, the distribution of purple beans count in Ghana could be grouped into lower and upper purple beans zones with the lower zone comprising of Central, Brong Ahafo and Eastern regions with the upper zone made up of Volta, Ashanti, Western north and south regions.

Table 1: Distribution of percentage of occurrence of purple beans in cocoa growing regions in Ghana for 2004/2005 cocoa crop season

Region	Mean Purple Bean counts (%)
Ashanti	33.0ab
Brong Ahafo	28.0b
Central	28.0b
Eastern	31.7b
Western south	34.7ab
Western north	32.7ab
Volta	38.7a
Mean	32.3
CV (%)	10.99
SE	2.0561

Means bearing identical letters are not significantly different from each other DMRT at 5% level.

Farmer perception of the causes of purple beans

Table 2a shows results of the perception of the farmer of the possible causes of purple beans in cocoa produced in Ghana. From the table, it can be seen that out of the 170 farmers interviewed, 27.6% were of the view that the occurrence of purple beans was due to under-fermentation; and 21.9% indicated it could be due to a combination of under-fermentation and the use of seeds from unripe pods for fermentation. Some of the farmers (15.3%) attributed purpleness of the beans to the use of seeds from unripe pods for fermentation. Three and

half percent of the farmers interviewed were of the view that purpleness problem was due to the genetic basis of the variety. Another 3.5% attributed the purpleness of the beans to the combined influence of genetic factors and the use of seeds from unripe pods. A third 3.5% of farmers were of the view that purpleness of the beans was due to the combined use of seeds from unripe pods for fermentation; genetic factors; improper drying; and the effect of agrochemicals applied on the parent trees. It can be seen from the table that the farmers who indicated that purpleness was due to improper fermentation and genetic factors were 4.7%. Those who attributed the problem to improper fermentation together with drying were 8.8%. A section of the farmers (1.4%) interviewed attributed purpleness of the beans to improper fermentation and the application of agrochemicals to the parent trees (Table 2a). A group of the farmers constituting, 1.2%, attributed the purple problem to drying and the use of seeds from unripe pods for fermentation. Farmers who indicated improper drying and the application of agro-chemicals to the cocoa trees as sole factors contributing to the high purple beans problem were 2.4% and 2.9% respectively. Only 0.6% of the farmers indicated that genetic factors together with improper drying were possible causes of the high purple bean counts in the country. A group of farmers, 2.9%, attributed the incidence of the high purple beans problem to factors such as the use of seeds from diseased pods and seasonal effects (Table 2a).

Table 2a: Farmer perception of the causes of purple beans

Cause(s) of purple beans	Freq.	%
Fermentation	47	27.6
Genetic	6	3.5
Drying	4	2.4
Agro-chemicals	5	2.9
Unripe pods	26	15.3
Fermentation & Genetics	8	4.7
Fermentation & drying	15	8.8
Fermentation & Agro-chemicals	2	1.4
Fermentation & unripe pods	37	21.9
Genetic & drying	1	0.6
Genetic & agro-chemicals	0	0
Genetic & unripe pods	6	3.5
Drying & agro-chemicals	0	0
Drying & unripe pods	2	1.2
Agro-chemicals & unripe pods	0	0
Fermentation, drying, genetic, agro-chemical & unripe pods	6	3.5
Others	5	2.9
Total	170	100

The perception of Purchasing Clerks of the causes of purple beans

From Table 2b it can be seen that of the 91 Purchasing Clerks interviewed, 50.5%, indicated that improper fermentation alone was the possible cause of the high purple beans in cocoa produced in Ghana. Some Purchasing Clerks interviewed, 22.0%, observed that improper fermentation and the use of beans from unripe pods for fermentation were the causes of the purple problem

in Ghana while 3.3% of the Purchasing Clerks identified genetic factors as the cause of the purple problem. From Table 2b, 9.9% of the Purchasing Clerks indicated that genetic and improper fermentation were the possible causes of purpleness. The Purchasing Clerks who identified the effects of improper fermentation and drying as the causes of purpleness of the beans were 5.5%. The other possible causes of purpleness of the beans indicated by the purchasing clerks were: 4.4% stated that it was due to a combined effects of improper fermentation and application of agro- chemicals to cocoa trees; 1.1% attributed it to improper drying; another 1.1% stated that it was due to the use of beans from an unripe pods; and a third 1.1% said it was the result of the combined effects of improper fermentation, drying, genetic factors, application of agro-chemicals and the use of beans from unripe pods. Table 2b shows that 1.1% of the Purchasing Clerks attributed the occurrence of the high purple beans problem to factors such as the use of seeds from diseased pods and seasonal effects.

Table 2b: The perception of Purchasing Clerks of the causes of purple beans

Cause(s) of purple beans	Freq.	%
Fermentation	46	50.5
Genetic	3	3.3
Drying	1	1.1
Agro-chemicals	0	0
Unripe pods	1	1.1
Fermentation & Genetics	9	9.9
Fermentation & drying	5	5.5
Fermentation & Agro-chemicals	4	4.4
Fermentation & unripe pods	20	22.0
Genetic & drying	0	0
Genetic & agro-chemicals	0	0
Genetic & unripe pods	0	0
Drying & agro-chemicals	0	0
Drying & unripe pods	0	0
Agro-chemicals & unripe pods	0	0
Fermentation, drying, genetic, agro-chemical & unripe pods	1	1.1
Others	1	1.1
Total	91	100

The perception of Grading Staff of the causes of purple beans

In Table 2c, 33.9% out of the 56 of the grading staff indicated improper fermentation as the cause of the high purple bean counts in cocoa produced in Ghana. The grading staff who were of the view that the high occurrence of purple beans in processed cocoa was as a result of improper fermentation and the use of beans from unripe pods were 16.1%. Another set of the grading staff,

16.1% indicated genetic factors alone as a possible cause of the purple beans. Those who indicated improper fermentation and genetic factors were 5.4%. Twelve and half percent of the grading staff attributed purpleness in the dry beans to both improper fermentation and drying; genetic factors; application of agro- chemicals; and the use of beans from unripe pods for fermentation. Each of the following pairs of factors was indicated by 1.8% of the interviewed grading staff as causes of purpleness in the processed cocoa bean. The pairs of factors from Table 2c were: improper drying and the use of agro-chemicals; genetic factors and the use of agro-chemicals; and improper drying and the use of agro-chemicals. Some grading staff 3.6% indicated the possible causes of purpleness to each of the following pairs of factors: improper fermentation and application of agro-chemicals to the cocoa trees and genetic factors and use of beans from unripe pods for fermentation.

Table 2c: The perception of Grading Staff of the causes of purple beans

Cause(s) of purple beans	Freq.	%
Fermentation	19	33.9
Genetic	9	16.1
Drying	1	1.8
Agro-chemicals	1	1.8
Unripe pods	0	0
Fermentation & Genetics	3	5.4
Fermentation & drying	0	0
Fermentation & Agro-chemicals	2	.3.6
Fermentation & unripe pods	9	16.1
Genetic & drying	0	0
Genetic & agro-chemicals	1	1.8
Genetic & unripe pods	2	3.6
Drying & agro-chemicals	1	1.8
Drying & unripe pods	0	0
Agro-chemicals & unripe pods	0	0
Fermentation, drying, genetic, agro-chemical & unripe pods	7	12.5
Others	1	1.8
Total	56	100

Harvesting intervals of cocoa pods

The harvesting intervals used by farmers are presented in Table 3. Out of the170 farmers, 60.6% stated that they harvested their cocoa pods at three weeks intervals while 26.5% and 12.4% indicated two and four weeks intervals respectively. Only 0.6% of the farmers stated that they harvested their pods after four weeks.

Table 3: Harvesting intervals of cocoa pods

Harvesting interval (weeks)	Freq.	%
2	46	26.5
3	103	60.6
4	21	12.4
>4	1	0.6
Total	170	100.0

Stage of pod ripeness for harvesting

Table 4 shows the stages of pod ripeness at which various farmers harvested their cocoa pods. Out of 170 farmers interviewed, 67.1% stated that they harvested only well ripe pods while 27.6% harvested both partly ripe and well ripe pods together. None of the farmers intentionally left the pods on the trees to over ripe but 1.8% of the farmers harvested partly, well and over ripe pods. Those who harvested both partly and over ripe pods constituted 0.6% while 2.9% harvested only partly ripe pods.

Table 4: Stages of pod ripeness for harvesting

Stage of pod ripeness	Freq.	%
Partly ripe	5	2.9
Well ripe	114	67.1
Overripe	0	0
Partly and well ripe	47	27.6
Partly and over ripe	1	0.6
Partly, well and over ripe	3	1.8
Total	170	100.00

Periods of pod storage before breaking

The pod storage periods before breaking for fermentation are presented in Table 5.

As shown in the table, 28.2% out of the 170 farmers interviewed stored the pods for three days before breaking for fermentation while 22.9%, 14.7%, 12.9% and 10.0% stored their pods for four, two, seven and five days respectively before breaking. Only 1.8% of the farmers stored the pods for six days before breaking them for fermentation. Whilst 7.6% of the farmers indicated that they stored their pods for only one day before breaking, 1.8% stated that they stored them for over a week before breaking for fermentation.

Table 5: Periods of pod storage before breaking

Periods of pod storage (days)	Freq.	%
1	13	7.6
2	25	14.7
3	48	28.2
4	39	22.9
5	17	10.0
6	3	1.8
7	22	12.9
> 7	3	1.8
Total	170	100.00

Regional distribution of cocoa types planted in Ghana

The regional distribution of cocoa types cultivated by farmers in Ghana is presented in Table 6. Out of the 170 farmers interviewed, 43.5% planted *Amazonia* cocoa while 31.2% planted a mixture of *Amelonado*, *Amazonia* and hybrid types of cocoa. Those who planted *Amelonado* and hybrid alone constituted 17.1% and 8.2% respectively.

On regional basis, 2.9%, 2.4% and 1.8% of the farmers from Volta, Brong Ahafo and Ashanti respectively planted *Amelonado*, referred to as Tetteh Quarshie. In Eastern and Western north regions, only 0.6% of farmers planted *Amelonado* in either region. From Table 6, it can be seen that no farmer from Central and Western north planted *Amelonado*. With the *Amazonia*, 11.8%,

9.4%, 6.4%, 5.9%, 5.3% and 4.7% of the farmers planted it in Western north and south, Brong Ahafo, Ashanti, Eastern and Central regions respectively. No farmer in the Volta region planted *Amazonia*. Table 6 shows that out of the number of farmers who planted the hybrid, 4% were from the Western north, 3.5% from Ashanti, 2.4% each from Central, Eastern and Western south and 1.2% from Brong Ahafo and Volta regions. Of the farmers who planted a mixture of *Amelonado*, *Amazonia* and hybrid cocoa types, 10.6%, 5.3%, 4.1% and 2.4% were in Western south; Brong Ahafo and Eastern; Ashanti, Volta regions respectively. In both Western south and the Central regions, 1.8% of the farmers planted the mixture. Surprisingly, only 17.1% planted hybrid against perception of Breeders that most farmers now plant hybrid cocoa in Ghana.

Table 6: Regional distribution of cocoa types planted in Ghana

Region	Cocoa types							
	Amelonado		*Amazonia*		Hybrid		Mixture	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Ashanti	3	1.8	10	5.9	6	3.5	7	4.1
B/Ahafo	4	2.4	11	6.4	2	1.2	9	5.3
Central	0	0	8	4.7	4	2.4	3	1.8
Eastern	1	0.6	9	5.3	4	2.4	9	5.3
W/North	1	0.6	20	11.8	7	4.0	3	1.8
W/South	0	0	16	9.4	4	2.4	18	10.6
Volta	5	2.9	0	0	2	1.2	4	2.4
Total	14	8.2	74	43.5	29	17.1	53	31.2

Source of planting material

In Table 7 are the sources of seed or/seedlings used by farmers for planting.

The table shows that the farmers who obtained seeds or seedlings from the recommended Cocoa stations (Seed Production Unit of COCOBOD) were 52.9% whereas those who obtained them from other sources (relations, friends, and self) were 47.1%.

Table 7: Source of planting material

Source of Seed / Seedling	Freq.	%
Cocoa Station	90	52.9
Relations / friends / Self	80	47.1
Total	170	100.00

Duration of fermentation of cocoa beans before and after 2003 / 2004 cocoa crop season

The number of days farmers fermented the cocoa beans before and after the 2003 / 2004 cocoa crop season are shown in Table 8. From the table it can be seen that of the farmers interviewed, only 35.9% fermented their beans for the six days recommended for fermenting cocoa in Ghana before 2003/2004 cocoa year while 49.4% fermented their cocoa for six days after the 2003/2004 educational programmes carried out by COCOBOD. From the table, it is clear that the percentage of farmers who fermented the beans for only four days before 2003/2004 educational programmes decreased from 22.4% to 0.6% after

the 2003/2004 cocoa season. Similarly, farmers who were fermenting their cocoa for five days before the high incidence of purple beans in 2003/2004 decreased from 32.9% to 2.9% after COCOBOD educational campaigns. Those farmers who fermented their cocoa for seven days after 2003/2004 crop season were 47.1%. Only 8.2% of farmers indicated that they fermented the cocoa beans for seven days before 2003/2004 crop season. Table 8 shows that none of the farmers fermented their beans for three days after 2003/2004. It was only 0.6% of the farmers who fermented their cocoa beans for three days before 2003/2004 cocoa year (Table 8).

Table 8: Duration of fermentation of cocoa beans before and after 2003 / 2004 cocoa crop season

Duration of fermentation (days)	Before 2003/2004		After 2003/2004	
	Freq.	%	Freq.	%
3	1	0.6	0	0
4	38	22.4	1	0.6
5	56	32.9	5	2.9
6	61	35.9	84	49.4
7	14	8.2	80	47.1
Total	170	100.0	170	100.0

Fermentation days

Presented in Table 9 are the procedures farmers used to assess the days for fermentation of cocoa beans. From the table 9, it can be seen that 78.8% out of farmers interviewed did not counted the number of days of fermentation on 24-hourly basis; while only 11.2% of the farmers count the fermentation days on 24- hourly basis. The table also shows that 10.0% of the farmers interviewed counted the number of days they ferment the beans from a day after heaping to when the heap was opened for drying.

Table 9: Fermentation days

Assessment of fermentation days	Freq.	%
Counting the days from pod breaking to when heaps were opened for drying.	134	78.8
Counting a day after heaping as day one.	17	10.0
Counting 24 hourly after covering the heap.	19	11.2
Total	170	100.00

Number of turning heaps during fermentation

Table 10 shows the number of times farmers turned the heaps of cocoa beans during fermentation. From the table, 57.1% of farmers interviewed, stated that they do not turn the heaps at all during fermentation period while 35.9% turned them but only once. While 5.9% of the farmers indicated that they turned the heaps twice, 1.2% stated that they turned the heaps three times during the fermentation period

Table 10: Number of turning heaps during fermentation

Number of turning	Freq.	%
Once	61	35.9
Twice	10	5.9
Thrice	2	1.2
Not at all	97	57.1
Total	170	100.00

Assessment of cocoa quality by farmers

The parameters which farmers used to assess the quality of their processed cocoa beans for sale are presented in Table11. From the table, 51.2% of the farmers considered thorough dryness of the beans as an indicator of good quality whilst 22.9% considered dryness and chocolate brown colour of the dry beans as good criteria. The farmers who considered chocolate brown colour of the beans alone as being good quality indicator were 11.8%. However, 8.2% of the farmers stated that dryness, chocolate brown colour, and aroma of the beans are the best traits of good beans. Dryness together with aroma, and aroma alone were considered as an acceptable measure of good cocoa quality by 4.1% and 0.6% of the farmers respectively. Farmers who considered other characteristics such as weight and purple colour of the cut surfaces of dry beans as good qualities were 1.2%.

Table 11: Assessment of cocoa quality by farmers

Qualities	Freq.	%
Dryness	87	51.2
Aroma	1	0.6
Chocolate brown	20	11.8
Dryness and aromas	7	4.1
Dryness and chocolate brown	39	22.9
Dryness, aroma and chocolate brown	14	8.2
Other	2	1.2
Total	170	100.00

Working experience of Grading Staff

The working experiences of Grading Staff of Quality Control Division (COCOBOD) who were interviewed are shown in Table 12. The table shows that most of the grading staff interviewed had long years of working experience. Majority of them representing 32.1% have been working between 26 and 30 years followed by 28.6% of those who had worked between 21 and 25 years. Those who had working experience between 16 and 20 years were 17.9% while officers who have worked for less than six years and between 11 and 15 years formed 3.6%. Out of the 56 Grading Staff interviewed, 14.3% had working experiences above 30 years.

Table 12: Working experience of Grading Staff

№ of years in service	Freq.	%
Less than 6	2	3.6
11—15	2	3.6
16—20	10	17.9
21—25	16	28.6
26—30	18	32.1
Over 30	8	14.3
Total	56	100.00

Assessment of purpleness of cocoa beans

The methods used to assess the purpleness of cocoa beans are presented in Table 13. The table shows that all the 56 grading staff interviewed used cut test method alone to determine purpleness in dry cocoa beans.

Table 13: Assessment of purpleness of cocoa beans

Method	Freq.	%
Cut test analysis	56	100.0
Chemical analysis	0	0
Empirical test	0	0
Experience	0	0
Total	56	100.0

Effects of fermentation duration on fermentability of cocoa

Figure 2 is the graphical representation of the conversion of anthocyanins to leucoanthocyanidins at wavelengths of 530nm and 460nm respectively. It can be seen from the figure that, total anthocyanin generally decreased while leucoanthocyanidins increased with increasing fermentation periods. The result also shows that Fermentation Index increased to a maximum of 1.2 on the fifth day of fermentation.

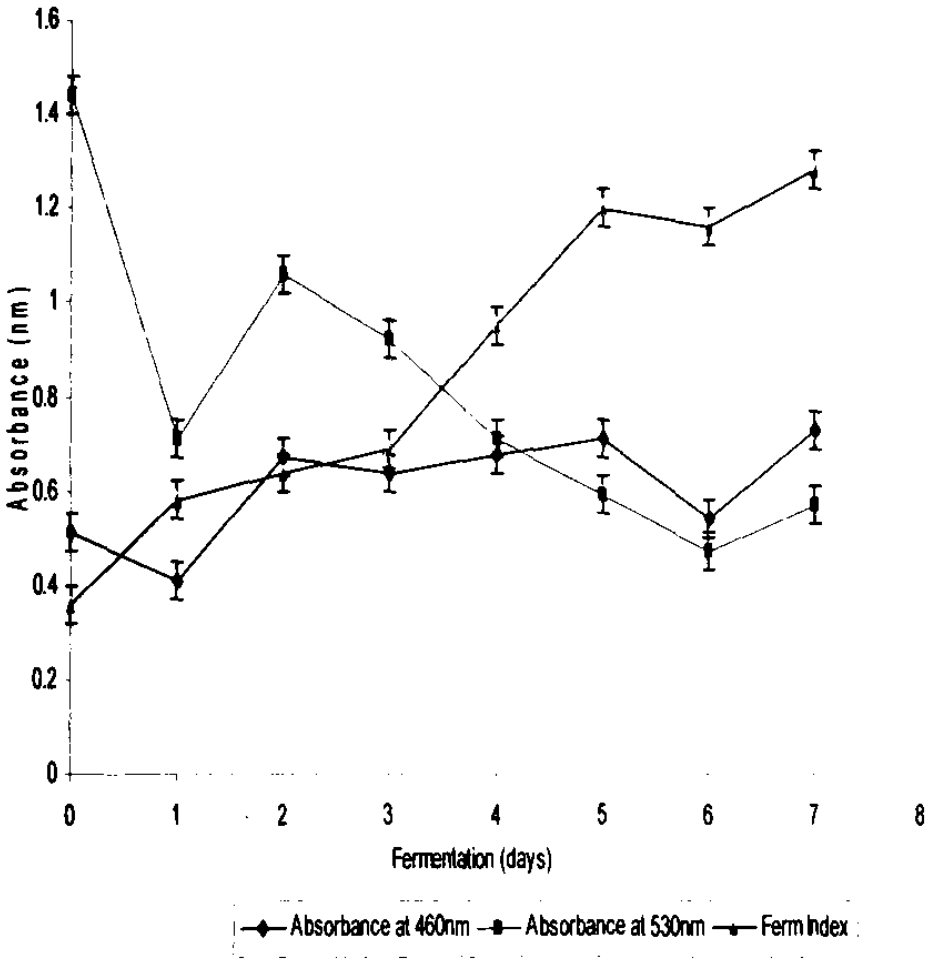

Fig. 2. Absorbance and Fermentation index of cocoa beans fermented for different durations

Effects of fermentation duration on total anthocyanins in commercial cocoa

Figure 3 shows the rate of degradation of anthocyanins in commercial cocoa fermented for different durations. From the figure, it is clear that there was rapid decline in total anthocyanins from 13.8mg/kg in unfermented cocoa (day 0) to 3.5mg/kg during the first two days of fermentation. Total anthocyanins in the beans fermented between the fifth and seventh days then became constant at 2.0mg/kg.

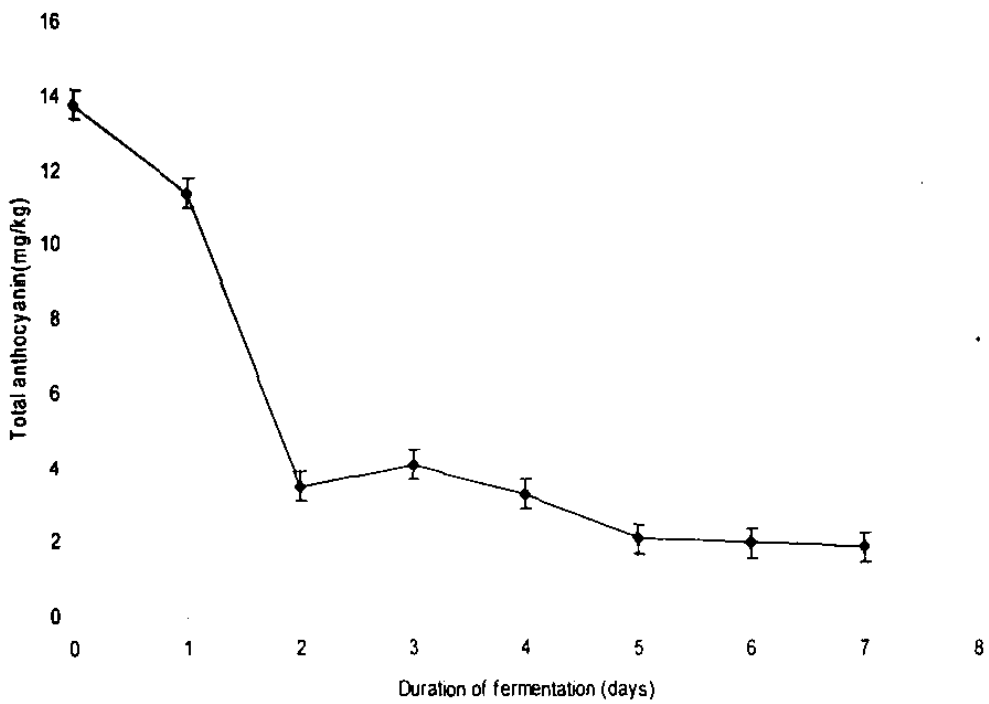

Fig.3 Total anthocyanins in commercial cocoa of different fermentation periods

Effects of pod storage and fermentation periods on purple beans occurrence

Percentages of the occurrence of purple beans in cocoa beans obtained from pods stored and fermented for different periods are presented in Table 14. The table shows that there was highly significant difference between the percentages of the occurrence of purple beans in cocoa beans from pods stored for different periods and those fermented for five days ranging from 48.3% to 42.0%. There were highly significant differences in the purpleness of beans stored for one, two, three and four days and in beans from a mixture of pods stored for the different periods and fermented for five days. From the table it is clear that there were no significant differences in the percentages of occurrence of purple beans in beans of pods stored for one, two, three and four days. There were highly significant differences in percentages of occurrence of purple beans in beans fermented for six days across the different pod storage periods. From the table, it can be seen that the percentages of occurrence of purple beans of beans fermented for six days generally decreased from 47.0%, in pods stored for one day to 32.0% in beans of pods stored for four days. It is interesting to note that the percentage of occurrence of purple beans of 32.0% of beans of pods stored for four days was significantly lower than percentage purple beans count of 40.3% of cocoa beans from a mixture of pods stored for different periods before breaking as practiced by the farmers. Similarly there was highly significant difference between percentage of purple beans count in beans from pods stored for one day and that of beans from pods stored for four days. The table again, shows that there was a highly significant difference between the

percentage of purple beans occurrence of beans fermented from a mixture of pods stored for different periods before breaking and those from pods stored for one day. However there were no significant differences in purpleness of beans from pods stored for two, three and four days, and fermented for six days. Similarly there was no significant difference in the percentage of purple beans occurrences in cocoa beans of pods stored for two, three and five days. There were also highly significant differences in purpleness of beans fermented for seven days from pods stored for different periods. From the table, it is clear that the percentage of purple beans count of 13.3% for beans from pods stored for four days was significantly lower than the percentage of purple beans counts of 20.7% and 32.3% for beans from pods stored for one and two days respectively and fermented for seven days. Also, the percentage of purpleness of beans from pods stored for four days, was also statistically lower than the percentage of 27.7% of beans obtained from a mixture of beans of pods stored for different periods. However, there was no significant difference in percentage of purple bean occurrence in beans from pods stored for two and three days.

Table 14: Percentage purple beans occurrence in cocoa pods stored and fermented for different periods

	Purple beans counts (%)		
	FMP		
PSP	Day5	Day6	Day7
Day1	48.3a	47.0a	20.7c
Day2	49.7a	34.0bc	32.3a
Day3	46.3ab	35.7bc	31.0ab
Day4	49.7a	32.0c	13.3d
Day5	42.0b	40.3b	27.7b
Mean	47.2a	39.3b	25.0c
CV (%)	4.44	6.38	5.34
SE	1.21	1.39	0.77

Means bearing identical letters are not significantly different from each other by Duncan's Multiple Range Test (DMRT) at 1% level.

FMP = Fermentation period **PSP** = Pods storage period

Total anthocyanin content of some approved cocoa cultivars used in Ghana

In Table 15 are presented the total anthocyanins content of different approved cocoa cultivars used in Ghana. From the table it is quite clear that the total anthocyanins contents in the different cocoa cultivars decreased with the increasing fermentation days. The total anthocyanins content across the different cultivars were highest in the unfermented cultivars ranging 3.30mg/kg in the *Criollo* to 9.68mg/kg in T60/887 x Pound 7. The total anthocyanins content was

least in the different cocoa cultivars after being fermented for seven days ranging from 1.23mg/kg in T85/799 x Sca 9 to 1.85mg/kg in T60/887 x Pound 7. It is interesting to note that total anthocyanin content was higher in the unfermented *Amelonado* than in the *Criollo* and *Amazonia* cultivars. However total anthocyanin content for *Amelonado* was lower than that for *Criollo* after fermentation for six days.

The observed differences in total anthocyanins contents of the cocoa cultivars tested were significantly different before and after fermentation for seven days. From the table, the total anthocyanins contents of the different cocoa cultivars were not significantly different from each other after fermentation for six days. It can also be seen from the table that generally the total anthocyanins content of the cultivars studied were more considerably reduced from the levels of that of each of the unfermented cocoa cultivars after being fermented for seven days than six days. Also it is clear that the reductions in the total anthocyanins contents were higher in cocoa cultivars with the initial higher total anthocyanins content before fermentation than those with relatively low initial total anthocyanins content. For instance, from the table, it can be seen that initial total anthocyanins content of the unfermented T60/887 x IMC 60 was 9.45mg/kg which had reduced to 2.19mg/kg after being fermented for seven days, about one-quarter of the initial content. However the unfermented *Criollo*, which among the cultivars studied had the least total anthocyanins content of 3.30mg/kg, had its total anthocyanins content reduced to 1.50mg/kg about half of the initial anthocyanins content after fermentation for seven days.

In spite of the considerable lowering of the total anthocyanins contents of the cocoa cultivars after fermentation for seven days, cultivars such as T60/887 x IMC 60; T60/887 x Be 8; and T60/887 x Pound 7 had relatively high total anthocyanins contents after the seven day fermentation and were very significantly different from the other cocoa cultivars.

Table 15: Total anthocyanins content (mg/kg) of different cocoa cultivars

Cultivars	Total anthocyanins (mg/kg)		
	Unfermented (day 0)	Six day fermentation	Seven day fermentation
Criollo	3.30f	2.76	1.50cde
Amelonado	6.98bc	2.51	1.62bcd
Amazonia	5.79cd	2.31	1.82bc
T85/799 x *Amelonado*	8.57ab	1.99	1.58bcd
T85/799 x T79/501	3.91ef	2.18	1.58bcd
T60/887 x *Amelonado*	6.03cd	2.00	1.49cde
T85/799 x Sca 9	7.26bc	2.46	1.23e
T85/799 x Pa 150	5.50cde	3.00	1.76bcd
T60/887 x Ma 12	5.94cd	3.02	1.70bcd
T60/887 x ICS 6	6.98bc	2.06	1.67bcd
T60/887 x IMC 60	9.45a	2.43	2.19a
T60/887 x Be 8	6.51cd	2.20	2.15a
T60/887 x Catongo	5.91cd	2.50	1.47de
T60/887 x Pound 7	9.68a	2.17	1.85b
T60/887 x Pound 10	4.93def	2.61	1.73bcd
Mean	6.45	2.41	1.69
CV (%)	10.76	20.01	7.62
SE	0.40	0.28	0.07

Means bearing identical letters are not significantly different from each other by Duncan's Multiple Range Test (DMRT) at 1% level in a given column

Purple beans occurrence in different cocoa cultivars

Table 16 shows the percentage of purple beans occurrence for different cocoa cultivars fermented for six and seven days. From the table it is quite clear that the occurrence of purple beans in the cultivars tested decreased with increasing periods of fermentation. Whereas the occurrence of purple beans in the cocoa cultivars fermented for six days ranged from 5.7% in *Criollo* to 52% T60/887 x Pound 10 with a mean of 21.9%, the same cultivars when fermented for seven days had purple beans occurrence ranging from 1.7% in *Criollo* to 18.0% in *Amazonia* with a mean of 8.3%. As it can be seen from the table, it was only in *Amazonia*, T85/799 x T79/501 and T60/887 x IMC 60 that the percentage occurrence of purple beans increased when the fermentation period was extended to seven days.

In spite of the decrease in the occurrence of purple beans when the fermentation day was increased from six to seven days, the differences in the prevalence of purple beans in the different cultivars after being subjected to the two fermentation periods were significantly high.

From the table, it is clear that, whether fermented for six or seven days, the occurrence of purple beans in T85/799 crosses was generally lower than in the T60/887 crosses. It is interesting to note that the occurrence of purple beans in the T60/887 crosses declined more drastically than in the T85/799 crosses when the fermentation period was increased from six to seven days. It can also be seen from the table that purpleness of the crosses of T60/887 and T85/799 were very significantly different from each other when they were fermented for six days.

The occurrence of purple beans in *Amazonia*, *Criollo* and *Amelonado* were not significantly different from that of the T85/799 crosses. It was only T60/887 x IMC 60 in which the occurrence of purple beans was not significantly different from those of T85/799 crosses. From the table it can be seen that *Criollo*, *Amelonado*, *Amazonia* and the cultivars with the T85/799 crosses contained less than 20.0% purple beans ranging 5.3 to 17.7% after fermentation for six days. It is also clear in Table 16 that purple beans occurrences in cultivars of T60/887 crosses with the exception of T60/887 x IMC 60 were all more than 20.0%. It is interesting to note that T60/887 x *Amelonado* contained about twice the percentage of purple beans (23.0%) than in *Amelonado* (10.7%) alone fermented for six days. Similarly, the percentage of purple beans occurrence in the cultivars of T85/799 x *Amelonado* crosses was only 5.3% which is about half the percentage of purple beans occurrence in *Amelonado* alone.

Table 16: % Purple beans occurrence in some cocoa cultivars

Cultivars	Purple beans counts (%)	
	Six day fermentation	Seven day fermentation
Criollo	5.7h	1.7g
Amelonado	10.7fg	4.7g
Amazonia	12.3f	18.0a
T85/799 x *Amelonado*	5.3h	2.3g
T85/799 x T79/501	13.3f	14.3b
T60/887 x *Amelonado*	23.0d	8.3de
T85/799 x Sca 9	13.7f	7.3def
T85/799 x Pa 150	17.7e	7.3def
T60/887 x Ma 12	32.7c	7.0def
T60/887 x ICS 6	48.3b	12.3bc
T60/887 x IMC 60	8.3gh	9.3cd
T60/887 x Be 8	24.0d	2.3g
T60/887 x Catongo	22.7d	4.0fg
T60/887 x Pound 7	34.7c	12.0bc
T60/887 x Pound 10	52.0a	13.0b
Mean	21.9	8.3
CV (%)	6.74	18.06
SE	0.84	0.86

Means bearing identical letters are not significantly different from each other by Duncan's Multiple Range Test (DMRT) at 1% level in a given column

Percentage degradation of anthocyanins in different cultivars of cocoa

In figure 4 is presented the percentage degradation of anthocyanins in cocoa cultivars fermented for six and seven days.

Figure 4 shows the rate of anthocyanin breakdown in cocoa beans of the different approved cocoa cultivars fermented for six and seven days respectively. Generally, it can be seen from the figure that the degradation of anthocyanins was high in the different cocoa cultivars when they were fermented for seven days than for six days. Also from the figure it is clear that the degradation in some cultivars after being fermented for seven days was higher than in other cultivars.

The cultivars T85/799 x *Amelonado* and T60/887 x Pound 7 fermented for six days had the highest rate of anthocyanin breakdown. *Criollo* had the lowest rate of anthocyanin breakdown after fermentation for six days. The rate of anthocyanin degradation of T85/799 x *Amelonado* and T60/887 x Pound 7 were the same after fermentation for six days. It can be seen from Figure 4 that the cultivars, T85/799 x T79/501 and T85/799 x Pa 150 also had the same rate of anthocyanin degradation after fermentation for six days. Similarly, T60/887 x *Amelonado*, T85/799 x Sca 9 and T60/887 x Be 8 also degraded at the same rate. It is clear from the figure that anthocyanin breakdown in T60/887 x IMC 60, T60/887 x Be 8 and T60/887 x Pound 7 were apparently the same irrespective of the six and seven days they were fermented respectively.

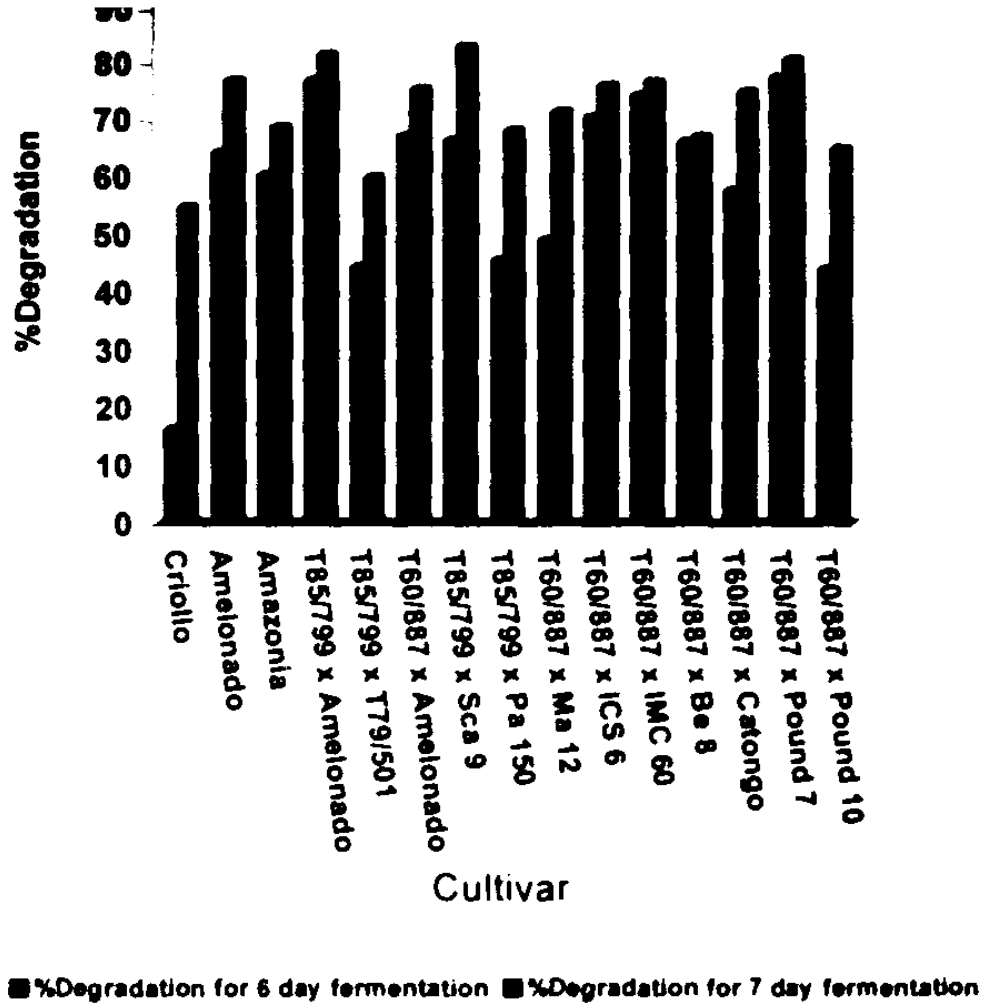

Figure 4: Percentage degradation of anthocyanins in some cultivars of cocoa

Anthocyanin contents of different purple colour classes of the dry cocoa beans from different locations

The total anthocyanin contents of cocoa beans with different shades of the purpleness collected from different locations are presented in Table 17. The table shows that the total anthocyanins content of cocoa beans of the various shades of purpleness were highly significantly different from each other. Also the degree of purpleness of cocoa beans collected from different locations can be seen to be significantly different. Table 17 shows that among the brown cocoa beans collected from the different locations, those collected from Koforidua had the least total anthocyanins content of 1.28mg/kg. The highest total anthocyanins content was recorded in brown cocoa beans obtained from CRIG.

The table also shows that the various shades of purpleness of cocoa beans collected from the different locations varied widely. Generally, it can be seen from the table that the least mean total anthocyanins content of 1.50mg/kg occurred in cocoa beans, which were brown in colour while the highest of 2.33mg/kg, occurred in deep purple cocoa beans. Inspite of the wide variation in the total anthocyanins content of the different shades of purpleness, their observed differences were not significant. Also the total anthocyanins content of the beans of brown colour was not significantly different from those of the beans of partly brown/partly purple and pale purple colours. It is clear from the table that it was only the means of the total anthocyanins contents of beans of the brown and deep purple colours, which were significantly different. It is interesting to note that total anthocyanins content of the intra colour variations were not significant across the locations.

Table 17: Anthocyanin contents of different purple colour classes of the dry cocoa beans from different locations

Location	Total anthocyanins (mg/kg)			
	Brown	Partlybrown/partly purple	Pale purple	Deep purple
CRIG	1.75	1.95	2.12	2.23
Koforidua	1.28	1.89	2.43	2.44
Kade	1.46	1.35	1.43	2.05
Suhum	1.62	1.93	2.38	2.64
C/Coast	1.40	1.64	2.21	2.34
Sankore	1.46	2.03	1.82	2.28
Mean	1.50b	1.80ab	2.07ab	2.33a
CV (%)		15.65		
SE		0.18		

Means bearing identical letters are not significantly different from each other by Duncan's Multiple Range Test (DMRT) at 1% levels.

Nib pH of different purple colour classes

Figure 5 is a graphical presentation of the pH of nibs of dry cocoa beans of different shades of the purple colour. It can be seen from Figure 5 that deep purple bean colour had the highest pH value of 5.7 while the lowest pH of 5.4 of nibs occurred in the brown bean colour. It is interesting to note that pale and partly brown/partly purple colours had the same value of 5.5.

nibs occurred in the brown bean colour. It is interesting to note that pale and partly brown/partly purple colours had the same value of 5.5.

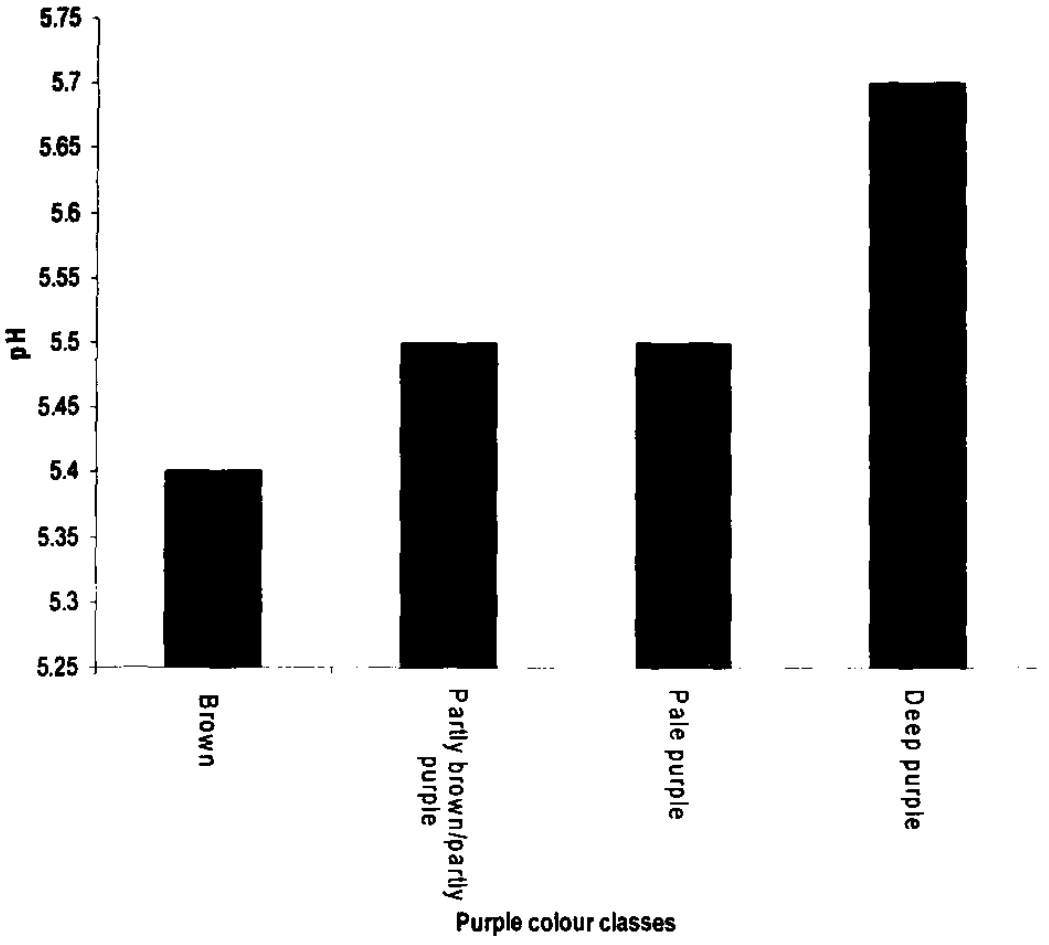

Fig 5: Nib pH for different purple colour classes

Longitudinal sections of cocoa beans showing different classes of purple and brown colour

Plates 5a-d shows the pictures of the various shades of purpleness observed in the cocoa beans studied. On the plates are also indicated the pH value and mean total anthocyanins content of cocoa beans of such purple colour.

Plate 6a: Deep purple colour beans (pH=5.7, mean total anthocyanin content=2.33mg/kg) (X 8)

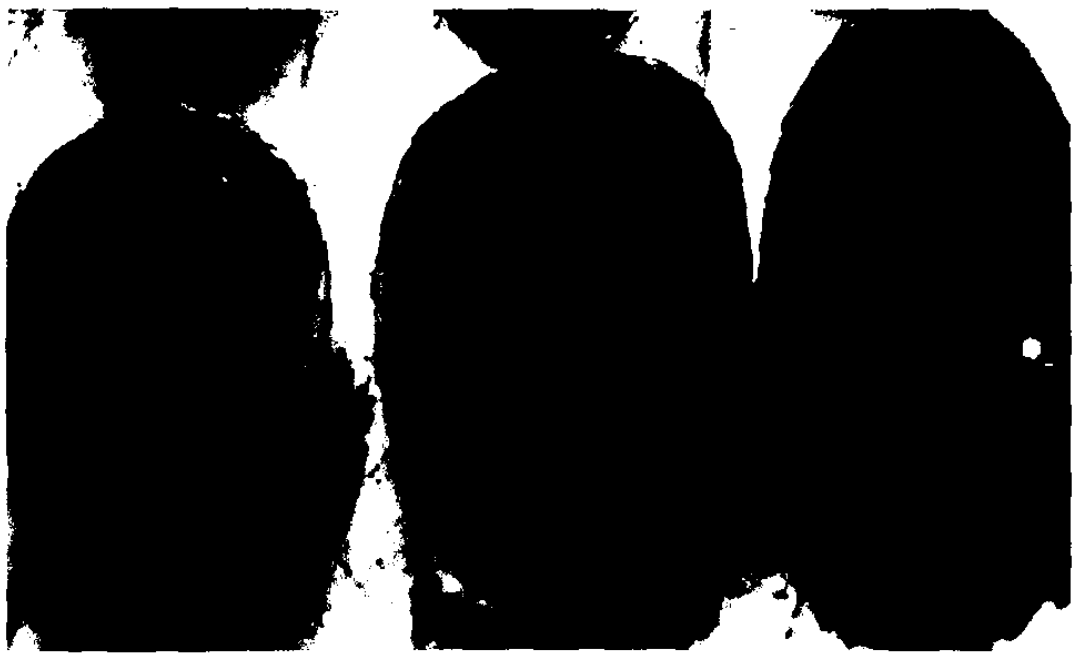

Plate 6b: Pale purple colour beans (pH=5.5, mean total anthocyanin content=2.07mg/kg) (X 8)

Plate 6c: Partly brown/partly purple colour beans (nib pH=5.5, mean total anthocyanin content =1.80mg/kg) (X 8)

Plate 6d: Brown colour beans (pH=5.4, mean total anthocyanin content = 1.50mg/kg) (X 8)

Influence of climatic factors on the occurrence of purple beans in dry cocoa beans

In Table 18 is presented correlation coefficient values between climatic factors and the occurrence of purple beans in dry commercial cocoa. As shown in the table, the occurrence of purpleness in cocoa beans processed from February to August 2006 was not correlated to rainfall, maximum temperature and sunshine, relative humidity at 9am and 3pm and minimum temperature .The correlation coefficients of the occurrence of purple beans and climatic factors were not significant at 5% levels.

Table 18: Correlation between climatic factors (independent) and the occurrence of purple beans (dependent) in dry commercial cocoa

Variable	X_1	X_2	X_3	X_4	X_5	X_6	X_7
X_1							
X_2	0.273						
X_3	-0.056	-0.702					
X_4	-0.171	-0.392	0.807				
X_5	0.427	0.653	-0.770	-0.835**			
X_6	-0.213	-0.169	-0.511	-0.603	0.435		
X_7	0.43	0.822*	-0.882	-759	0.945**	0.341	

N= 7 *P= 0.05 **P= 0.01

Keys

X_1 = Purple bean counts **X_2** = Rainfall **X_3** = Relative humidity at 9am **X_4** = Relative humidity at 3pm **X_5** = Temperature maximum **X_6** = Temperature minimum **X_7** = Sunshine hour

CHAPTER FIVE

DISCUSSION

The survey studies have clearly established the occurrence of purple beans in cocoa produced in Ghana. The high percentage purple beans occurrence for Volta could be attributed to the predominantly *Amelonado* type of cocoa grown in the region. According to Mossu (1992) the cotyledon of *Amelonado* cocoa is darker in purple colour than in *Criollo* and *Trinitario* cocoa. It is most likely that because of high rate of smuggling of cocoa from Volta to Togo where not much emphasis is placed on the quality of cocoa purchased, cocoa farmers in Volta region do not take the trouble to ferment and dry their cocoa properly. This might have accounted for the high occurrence of the purple beans in cocoa produced in that region. It is also possible that because of the extremely low production of the produce in Volta, farmers might be compromising on some of the primary processing methods of the beans for economic reasons.

The occurrence of high percentage purple beans in the cocoa produced in Ashanti, Western south and north regions could be due to large quantities of wet beans which the farmers load on small drying mats. Hii Ching Lik *et al* (2003) made a similar observation and reported that loading drying mats with large quantities of wet cocoa beans resulted in high occurrence of purple colour in the fermented cocoa beans. The results also support that of Wood and Lass (1985)

who stated that heavy loading of drying mats with large quantities of fermented cocoa beans leads to poor aeration of the beans which slow down the oxidative stage of fermentation which is so vital in the development of the chocolate brown colour of well fermented beans. The observed high awareness of Farmers, Purchasing Clerks and Grading Staff of improper fermentation in Table 2(a-c) contributing to the occurrence of purple beans in the processed beans, is an indication of the effectiveness of the educational programme organized on fermentation by COCOBOD during the study period.

From the survey results, the majority of farmers complying with harvesting regulations further indicated the usefulness and effectiveness of COCOBOD educational programmes. The study revealed that *Amazonia* cocoa is predominantly grown in Ghana. However, cocoa farms that are heterogeneous in cocoa types planted namely *Amelonado*, *Amazonia* and hybrids constitute the second largest in Ghana (Table 6) The survey results revealed that majority of the farmers are still using materials from friends and relations. This suggests that the COCOBOD educational programmes should be intensified and embarked upon vigorously to create awareness of the importance of farmers obtaining cocoa planting materials from COCOBOD approved seed garden. It is possible that the high incidence of purple beans occurrence could be due to the use of planting materials by farmers from friends and relations which may be heterogeneous in genetic make up. Clapperton (1993) made a similar observation and reported that the type of planting material used by farmers has a major influence on the flavour and colour of the cocoa beans. Majority of the farmers, now ferment the beans for the recommended six and seven days

respectively. However the studies also revealed that majority of them were not able to count the number of days for fermenting cocoa beans accurately. (Table 9). This could be due to the high number of illiterate caretaker farmers engaged on the farms by some absentee cocoa farmers. It is also possible that education on how to count the recommended number of days for fermenting cocoa was not well emphasized in the educational programme for the farmers. The results indicating that some farmers counted the fermentation period on 24-hourly basis implies that they were the only few farmers who actually fermented the beans for the recommended six days. This might have contributed to the high incidence of purple beans in cocoa produced in Ghana.

The high percentage of farmers, who could not mix or turn the beans during fermentation (Table 10), could be due to the shortage of farm hands. The shortage of labour force in the farms is largely due to the rural urban drift. It is possible that the non-turning or mixing of the beans during fermentation by the farmers could have led to poor aeration of the fermenting heaps and consequently poor fermentation, and hence the presence of the high proportion of purple colour of the cocoa beans produced in Ghana of late. This finding confirms that of Rohan (1957a) who found that cocoa beans that are not sufficiently fermented as a result of poor aeration develop deep purple colour.

The high percentage of the farmers, who considered dryness of the cocoa beans as good quality criterion, held the view that properly dry cocoa beans are less purple. This confirms the assertion of Rohan (1963) that the cotyledons of the beans brown during drying, a period in which further oxidation of cocoa polyphenols by polyphenol-oxidases takes place.

The longer working experience of the grading staff of the Quality Control Division of COCOBOD, which was between 21 and 30 years, was a clear indication that the high purple beans occurrence in cocoa produced in Ghana cannot be due to inexperience of the Grading Staff. However, the over reliance on cut test alone in the assessment of the purpleness of dry cocoa beans which is very subjective, could have contributed to the high incidence of the purple beans problem. Dand (1993) made similar observation on the subjectivity of the cut test and also observed that the test totally depends on the sight of the analyst while noting that nobody's sight can be used as a standard for such measurement.

The decrease in anthocyanin and the increase in leucoanthocyanidin contents with increasing fermentation periods suggest the conversion of anthocyanins to leucoanthocyanidins during fermentation (Fig. 2). A similar observation has been made by Rohan (1963); Kim and Keeney (1984) and Bonvehi and Coll (2000), who noted that anthocyanins are hydrolyzed during fermentation and change to colourless leucoanthocyanidins. The occurrence of a Fermentation Index of 1.2 after the fifth day of fermentation indicates that with proper harvesting procedure and adequate pod storage periods before breaking, good fermentation could even be achieved within five days period. This finding agrees with that of Jinap *et al.* (2000) who reported that a Fermentation Index greater than one to mean well fermented beans.

The repeated occurrence of 2.0mg/kg anthocyanins content in cocoa beans fermented for five, six or seven days seems to suggest that 2.0mg/kg (Fig.3) could be the required anthocyanins content in well fermented cocoa

beans. These findings are indeed indications that desired chocolate flavour and aroma could be obtained if cocoa beans are fermented for five, six or seven days.

The highly significant reduction in the percentage purple beans occurrence in beans fermented for six day from pods stored for four days than those from a mixture of pods stored for different periods before breaking (Table 14), suggest that beans from pods harvested and stored at different periods should be broken and fermented separately for better fermentation of the beans. These observations are similar to those of MacLean and Wickens (1951) who noted that West African *Amelonado* pods stored for three or more days before breaking had its purple colour reduced from 82.0 to 39.6 percent.

The highly significant difference observed between the percentages of purple bean counts in the beans of pods stored for one and four days respectively, after being fermented for six days, could be due to the decrease in pulp volume and the increase sugar levels after the four- day storage. A similar observation was made between the percentages of purple bean counts in the beans of pods stored for one and five days respectively. Tomlins *et al.* (1993) have reported that pulp volume decreased in Malaysian cocoa beans from pods stored for seven days. MacLean and Wickens (1951) have observed that pods stored for four days before breaking had significantly lower temperatures after 96 hours of fermentation than those stored for only one day and this lead to a significant reduction in percentage of purple and wrinkle bean counts. It is possible that the observed significant differences between the percentage counts of pulp beans in the beans of pods stored for one and four days respectively,

before fermentation, could be due to the decrease in the pulp volume and increase in the sugar levels in pods stored for four days, which probably enhance fermentation (MacLean and Wickens 1951). Similarly the least percentage of purple beans in the beans from a mixture of pods stored for different periods (day 5) before and fermented for six days, in this study (Table 14), could also be attributed to fermentation enhanced by the decrease in pulp volume and increase sugar levels of the beans from the mixture of pods stored for different periods.

The significantly high percentage of purple beans counts in cocoa beans of pods stored for one, four and five days respectively and fermented for six days (Table 14) may be due to loss of pulp and increase in sugar levels in pod stored for four days.

The highly significant differences observed in total anthocyanins content of unfermented cocoa beans of the different cocoa cultivars (Table 15) is suggestive of cultivars being a possible contributor to the occurrence of purple beans in cocoa produced in Ghana recently. This result is consistent with that of Niemenak *et al.* (2006) who have observed that total phenols; catechin, epicatechin and anthocyanin in fresh and fermented-like beans were genotype dependent. Clapperton (1993) also made similar observation when he reported that flavour and colour of cocoa beans is greatly influenced by the planting material used. The considerable reduction of anthocyanins content in the unfermented cocoa cultivars studied after being fermented for seven days than six days is an indication that anthocyanins could break down to acceptable levels when the cocoa beans are adequately fermented. The highly significant reduction in total anthocyanins content in the cocoa cultivars with the initial

higher total anthocyanins content before fermentation than those with relatively low initial total anthocyanins content after six and seven days fermentation could be due to different rate of anthocyanins breakdown in the different cocoa cultivars. These observations are indications that cultivars with different rate of anthocyanins break down should be fermented separately. This is in conformity with the recommendation of Wood and Lass (1985) that mixed fermentation should be avoided. The highly significant differences of total anthocyanin in the beans of different cocoa cultivars fermented for seven days further explain the different rates of breakdown of anthocyanins of the different cultivars tested. This further indicates that cultivars could be one of the possible factors responsible for the development of purpleness of the cocoa beans. The strong correlation between purple beans occurrence and total anthocyanins contents in the dry beans of some cocoa cultivars, is also a further prove that purpleness of the cocoa beans is cultivar dependent.

The significantly higher percentage of purple beans count recorded for the cultivars T60/887 x Pound 10, 52.0%, and T60/887 x ICS 6, 48.3%, whose beans were fermented for six days (Table 16) could be attributed to the size of the beans. It was observed during the study that the sizes of the beans for cultivars T60/887 x Pound 10 and T60/887 x ICS 6 were larger than the other cultivars used. It is most likely that the larger sized beans affected proper fermentation of these cultivars within the six-day period. This finding is suggestive of the need for larger beans to be fermented for longer periods to reduce the occurrence of purple beans in such cultivars to acceptable levels. This result confirms the work of Bellefroid (1935) and Palma (1951) who reported

that the size of cocoa beans influences their fermentation duration. Bellefroid (1935) further noted that the thin Venezuelan cocoa beans ferment quicker than the thick *Forastero* types. The lower percentage purple beans count of less than 20.0% observed in *Criollo*, *Amelonado*, *Amazonia* and all cultivars with T85/799 crosses than in cultivars with the T60/887 crosses fermented for six days suggest that the former group of cocoa cultivars could be fermented together but separately from the T60/887 crosses to ensure uniform fermentation. The general reduction in the purpleness of the beans of T60/887 with increasing periods of fermentation is suggestive of the need for the beans of T60/887 crosses to be fermented for longer periods than usually fermented if good quality beans are to be obtained.

The different rates of anthocyanins breakdown in the different cultivars of cocoa may be responsible for the different purple colour classes observed. The apparently equal rates of anthocyanin degradation in the T60/887 x *Amelonado*, T85/799 x Sca 9 and T60/877 x Be 8 crosses fermented for six days suggests that these cultivars could be fermented together to obtain good quality beans. T85/799 x T79/501 and T85/799 x Pa 150 crosses also with equal rates of anthocyanins degradation is indicative that they could be fermented together to obtain good quality produce.

The lack of significant differences between the total anthocyanins contents of both the partly brown/ partly purple and pale purple beans and that of the control suggest that partly brown/ partly purple and pale purple beans could be grouped together with the brown beans during assessment without the

quality of the produce of such mixtures being affected. Anon (1968) also has observed that partly brown/ partly purple beans are not defective beans and recommended that it could constitute at most 20% of commercial cocoa. Brown (1957) has reported that pale purple beans could be used to produce good quality cocoa products in addition to serving as a safeguard against over fermentation.

The highly significant differences observed between the total anthocyanins content of the brown and deep purple beans is indicative that deep purple beans contain high anthocyanins content and might require longer fermentation periods for the excess anthocyanins to break down. This observation is in line with the finding of Duthie (1937) who noted that deep purple coloured cocoa beans required longer periods of fermentation. Tomlins (1993) and Forsyth and Quesnel (1957) made similar observations and reported that unchanged anthocyanin pigments were responsible for most of the deep purple colour of fresh *Forastero* cocoa beans.

The same pH values obtained for both pale purple and partly brown/partly purple coloured beans are indicative that these purple colours which were closer to pH of the control brown could be considered as being well fermented beans and of acceptable quality. The pH value of 5.5, falling within the acceptable pH range (5.2-5.5) of a good quality cocoa bean as reported by Jinap and Dimick (1991, 1994) and Lopez (1983) further confirms the acceptability of pale purple and partly brown/partly purple beans as of commercial value. This result is in line with that of Biehl et al., (1982, 1985) who has observed that the quality of cocoa depends largely on its final pH since

according to him the formation of cocoa specific aroma precursors is strongly dependent on the degree of acidification of the nib during fermentation. Takrama and Aculey (2001), and Kuebutornye *et al.* (2003) also reported that a nib pH of 5.3 is an indication of a successful fermentation. The presence of high anthocyanin content present in deep purple beans is likely to be accountable for its high pH of 5.7. This indicates that cocoa beans with such colours are not well fermented. This result is similar to Jinap and Dimick (1991, 1994) and Lopez (1983) who reported that cocoa beans with pH of 6 are not well fermented and produce purple colour in the dry beans. In spite of the lack of significant differences among purpleness of the cocoa beans collected from the different locations, the apparent variations in their total anthocyanins content could be due to geographical variations.

The lack of a significant effect of rainfall, temperature, relative humidity and sunshine hours on purple beans occurrence in cocoa beans observed in this study (Table 18) is in contrast with the findings of Roubelakis-Angelakis and Kliewer (1986), Kliewer (1977), Cobbina and Miller (1987), Wang and Zheng (2001) who noted that light, relative humidity and temperature control accumulation of anthocyanins in cocoa beans, which is the cause of the purple colour of the cocoa beans.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The findings of this study are summarized as follows:

1. The national percentage purple beans count is 32.3%. Volta region had the highest percentage purple beans count of 38.7% with the least occurrence recorded in the Central region as 28.0%.
2. Under-fermentation of the cocoa beans was a major contributor to the occurrences of purple beans in cocoa produced in Ghana.
3. *Amazonia* cocoa type was cultivated by majority of cocoa farmers in Ghana followed by those who cultivated a mixture of *Amelonado*, *Amazonia* and hybrid cocoa types.
4. Farmers still using planting materials from friends and relations were 47.1% of the cocoa farmers in Ghana.
5. The cut test analysis was the only method used for assessing the quality of cocoa in Ghana.
6. A well fermented cocoa should have a Fermentation Index of one and above within fermentation period of five and six days.
7. Good quality cocoa beans should have residual anthocyanins of 2.0mg/kg of dry cocoa beans and a pH range of 5.4 to 5.5.
8. The pale and partly brown/partly purple beans were as good as the

acceptable cocoa beans of brown colour considering their pH and anthocyanin levels.

9. Dry cocoa beans with brown colour had residual anthocyanins of 1.5mg/kg; that of partly brown/partly purple beans was 1.80mg/kg; in the pale purple beans it was 2.07mg/kg. The deep purple beans had residual anthocyanins of 2.33mg/kg.
10. The anthocyanins content and the occurrence of purple beans varied with cultivar.
11. The rate of anthoicyanins breakdown varied with the cultivar.
12. Among all the cultivars tested for their anthocyanins contents after six days of fermentation, onlyT85/799 x *Amelonado* and T60/887 x *Amelonado* contains 1.99mg/kg and 2.00mg/kg. The other cultivars contained more than 2.00mg/kg.
13. Cultivars with anthocyanin contents of more than 2.0mg/kg had their anthocyanin contents reduced to less than 2.0mg/kg after seven days fermentation except for T60/887 x IMC 60 and T60/887 x Be 8.
14. *Criollo, Amelonado, Amazonia* and all T85/799 crosses had purple beans of less than 20.0% while all T60/887 crosses had purple beans more than 20.0% after six days fermentation except for T60/887 x IMC 60.

Conclusions

The results of this study have strongly indicated the occurrence of purple beans in cocoa produced in Ghana to be a national problem. It was evident from the studies that the genetic composition of cocoa varieties released to farmers and the practice of under fermentation of cocoa beans by farmers contributed considerably to the high occurrence of purple beans in cocoa produced in Ghana. The indications in the studies that the partly brown/partly purple and pale purple beans have acceptable anthocyanin contents and pH values of the brown cocoa suggests the beans of the two shades of purpleness could be mixed with the brown beans to produce cocoa products of good quality.

Recommendations

On the basis of the findings of this study, it is recommended that:

1. The education on proper fermentation of cocoa by farmers should be well emphasized in educational programmes for farmers.
2. The studies on the occurrence of purple colour pattern should be carried out with much emphasis on chocolate flavour and aroma as parameters.
3. Cultivars with high rate of anthocyanin degradation should be selected and tested for their disease resistance and when found suitable should be supplied to farmers.
4. Studies into microfloral changes during fermentation of different cocoa cultivars released to farmers and its effects on the purple colour of the beans should be carried out.
5. Cocoa pods harvested at different periods should not be broken and fermented together by farmers
6. Further work should be done on anthocyanin content of different shades of purple colour of cocoa beans collected from wide range of areas.
7. Mix fermentation of T85/799 and T60/887 cross hybrids should be avoided. However, *Criollo, Amelonado* and *Amazonia* cocoa varieties can be fermented together with cultivars with T85/799 crosses.
8. Farmers should ferment cocoa cultivars from T85/799 crosses for six days and extend the fermentation period for cultivars of T60/887 crosses to seven days.

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APPENDIX I

A SURVEY OF OCCURRENCE OF HIGH PURPLE BEANS IN COCOA PRODUCED IN GHANA.

Introduction

I am an M. Phil. Student from the University of Cape Coast. I am here to ask you some questions pertaining to preparation of cocoa beans on your farm and quality assessment of dried cocoa beans. The information is needed for studies into the occurrence of purple beans in cocoa produced in Ghana.

I. (To be answered by cocoa farmers only)

A. Background information.

1.0.

Location.....

.....

2.0.Nationality.....

.....

3.0. Sex: a) male [ ] b) female [ ]

4.0. Age: a) 18-30 [ ] b) 31-40 [ ]c) 41-50 [ ] d) above

50 [ ]

5.0. Number of year in farming: a) <5 [ ] b) 6-10 [ ] c) 11-15 [ ] d)16-20 [ ]

e) 21-25 [ ] f) 26-30 [ ] g) 31-35 [ ] h) over 35 [ ]

6.0. Educational status ?

a) basic education [ ] b) secondary cycle [ ] c) tertiary [ ]

d) no formal education [ ]

7.0. Who owns the farm(s)?

a) self [ ] b) caretaker [ ]

8.0. What labour type do you use on the farm?

a) family labour [ ] b) permanent labour [ ] c) seasonal labour [ ]

9.0. How many farms do you have?

.....

Complete the following table.

	Farm 1	Farm 2	Farms 3	Farm 4
Cocoa type				
Source seeds and/or seedlings				
Farm size. (acres)				
Production/farm yield (bags).				

Harvesting

10.0. What type of pods do you harvest for processing?

a) partly ripe [ ] b. well ripe [ ] c). over ripe

d). other (specify).....

11.0. Give reasons for your choice (s) in 10 above.....

12.0. At what intervals do you harvest the pod? a) one week [ ] b) two weeks [ ] c) three weeks [ ] d) Monthly [ ]

13.0. How long does it take after harvesting for you to start pod breaking?

a) One day [ ] b) two days [ ] c. three days [ ] d). four days [ ] e). other (specify).....

Cocoa fermentation

14.0. How many days were you fermenting the beans before 2003/2004 cocoa season?

a) three [ ] b) four [ ] c) five d) six [ ] e) seven [ ] f. not al all [ ]

15.0. Give reasons for your choice in 14 above.....

16.0. How many days do you now ferment your cocoa?

a) three [ ] b) four [ ] c) five [ ] d) six [ ] e) seven [ ]f) not at all [ ]

17.0. Give reasons for choice in 16 above.....

18.0. How do you count the number of days in 14 and 16? .....

19.0. How many times do you usually turn the cocoa beans during fermentation?

a) once [ ] b) twice [ ] c) thrice [ ] d) not at all [ ]

20.0. Give reason (s) for your choice in 19

.....

21.0 What methods of fermentation do you adopt on your farm?

a) heap [ ] b) tray [ ] c) basket [ ] d) box [ ]

e) other (specify).....

22.0. Give reasons for your choice of in 21 above

.....

23.0. What materials do you use in the fermentation of the wet beans?

a) banana leaves [ ] b) cocoa sacks/ fertilizer bags [ ] c) polythene sheet [ ] d) other (specify).....

24.0. Give reason for your choice in 23 above.....

Drying of cocoa

25.0. How long do you dry your fermented beans?

a) 3 days [ ] b) 5 days [ ] c) 7 days [ ] d) days [ ] e) 11 days [ ] f) others (specify).....

26.0. How do you dry your cocoa on your farm?

a) on drying mats [ ] b) on polythene sheets [ ] c) on cement floor [ ] d) others (specify).....

27.0. How do you know that your beans are dried?

a) empirical test [ ] b) use of moisture meter [ ] c) experience [ ]

Problems and constraints

28.0. What do you consider the most difficult problem(s) in the fermentation of cocoa?

a) lack of planting/ banana leaves [ ] b) theft [ ] c) others [ ]

d) others specify).....

Cocoa quality

29.0. What are /is the feature(s) of a good quality beans?

a) dryness [ ] b) flavour [ ] c) brown / chocolate [ ]

d) others (specify).....

30.0. What are the measures or strategies you adopt to obtain the good quality features you have mentioned in 29 above?

a) proper fermentation [ ] b) proper drying[ ] c) harvesting of mature ripe pods [ ] d) others (specify).....

31.0. Have you ever noticed any difference(s) in the contents of the ripe pods of the cocoa type you have on your farm?

a) yes [ ] b) no [ ]

32.0. If yes, what are the differences?.....

.....

33.0. Do these differences affect the fermentation of the wet beans on your farm? a) yes [ ] b) no [ ]

34.0. If yes, how do they affect fermentation of the wet cocoa?

.....

35.0. Have you ever seen purple colour in your dried cocoa beans when broken before the 2003/ 2004 main crop season?

a) yes [ ] b) no [ ]

36.0. If yes, since when?

a) 190 - 1980 [ ] b) 1981- 1990 [ ] c) 1991- 2000 [ ] d) 2001 to date [ ]

37.0. Have you ever been told by any marketing clerk that your cocoa contained high unacceptable levels of purple beans?

a) yes [ ] b) no [ ]

38.0. If yes, how do you recondition such beans?

a) re-fermentation [ ] b) picking [ ] c) Re-drying [ ]

d) others (specify).....

39.0. Have you noticed any colour change after re-conditioning purple beans?

a) yes [ ] b) no [ ]

40.0. If yes, what is the new colour?

a) partly brown/chocolate [ ] b) partly purple [ ] c) purple [ ]

d) brown/ chocolate [ ]

41.0. What are/is the cause(s) of the purple colour in the beans?

a) improper fermentation [ ] b) cocoa variety [ ] c) improper drying [ ]
d) application of agro- chemicals [ ] e) harvesting of unripe pods [ ]

f) others (specify).....

42.0. What is the colour of ideal dried quality cocoa beans when broken?

.....

II. (To be answered by Purchasing Clerks of Licensed Buying Companies)

A. background information.

43.0. Region:.....

44.0. District:.....

45.0. Sex:

a) Male [ ] b) Female [ ]

46.0 Age:

a) 18-30 [ ] b) 31-40 [ ] c) 41-50 [ ] d) Above 50 [ ]

47 Rank:

a) District Manager [ ] b) Depot Keeper [ ] c) Marketing Clerk I [ ]

d) Marketing Clerk II [ ] e) Purchasing Clerk [ ]

48.0. Nationality:

a) Ghanaian [ ] b) other (Specify).....

49.0. Length of service:

a) less than 6 [ ] b) 6-10 [ ] c) 11-15 [ ]

d) 16-20 [ ] e) 21-25 [ ] f) 26-30 [ ]

g) above 30 [ ]

50.0 What is your level of education?

a) no formal education [ ] b) basic education [ ]

c) second cycle [ ] d)tertiary [ ]

Pre-sale inspection

51.0. Do you carry out any pre-sale inspection of beans offered to you by your farmers?

a) yes [ ] b) no [ ]

52.0. If yes, how do you do it?

a) physical examination [ ] b) empirical and cut tests [ ]

c) no reason [ ] d) other (specify).....

53.0. What are some constraints associated with pre-sale inspection?

a) takes a lot of time [ ] b) very difficult task [ ]

c) fear of loosing farmers [ ] d) lack of inputs /money [ ]

e) no reason [ ] f) competition among L.B.C.'s [ ]

g. other (Specify).....

54.0. What are the possible solutions to the constraints you have stated in 53?

a) intensify education on the need for pre-sale inspection [ ]

b) empowerment of L.B.C.'s to confiscate inferior cocoa [ ]

c) soft loans for inputs [ ]

d) reduction in the number of L.B.C.'s [ ]

e) no reason [ ]

f) other (Specify).....

55.0. What quality parameters do you consider in determining quality of cocoa beans?

a) bean uniformity (size and colour) [ ] b) dryness [ ]

c) defects [ ] d) uniformity and dryness [ ]

e) dryness and defects [ ] f) uniformity, dryness and defects [ ]

g)other (specify).....

56.0. How do you assess the quality parameters you have stated in 55 above?

a) empirical test [ ] b) cut test [ ]

c) experience [ ] d) empirical and cut tests [ ]

e) empirical test and experience [ ] f) cut test and experience [ ]

g) empirical, cut tests and experience [ ]

h) others (specify) .....

Experience with purple beans

57.0. Have you ever seen purple colour in dried cocoa beans before 2003/2004 cocoa season?

a) yes [ ] b) no [ ]

58.0. If yes, since when?

a) 1960s [ ] b) 1970s [ ]

c) 1980s [ ] d) 1990s [ ]

e) 2000 to date [ ]

59.0. Have you ever considered purple colour in dried cocoa beans as a defect?.

a) yes [ ] b) no [ ]

60.0. If yes, since when?

a) 2000/2001 crop season [ ] b) 2001/2002 crop season [ ]

c) 2002/2003 crop season [ ] d) 2003/2004 crop season [ ]

61.0. How do you determine purple level in the dried beans presented to you by your farmers?

a) cut test [ ] b) experience [ ] c) empirical test [ ]

d) cut test and experience [ ] e) cut and empirical tests [ ]

f) experience and empirical test [ ] g) cut test, experience and empirical test [ ]

h) other (specify) .....

62.0. Can purple colour in the dried cocoa beans be changed through any form of re-conditioning?

a) yes [ ] (If yes, go to 2.10) b) no [ ]

63.0. If no, why?

a) no proven research on purple [ ] b) it is an intrinsic defect [ ]

c) no reason [ ]

d) other (specify).....

64.0. If yes, how can purple beans be reconditioned?

a) re-fermentation [ ] b) picking [ ]

c) re-drying [ ] d) re- fermentation and picking [ ]

e) re-fermentation and re-drying [ ] f) picking and re-drying [ ]

g) re- fermentation, picking and re-drying [ ]

h) other (specify).....

65.0. What are/is the cause(s) of the purple colour in the beans?

a) improper fermentation [ ] b) cocoa variety [ ] c) improper drying [ ]

d) application of agro- chemicals [ ] e) harvesting of unripe pods [ ]

f) others (specify).....

III. (To be answered by Quality Control Officers only)

a. Background information.

66.0. Region:.....

67.0. District:.....

68.0. Age group: a.18-30[ ] b.31-40 [ ] c. 41-50 [ ] d. above 50.

69.0. Sex: a) male [ ] b) female [ ]

70.0. Rank:.....

a) Q.C.A. [ ] b) S.Q.C.A. [ ] c) P.Q.C.A.[ ] d) C.Q.C.A. [ ]

71.0. Nationality:

a) Ghanaian [ ] b) other (specify).....

72.0. Length of service: a) less than 6 [ ] b)6 – 10 [ ] c)11 – 15 [ ] d)16 – 20 [ ] e)21 – 25 [ ] f)26 – 30 [ ] g) above30 [ ]

73.0. What your level of education?

a) no formal education [ ] b) basic education [ ]

c) second cycle [ ] d) tertiary [ ]

Quality assessment

74.0. What quality parameters do you consider in determining quality of cocoa beans?

a) bean uniformity (size and colour) [ ] b) defects [ ]

c) dryness [ ] d) beans uniformity and dryness [ ]

e) beans uniformity and defects f) dryness and defects [ ]

g) beans uniformity, dryness and defects

h)others (specify).....

Grading of purple beans

75.0. Have you ever seen purple colour in dried cocoa beans during cut test before the 2003/2004 cocoa season?

a) yes [ ] b) no [ ]

76.0. If yes, since when?

a) 1960s [ ] b) 1970s [ ] c) 1980s [ ] d) 1990s [ ] e) 2000 to date [ ]

77.0. Have you ever considered purple colour in the dried cocoa beans as a defect in determining cocoa grade?

a) yes [ ] b) no [ ] (If no, go to 2.7)

78.0. If yes, since when has purple colour in cocoa beans become a defect?

a) 2000/2001 season [ ] b) 2001/2002 season [ ] c) 2002/2003 season [ ] d) 2003/2004 season [ ] e) 2004/2005 [ ]

f) other (specify).....

79.0. How do you determine the purple defects in dried beans?

a) cut test [ ] b) empirical test [ ] c) by experience [ ]

d) empirical and cut tests [ ] e) empirical test and experience [ ]

f) cut test and experience [ ] g) cut, empirical tests and experience [ ]

h) other (specify).....

80.0. Why don't you consider purple beans defect in your quality assessment?

a) not part of defects taught in training school [ ]

b) not backed by any law [ ]

c) no reason [ ]

d) other (specify).....

81.0.Do you advice on re-conditioning of purple beans?

a) yes [ ] b) no [ ] (if no, go to 2.10)

82.0. If yes, what advices do you give?

a) re-fermentation [ ] b) picking [ ]

c) drying [ ] d) re- fermentation and picking

e) re-fermentation and re-drying [ ] f) picking and re-drying [ ]

g) re-fermentation, picking and drying [ ]

h) other (specify).....

83.0. Why don't you advice on reconditioning of purple beans?

a) it is intrinsic/ inherent b)could deteriorate other quality parameters [ ]

c) no reason

d) other (specify).....

84.0. What is /are the cause(s) of purple colour in dried in cocoa beans?

a) improper fermentation [ ] b) cocoa variety [ ] c) improper drying [ ]

d) application of agro- chemicals [ ] e) harvesting of unripe pods [ ]

f) others (specify).....

85.0. What is the highest level of purple beans you have graded and sealed during the 2004/2005 main crop season?

a)100[ ] b)200[ ] c)300[ ] d)400[ ] e)500 [ ] f) 600[ ] g) SS [ ]

86.0. What is the lowest level of purple beans you have graded and sealed during the 2004/2005 main crop seasons?

a)100[ ] b)200[ ] c)300[ ] d)400[ ] e)500[ ] f)600[ ] g) SS [ ]

87.0.What is the highest level of purple beans you have graded and sealed during the 2005 light crop season?

a)100[ ] b)200 [ ] c)300[ ] d)400[ ] e)500 [ ] f) 600[ ] g) SS[ ]

88.0. What is the lowest level of purple beans you have graded and sealed during the 2005 light crop season?

a) 100 [ ] b) 200[ ] c) 300[ ] d) 400[ ] e) 500[ ] f) 600[ ] g) SS[ ]

89.0. What do you suggest could be done to eliminate or reduce the purple

incidence in prepared cocoa beans?

a) Intensify education on proper primary processing of cocoa [ ]

b) Supply of materials for alternative fermentation methods [ ]

c) Other (Specify).....

Thank you!

APPENDIX 2

Analysis of variance for the mean Regional percentage purple beans occurrence for 2004/2005 cocoa season

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	37.850	18.925	1.49.22	0.2638
Regions	6	245.818	40.970	3.2304	0.0397
Error	12	152.189	12.682		
Total	20	435.858			

APPENDIX 3a

Analysis of variance for the mean percentage purple beans occurrence of beans obtained from pods stored at different periods and fermented for five days

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	31.600	15.800	3.6046	0.0765
Pod storage	4	123.733	30.933	7.0570	0.0098
Error	8	35.067	4.383		
Total	14	190.400			

APPENDIX 3b

Analysis of variance for the mean percentage purple beans occurrence of beans obtained from pods stored at different periods and fermented for six days

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	4.800	2.400	0.4126	
Pod storage	4	431.067	107.767	18.5272	0.0004
Error	8	46.533	5.817		
Total	14	482.400			

APPENDIX 3c

Analysis of variance for the mean percentage purple beans occurrence of beans obtained from pods stored at different periods and fermented for seven days

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	6.400	3.200	1.7944	0.2271
Pod storage	4	755.333	188.833	105.8879	0.0000
Error	8	14.267	1.783		
Total	14	776.000			

APPENDIX 4a

Analysis of variance for the mean total anthocyanins contents in unfermented (day 0) dry cocoa beans

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	0.008	0.004	0.0083	
Cultivars	14	137.478	9.820	20.3613	0.0000
Error	28	13.504	0.482		
Total	44	150.990			

APPENDIX 4b

Analysis of variance for the mean total anthocyanins contents in fermented (6 days) dry cocoa beans

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	0.351	0.175	0.7523	
Cultivars	14	4.619	0.330	1.4161	0.2099
Error	28	6.524	0.233		
Total	44	11.494			

APPENDIX 4c

Analysis of variance for the mean total anthocyanins contents in fermented (7 days) dry cocoa beans

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	0.089	0.045	2.6989	0.0848
Cultivars	14	2.623	0.187	11.3066	0.0000
Error	28	0.464	0.017		
Total	44	3.176			

APPENDIX 5a

Analysis of variance for the mean percentage purple beans occurrence in fermented (6 days) dry cocoa beans

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	4.578	2.289	1.0785	0.3538
Cultivars	14	8962.578	640.184	301.6574	0.0000
Error	28	59.422	2.122		
Total	44	9026.578			

APPENDIX 5b

Analysis of variance for the mean percentage purple beans occurrence of fermented (7 days) dry cocoa beans

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F- value	Probability
Replication	2	6.578	3.289	1.4674	0.2477
Cultivars	14	1011.911	72.279	32.2493	0.0000
Error	28	62.756	2.241		
Total	44	1081.244			

APPENDIX 6

Correlation coefficient between the occurrences of purple beans and total anthocyanins content in some cocoa cultivars

	Purple beans count (Dependent)	Total anthocyanin (Dependent)
Cultivars (Independent)	0.993**	1.000**

** *Correlation is significant at 0.01 levels.*

APPENDIX 7

Percentages for the mean total purple; mouldy; slaty; and other defective beans occurrence in districts surveyed in 2004/2005 cocoa season

Districts	Total purple	Mouldy	Slaty	Other defects. (germ, weevil, flat b
New Edubiase	25.7	0.3	1.7	0.7
Obuasi	34.7	0.3	1.7	0.3
Antoakrom	38.7	0.0	1.7	0.3
Sankore	23.7	0.0	1.7	1.0
Kukuom	31.0	0.3	1.3	0.3
Goaso	29.7	0.0	1.0	0.7
Cape Coast	31.7	0.0	1.0	0.7
Assin Breku	26.0	0.0	1.3	0.3
Twifo Praso	26.3	0.3	0.7	0.0
Suhum	33.3	0.0	1.3	1.0
Kade	32.7	0.0	1.7	1.0
Nkawkaw	29.0	0.0	1.7	1.0
Awaso	30.3	0.0	0.7	0.7
Sefwi Akotombra	35.0	0.0	1.0	0.3
Juabeso	38.0	0.0	1.7	1.0
Wassa Akropong	31.0	0.7	1.3	1.0
Agona Amenfi	32.7	0.3	1.0	0.3
Asankragwa	34.0	0.0	1.3	0.0
Hohoe	39.3	0.3	1.7	0.3

APPENDIX 8

Percentages for the mean total purple; mouldy; slaty; and other defective beans as a result of changes in rainfall; relative humidity; temperature and sunshine hours

Month	Total purple	Mouldy	Slaty	Other defects (germinated, weevil, flat b
February	30.3	0.0	0.7	0.0
March	31.3	0.0	0.3	0.0
April	40.3	0.3	0.7	0.0
May	42.7	0.0	1.3	0.0
June	26.3	0.0	0.0	0.3
July	22.0	0.0	0.3	0.0
August	34.0	0.3	0.3	0.0