EFFECT OF MECHANICAL DEPULPING ON THE BIOCHEMICAL, PHYSICOCHEMICALAND POLYPHENOLIC CONSTITUENTS DURING FERMENTATION AND DRYING OF GHANAIAN COCOA BEANS

BY

DANIEL TETTEH OTU AMANQUAH

(10220709)

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DECLARATION

I declare that this thesis presented herein was conducted by me under supervision at the Department of Nutrition and Food Science, University of Ghana, Legon in Accra, Ghana. All references have been duly cited and listed.

Daniel Tetteh Otu Amanquah

(Student)

Supervisory Committee

Prof. Emmanuel Ohene Afoakwa

(Principal Supervisor)

Dr. Agnes Budu

(Co-supervisor)

DEDICATION

This treatise is dedicated to God Almighty for His protection and grace, to my parents Dr. Seth Amanquah and Mrs. Margaret Amanquah for their continuous commitment and faithfulness towards my education and the last but not the least to my siblings Richard Amanquah and Margaret Corquaye for their support and prayers.

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ABSTRACT

This work investigated the effects of mechanical depulping (asa means of pulp pre-conditioning) on thechemical, physicochemical and polyphenolic constituents during fermentation and drying of Ghanaian cocoa beans. The fermentation study and the drying study were conducted using a 5x4 factorial experiment with the principal factors being;(a) concentration of depulped beans (0, 25, 50, 75 and 100%),(b) fermentation time (0, 2, 4 and 6 days) and(a) concentration of depulped beans (0, 25, 50, 75 and 100%), (b) drying time (0, 2, 4 and 6 days) respectively. Ripe cocoa pods were depulped using a mechanical depulper and the depulped beans mixed with undepulped beans in a pre-determined ratio prior to fermentation. All parameters were studied using standard methods.

Mechanical depulping and fermentation influenced all the studied parameters.Protein content decreased from 16.23% at the beginning of fermentation to 13.64% by the sixth day of fermentation in undepulped beans.Potassium was the dominant mineral and iron the least.Depulping caused a decrease in the potassium (K) content from 1000.43 mg/100g at the start of fermentation to 718.18 mg/100g at the end of fermentation in undepulped beans. Changes in biochemical composition and physicochemical properties were variable during fermentation of the depulped beans. The pH decreased from 6.5 to 6.3 for samples containing 0% to 100% respectively at the beginning of fermentation.Depulping caused slight increases in pH from 5.7 at the end of fermentation in 0% depulped beans to 6.02 at the end of fermentation in samples containing 100% depulped beans. Reducing sugar increased from 157.33 mg/g at the start of fermentation to 304.95 mg/gbythe sixth day of fermentation in 0% depulped beans.Depulping caused slight decreases in reducing sugar content. Increasing fermentation and depulping resulted in an increase in FFA. The FFA

content increased from 0.89% in 0% depulping to 0.94% in samples containing 25% depulped beans.All thetreatments (0%, 25%, 50%, 75% and 100%) depulped beansobtainedfermentation index of one (1) by the forth (4^{th}) day of fermentation.Total polyphenols and *o*-diphenols reduced with respect to depulping and fermentation time.Total polyphenols decreased from 31.29 mg/gat the start of fermentation to 22.23 mg/gat the end of the fermentation process in undepulped beans.Depulping caused a reduction in total polyphenols from 22.23 mg/g in undepulped beans to 19.9 mg/gin 25% depulped beans.

Depulping of cocoa beans caused an increase in pHfrom 5 to 6 during drying. Depulping caused a slight increase in pH from 5.46 by the fourth day of fermentation in undepulped beans to 5.78 by the fourth day of fermentation in samples containing 100% depulped beans, however the FFA content after drying was far below the stipulated 1.75%.The anthocyanins content of the beans varied significantly with increasing dryingtime and depulping. It decreased from 7.60 mg/gat the start of drying to 5.35 mg/gby the fourth day of drying in undepulped beans.

Drying of cocoa beans showed no distinct trendfor lightness (L), redness (a) and yellowness (b) for all levels of depulped beans.

Cut test on the samples showed that percentage of purple beans generally reduced with increasing depulping.

These findings suggest that mechanical depulping influenced to a varying degree the chemical, biochemical and polyphenolic content of fermented and dried Ghanaian cocoa beans.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Cocoa beans (*Theobroma cacao*), which are the primary raw material for the manufacture of chocolate and other confectionary products depend predominantly on fermentation as a critical step as well as other important processes such as drying and roasting to develop its unique flavour (Beckett, 2009; Afoakwa, 2010). Cocoa is noted for its flavour properties due to aroma precursors inherent in the beans, which are formed during fermentation and drying and are transformed during roasting into various flavour characteristics. Cocoa is processed into other semi-finished products such as cocoa butter, cocoa liquor, cocoa cake and cocoa powder for further processing into a wide variety of products such as chocolate, sweetened cocoa powder, cakes, alcoholic beverages, wines, soaps and cosmetics (Amoa-Awua *et al.*, 2006; Afoakwa and Paterson, 2010).

Cocoa is the main cash crop in Ghana and also the major economic crop in most West African countries. Currently, Africa is the world's largest producer of cocoa with an estimated 71% of the world's production, and accounted for 2.826 of 4.0 million tonnes of cocoa in 2011/2012 projections (ICCO, 2012a). Ghana however, continues to maintain its position as the second largest exporter of cocoa after Ivory Coast with a record high of 1,004,190 tonnes in 2010/2011 (Ashitey, 2012).Chocolate manufacturers recognize the Ghanaian cocoa beanas the standard for bulk cocoa with respect to chocolate flavour and due to this, it commands a premium price on the international market (Fowler, 2009; Afoakwa and Paterson, 2010).

In Ghana,cocoa is usually cultivated by smallholder farmers and some individuals on small to large scale;mainly in the Eastern, Western, Ashanti, Brong Ahafo, Central and Volta

regions (Baker *et al.*, 1994). There are two main harvesting seasons in Ghana, the main crop is harvested in October to December and the mid crop is harvested in March (Afoakwa, 2010). The predominant cultivars of cocoa grown in Ghana include Amelonado,Upper Amazon (Amazonica) selections and Hybrid (Amelonado and Amazoinca) cocoa (Amoa-Awua *et al.*, 2006; Afoakwa, 2010).

Fermentation is a major postharvest treatment, which involves a series of chemical, biochemical, physicochemical and polyphenolic changes due to the breakdown of sugars, proteins and polyphenols. These changes are due to various activities of microorganisms such as yeasts, lactic acid bacteria and acetic acid bacteria and also a myriad of enzymatic reactions by proteases, invertase and polyphenol oxidases (Thompson *et al*., 2001; Schwan and Wheals, 2004;Nielsen*et al*., 2007).The pulp of the cocoa bean acts as a substrate for microorganismsduring fermentation due to its high sugar content. In a sequential order of yeasts and bacteria they consume the pulp and produce acids and heat in the process, and consequently leading to the eventual death of the bean (Nielsen *et al.*, 2007; Beckett, 2009; Afoakwa, 2010). The changes that occur during fermentation as observed by Afoakwa *et al*. (2012a) has consequential effect on the nib acidification, reduction in bitterness and astringency, colour development and flavour precursor formation as compared to unfermented cocoa beans.

Drying of cocoa beans is imperative to prevent postharvest losses such as the growth of mould and infestation from insects (Thompson *et al*., 2001; Afoakwa, 2010). In Ghana the common method used is the drying of cocoa beans on raised platforms covered with mats made of bamboo (Tomlins *et al*., 1993). Moisture content of the beans rise to about 40 - 50% during fermentation (Thompson *et al*., 2001), and therefore needs to be reduced to about 7 - 8% during drying as the higher moisture content presents great economic and safety issues to the beans (Beckett, 2009; Afoakwa and Paterson, 2010). The beans are usually fully dried

within a week with regular sunshine, but this can be protracted to about two or more weeks with cloudy and rainy weather. Drying also plays an all-important role in flavour formation. Afoakwa (2010) stated that during drying majorpolyphenol oxidizing reactions are catalyzed by polyphenol oxidases giving rise to newflavour components. Sun drying allows for slow migration of moisture throughout the bean, which transports flavour precursors that has been formed during fermentation (Thompson *et al*., 2001).

Prior to the fermentation process, harvested cocoa pods areusually stored for a few days before they are opened, and this is referred to as pod storage. This practice is performed by farmers unknowingly of its importance. The technique of pod storage has been identified as a means of pulp pre-conditioning of cocoa beans prior to fermentation and has been reported to have beneficial effect on the cocoa bean colour and flavour (Nazaruddin*et al*., 2006; Afoakwa *et al*., 2011a; Afoakwa *et al*., 2012a). Pulp pre-conditioning can also be done by either depulping (mechanical or enzymatic) or bean spreading (Rohan, 1963; Schwan and Wheals, 2004; Afoakwa *et al*., 2011a) and these techniques might have important bearing on the chemical, biochemical and polyphenolic constituents of fermented Ghanaian cocoa beans. However, the use of mechanical depulping as means of pulp preconditioning of Ghanaian cocoa beans is yet to be fully exploited

1.2 Rationale

Cocoa fermentation and drying are critical to the development of quality beans and these are influenced by factors such as pulp pre-conditioning, fermentation method and drying conditions.Several works have been done on pod storage as a means of pulp pre-conditioning cocoa beans. Findings from these works have established that pod storage as a means of pulp pre-conditioning prior to fermentation lead to reduction in pulp volume, bean acidity and fermentation time; resulting in enhanced cocoa bean flavours(Nazaruddin*et al*., 2006; Afoakwa *et al*., 2011ab; Afoakwa *et al*., 2012ab).

Excessive pulp covering the beans leads to high acid production that is sometimes detrimental to the quality of the cocoa beans (Afoakwa and Paterson, 2010). Mechanical depulping is one of three ways of pulp pre-conditioning cocoa beans prior to fermentation and is employed to separate a portion of the pulp content of the fresh beans to reduce the amount of pulp available as fermentation substrate.The process causes bruising of the beans and its inherent cell structures leading to activation of enzymes, which might influence various biochemical processes during fermentation.However, the extent to which this technique of pulp pre-conditioning would influence the chemical, biochemical, physicochemical and polyphenolic constituents of the cocoa bean during fermentationis yet to be fully exploited and comprehended. To date,no workhas been done to investigate the use of mechanical depulping as a form of pulp pre-conditioning and the extent to which this techniquewould influence the quality of Ghanaian cocoas.

1.2 Main objective

To investigate the effect of mechanical depulping on the biochemical, physicochemical and polyphenolic constituents of Ghanaian cocoa beans during fermentation and drying.

1.3 Specific objectives

The specific objectives of the study were:

i. To evaluate the effect of mechanical depulping on the chemical composition of fermented Ghanaian cocoa beans.
ii. To investigatethe influence of mechanical depulping on the biochemical and physicochemical composition during the fermentation process of Ghanaian cocoa beans.

iii. To determine the effect of mechanical depulping on the polyphenolic compounds concentration, fermentation index and appearance properties of Ghanaian cocoa beansduring fermentation.

iv. To determine the influence of mechanical depulping on the acidification, browning index, anthocyanins, colour and free fatty acids (FFA)levels during drying of fermented Ghanaian cocoa beans.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and classification of cocoa

Cocoa (*Theobroma cacao*) is known to have originated from Southern and Central America and is native to the Amazon and Orinoco valleys of South America (Thompson *et al*.,2001, Beckett, 2009; Afoakwa, 2010). History has it that about 600 AD ago the Maya and Aztecs were the first to grow and consume cacao, what is now widely known as cocoa (Beckett, 2009). The word *Theobroma* means food of the gods thus *theos*meaning 'god' and *broma* meaning 'food' (Afoakwa, 2010;Nair, 2010). The Spanish introduced cocoa to Europe and also to Fernando Po now called Bioko, an island in Equatorial Guinea in 1840 and by 1897 Tetteh Quarshie a Ghanaian, brought cocoa from Bioko and successfully cultivated it in Ghana. Cocoa grown in other West African countries such as Nigeria, Togo, Ivory Coast and others originated from Ghana (Nair, 2010).

There are about twenty-two (22) species of the genus *Theobroma* (Nair, 2010), out of which only one, *Theobroma cacao*, a member of the family *Sterculiaceae* is of economic importance (Thompson *et al*.,2001, Afoakwa, 2010;Nair, 2010). Other species according to Nair (2010), including *Theobroma bicolor*, *Theobroma angustifolium* and *Theobroma grandiflorum* are usually cultivated and consumed because of their sweet edible pulp and used as drinks in some parts of Brazil.Currently, there are four (4) types of commercial cocoa (*Theobroma cacao*) belonging to the family *Sterculiaceae* that are cultivated and these include;*Forastero*, *Criollo*, *Nacional* and *Trinitario* (Fowler, 2009; Afoakwa, 2010;Nair, 2010). The cocoa of commerce are grouped under two (2) broad umbrellas; the "bulk"and "fine" cocoa, which according to Lopez and Dimick (1995), the Forastero-Amelonado variety make up the "bulk" cocoa because of their strong cocoa flavour and "fine" cocoa constitutes Criollos, *Nacional* and Trinitarios which have mild and floral flavours.

The *Criollo*variety has a mild nutty cocoa flavour but is highly susceptible to diseases while *Trinitario*, a hybrid of *Forastero*and*Criollo* is more disease resistant (Beckett, 2009). The *Forastero* cocoa and its varieties are ubiquitous and widely grown,accounting for approximately 95% of the world cocoa production (Thompson *et al*.,2001;Afoakwa, 2010). The *Forastero* variety is also referred to as *Amelonado* because of the melon shape of its seeds. The cultivation of the *Nacional*cocoa variety is restricted to Ecuador. It is noted for its fine "Arriba" flavour (Beckett, 2009;Afoakwa and Paterson, 2010).

2.2 Cocoa cultivation

Prior to planting, certain environmental and edaphic conditions must be at their optimum for a successful growth of the cocoa plant. Temperature, rainfall and humidity are the three most important environmental factors that affect growth. According to Afoakwa (2010) and Fowler (2009), cultivation of cocoa requires temperatures ranging between 18–32˚C andrainfall within 1500 and 2500 mm well distributed throughout the year. During dry seasons or rainfall below 100 mm other sources for replenishing water lost via transpiration of the plant such as irrigation must be seriously considered to prevent the cocoa plant from wilting and from rapid senescence. During the early stages of the growth of cocoa plants they are mostly intercropped with other food crops such as plantain and oil palm to act as windshields and also for shade provision. The cocoa trees are grown to cover a total density of 600–1200 trees/acre (1500–3000 trees/ha), this ensures that humidity is typically maintained between the ranges of 70–80% duringthe day and 90–100% at night (Fowler (2009; Afoakwa, 2010).

Cocoa can grow in a vast array of soils, however edaphic factors such as pH must be neutral to partially acidic with a range of 5–7.5, and the soil must also possess a good drainage and well aerated system(Fowler, 2009; Afoakwa, 2010). The rooting of cocoa is that of the tap root system with few lateral roots hence the soil must be approximately 1.5 m deep

or more to facilitate the uptake of water and nutrients (Fowler, 2009; Nair, 2010). Cocoa husbandry, pest and disease control should be done meticulously to achieve substantial yield (Fowler, 2009; Afoakwa, 2010). Some pests and diseases include black pod, witches' broom, frosty pod rot, swollen shoot, capsids and mirids, and the cocoa pod borer.Squirrels, rats and monkeys also consume and destroy a chuck of ripe pods (Fowler, 2009; Nair, 2010).

The cocoa tree comes into fruition after two to three years of maturity and reach their full yield potential when it's six to seven years old (Fowler, 2009). With a growing height of approximately 10 m at maturity, harvesting is usually difficult especially with fruits growing at its apex. Afoakwa (2010) stated that"modern breeding methodshave led to the development of trees to a standard of approximately 3 m tall to allow for easy harvesting". The cocoa plant is said to have an economic viable life span of about 25 – 30 years (Fowler, 2009).After a successful pollination and fertilization, the pods reaching maximum and fully matureabout 140 days. The fruits are then allowed to ripen for about 10 days and the pods are harvested (Afoakwa, 2010; Fowler, 2009).The matured cocoa fruits measure between 100 and 350 mm long and have awet weight of approximately 200 g to 1 kg (Fowler, 2009). Colour change is a major factor in determining the ripeness of cocoa pods. However, due to genotypic differences there areconsiderable variations in the shape, surface texture and colour. Cocoa colour generally varies from green or purple to various shades of red, orange or yellow pods (Afoakwa, 2010).

2.3 Cocoa production, supply and consumption

2.3.1 Global production of cocoa

Global production of cocoa beans was reported to be 3.6 million tonnes in the 2009/2010 fiscal years, a marginal increase compared with the previous seasons (ICCO, 2012). Production also increased to about 4.3 million tonnes in 2010/2011 and a marginal decline in 2011/2012 with 3.9 million tonnes (Table 1). According to statistics from ICCO

(2012), cocoa beans from smallholder farms account for approximately 90–95% globally. In Ghana, mainly smallholder farmers undertake production of cocoa with only about 5% from large plantations such as those owned by the Government subsidiary, Ghana Cocoa Board (COCOBOD) via its institutions and some private investors. It is estimated that Ghana has a market share of about 28 - 31% second to the Ivory Coast which, has a market share of over 46.8% and thus Africa produces about 70 - 75% of the worlds cocoa (ICCO, 2012).

FromTable 1 below, productionof cocoa declined by 400,000 tonnes from 2010/2011 to 2.826 million tonnes in 2011/2012 in Africa, but increased by 15,000 tonnes in the Americas amounting to 574,000 tonnes while in Asia and Oceania, there was a drastic increase by66,000 tonnes to 590,000 tonnes.Nonetheless, Africa still remainsthe largest cocoa-producing region, accounting for 70.8% of world cocoa output in 2011 - 2012, followed by Asia and Oceania at 14.8% and the Americas at 14.4%.

While several factors affect cocoa production worldwide, drastic changes in the production of cocoa are largely affected by changes in weather conditionssuch as duration and intensity ofsunshine and rainfall as well as soil moisture and temperature (ICCO, 1997). One of such weather changes is known as the Southern Oscillation often referred to as ENSO (*El Niño*/*La Niña*-Southern Oscillation). It originates from the tropical Pacific and occurs on average every four years. *El Niño* and *La Niña* events are the extremes in a vast repeating cycle of large-scale fluctuationsin air pressure with *El Niño* being the warm extreme and *La Niña* the cold extreme (IRI, 2009).

Table 1: Global productionof cocoa beans in thousand tonnes from 2009 to 2012

Production	2009/2010	Estimates	Forecasts

Regions			2010/2011		2011/2012	
Africa	**2486**	**68.4%**	**3226**	**74.9%**	**2826**	**70.8%**
Cameroon	209		229		210	
Côte d'Ivoire	1242		1511		1410	
Ghana	632		1025		890	
Nigeria	235		240		220	
Others	168		221		96	
America	**516**	**14.2%**	**559**	**13.0%**	**574**	**14.4%**
Brazil	161		200		190	
Ecuador	150		161		175	
Others	205		199		209	
Asia & Oceania	**633**	**17.4%**	**524**	**12.2%**	**590**	**14.8%**
Indonesia	550		440		500	
Papua New Guinea	39		47		48	
Others	44		37		42	
World total	**3635**	**100.0%**	**4309**	**100.0%**	**3990**	**100.0%**

Source: ICCO (2012). Note: *Totals may differ from sum of constituents due to rounding.*

ICCO(1997) stated that the *El Niño* weather events reduced cocoa production, on average, by 2.4% at world level however due to this reduction, world cocoa prices increased by 1.66% on average. Theimpact of the *El Niño* event is not homogenous across cocoa producing countries. Cocoa production in Ghana, Nigeria and Cameroon are not systematically affected by*El Niño* events (IRI, 2009).

2.3.2 Cocoa production in Ghana

Cocoa is produced in Ghana mainly in the forest areas, thus, Western, Ashanti, Brong-Ahafo, Central, Eastern, and Volta regions, where the rainfall is usually between 1,000 and 1,500 mm per year (Ashitey, 2012).In a bid to establish sustainable ways to increase yield and improvequality of cocoa beans, the government of Ghana via concerted effortshas empowered its institutions such as COCOBODand its subsidiaries including Cocoa Research Institute of Ghana (CRIG) and major stakeholders including farmers and some private bodies

to provide extension services to the farmers (Ashitey, 2012).The purpose of these extension services is to improve agronomic practices, provide fertilizer and chemicals for pest and disease control.The Government is also committed to higher remuneration for farmers as an incentive for high productionand also providing funds for the rehabilitation and replanting of old farms. As well, institutions such as CRIG and other research stations have the mandate to develop hybrid cocoa seedlings with higher yields.

2.3.4 Cocoa supply chain in Ghana

Despite several interventions by the government of Ghana, cocoa farmers in Ghana continue to rely on the traditional methods such as the use of hoes and cutlasses for farming. Mohammed *et al*. (2012) reported that competition among farmers is non-existent becausegovernment of Ghana is the sole exporter of cocoa. Ghana cocoa value chain consists of three (3) main players; farmers, license buying companies (LBCs) and COCOBOD. Like most cocoa producing nations in Africa and Asia, cocoa is a major contributor to Ghana's gross domestic product (GDP) and foreign exchange earner for the country.

Cocoa production in Ghana and most West African countries is mainly on smallholder or family farms usinglabour-intensive methods.The vast majority of labour employed oncocoa farms in Ghana is adult and it can be either fulltime or casual basis (Beckett, 2009). During busy harvest periods children are made to help with farm work such as plucking of cocoa pods and carrying baskets of cocoa pods to breaking sites. In as much as cocoa growing provides significant benefits to the Ghanaian rural economies and the national economy at large, much can be done to improve the conditions and livelihood of cocoa farmers since majority of these farmers continue to use primitive farming methods (Mohammed *et al*., 2012).

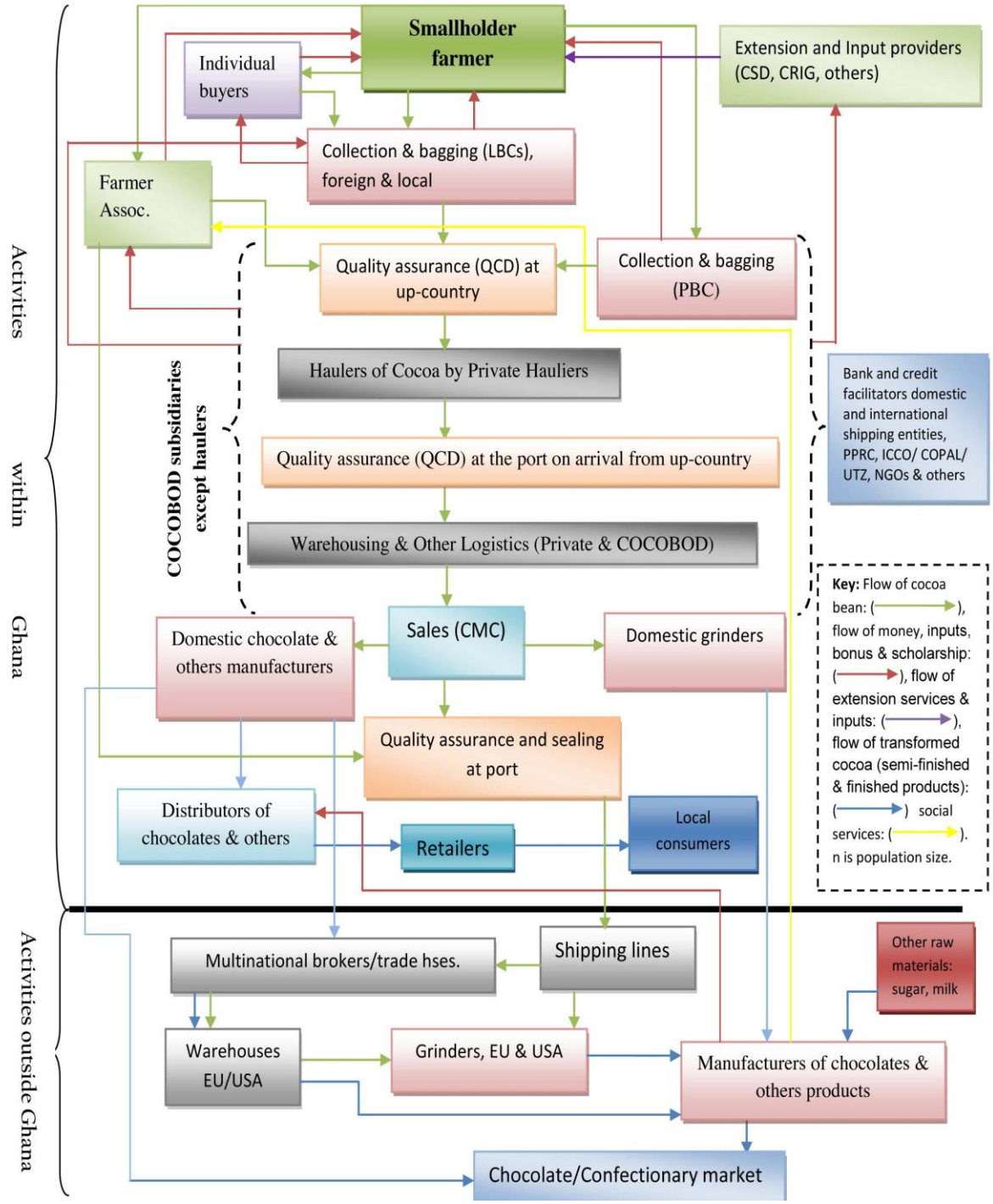

Figure 1: Value Chain of Ghana cocoa

Source: Mohammed *et al.* (2012)

From the detailed map of Ghana's cocoa value chain (Figure 1), the smallholder farmers after fermentation and drying of their cocoa beans sell their dried cocoa beans to the cocoamarketing company (CMC) via the license buying companies (LBCs). However there

are some individuals (middle men) who are not aligned to any institution who buy from the farmers when they need money and they intend sell to the LBCs.Quality control checks are conducted on all cocoa by the Quality Control Division of COCOBOD.

2.2.4 Cocoa Commerce: demand, consumption and market for cocoa

Grindings of cocoa beans serves as a proxy for the estimation of the demand for the commodity across the world. Cocoa processing, or grinding, entails the transformation of dried cocoa beans into a variety of processed products including cocoa paste or liquor, cake, powder and butter (Afoakwa, 2010).The foregoing discussion gives a view that two-thirds of the world total grindings and consumption of cocoa beans occurs outside the production zones, demand is highest in Europe with 39.1% and the Americas with 21.8% (www.worldcocoafoundation.com). Nonetheless due to the increase in demand and consumption, grindings has also increased in some producing countries such as Ghana and Ivory Coast of which has necessitated the expansion of Cargill Cocoa and Chocolate processing factory in the later (www.cargill.com).

Due to a growing number of Fairtrade and organic crop organizations around the world especially in Europe and America, there has been a marked increase in the demand and consumption of specialty cocoa products. Current market trend shows that Fairtrade cocoa commands a very small share of the cocoa market (0.5%). The typical significance of Fairtrade cocoa is that the farmers via their respective organizations receive a higher price for their cocoa beans.It is calculated on the basis of world market prices, plus Fairtradepremiums. The Fairtrade premium for standard quality cocoa is US$ 150 per tonne. The minimum price for Fairtradestandard quality cocoa, including the premium, is US$ 1,750 per tonne. However, based on the steady growth of Fairtrade and support from activists and the general public, some Fairtradeparticipants claim that the idea were eventually grown from a niche market and become more mainstream (ICCO, 2013).

The organic cocoa market represents a very small share of the total cocoa market, estimated at less than 0.5% of totalproduction. ICCO (2012) projectionsfor production of certified organic cocoa at 15,500 tonnes, mainly from the following countries:Madagascar, Tanzania, Uganda, Belize, Bolivia, Brazil, Costa Rica, Dominican Republic, El Salvador, Mexico, Nicaragua,Panama, Peru, Venezuela, Fiji, India, Sri Lanka and Vanuatu.

However, similar to Fairtrade cocoa, the demand for organic cocoa products is growing at a very strong pace, as consumers are increasingly concernedabout the safety of their food supply along with other environmental issues chiefly global warming. Certified organic cocoa producers must comply with all requirements associated with the legislation of importing countries onproduction of organic products. The benefit for cocoa farmers is that organic cocoa commands a higher price thanconventional cocoa, usually ranging from US$ 100 to US$ 300 per tonne. It is inspiring to note that originating countries with smaller volumescan fetch much higher premiums. This premium should cover both the cost of fulfilling organic cocoa production requirementsand certification fees paid to certification bodies (ICCO, 2012).

Table 2: Grindingsof cocoa beans in thousand tonnes from 2009 to 2012

Grinding Regions	2009/ 2010		Estimates 2010/2011		Forecasts 2011/2012	
Europe	**1524**	**40.8%**	**1615**	**41.1%**	**1536**	**39.0%**
Germany	361		439		420	

Netherlands	525		540		510	
Others	638		636		606	
Africa	**685**	**18.3%**	**658**	**16.7%**	**717**	**18.2%**
Côte d'Ivoire	411		361		440	
Ghana	212		230		215	
Others	61		67		62	
America	**815**	**21.8%**	**860**	**21.9%**	**839**	**21.3%**
Brazil	226		239		240	
United States	382		401		385	
Others	207		219		214	
Asia & Oceania	**708**	**19.0%**	**795**	**20.2%**	**850**	**21.6%**
Indonesia	130		190		240	
Malaysia	298		305		295	
Others	280		299		315	
World total	**3731**	**100.0%**	**3927**	**100.0%**	**3941**	**100.0%**
Origin grindings	**1527**	**40.9%**	**1598**	**40.7%**	**1693**	**43.0%**

Source: ICCO (2012). ***Note: Totals may differ from sum of constituents due to rounding.***

Ghana processes about 215 thousand tonnes (Table 2) (about 40 percent of its cocoa bean production) domestically and exports the processed materials (ICCO, 2012).

2.3.1 Pulp composition

The pulp is white, sweet and a mucilaginous substance surrounding the beans. It is the fermentation substrate and its composition is therefore a critical factor on the outcome of fermentation (Beckett, 2009; Thompson *et al.*, 2001; Nielsen, 2006; Afoakwa, 2010). Water dominates its composition with about 82 – 87% with the remaining percentage consisting of approximately 10 – 15% sugars (glucose, fructose and sucrose), 2 – 3% pentosans, 1 – 3% citric acid and 1 – 1.5% pectin (Fig. 4) (Lopez and Dimick, 1995; Afoakwa, 2010). This composition clearly makes the pulp an ideal medium for a medley of microorganisms to

proliferate. Proteins, amino acids, vitamins (predominantly vitamin C) and minerals are in the minority.

Earlier research conducted on Ghanaian *Forastero* cocoa by Nielsen (2006) noted that the main sugars of the fresh pulp were glucose (5.4 – 6.6%) and fructose (6.3 – 7.4%) with only small amounts of sucrose (less than 0.3%) present.The glucose and fructose (monosaccharide's) to sucrose ratio is a function of the maturity of the cocoa fruit thus changes with the degree of maturity withunripe pods containing a higher proportion of sucrose and ripe pods containing mainlyfructose and glucose. This clearly suggests that pulp preconditioning (pod storage) plays a central role in the permutation of pulp composition (Afoakwa *et al.*, 2012). The pulp is not only viscous due to its sugar content but also a relatively high content of pectin and other polysaccharides 1 – 2% (Nielsen, 2006 and Afoakwa, 2010).

With a relatively low pH of the fresh pulp of about 3.94 – 4.12, which might be due to the citric acid content, approximately 0.6 – 0.7% and absent or only low amounts (less than0.2 %) of acetic acid, lactic acid and ethanol were detected in the fresh pulp (Nielsen, 2006).

2.3.2 Bean composition

The size of cocoa beans is of practical significance as flat beans are not viable. The minimum average bean size is 1 g (Nair, 2010). A transverse section via a cocoa beanrevealstwo cotyledons (nibs) and a small germ or embryo,all enclosed in a leathery seed coat or testa (shell) (Figure 2), which is impermeable to large molecules however, small molecules such ethanol and acetic acid are capable of diffusing into the bean.The cotyledons have two main functions, as thestorage organs containing nutrients for the development of the seedling and as the premier leaves of the plant when the seed germinates (Nielsen, 2006; Nair 2010; Afoakwa, 2010).

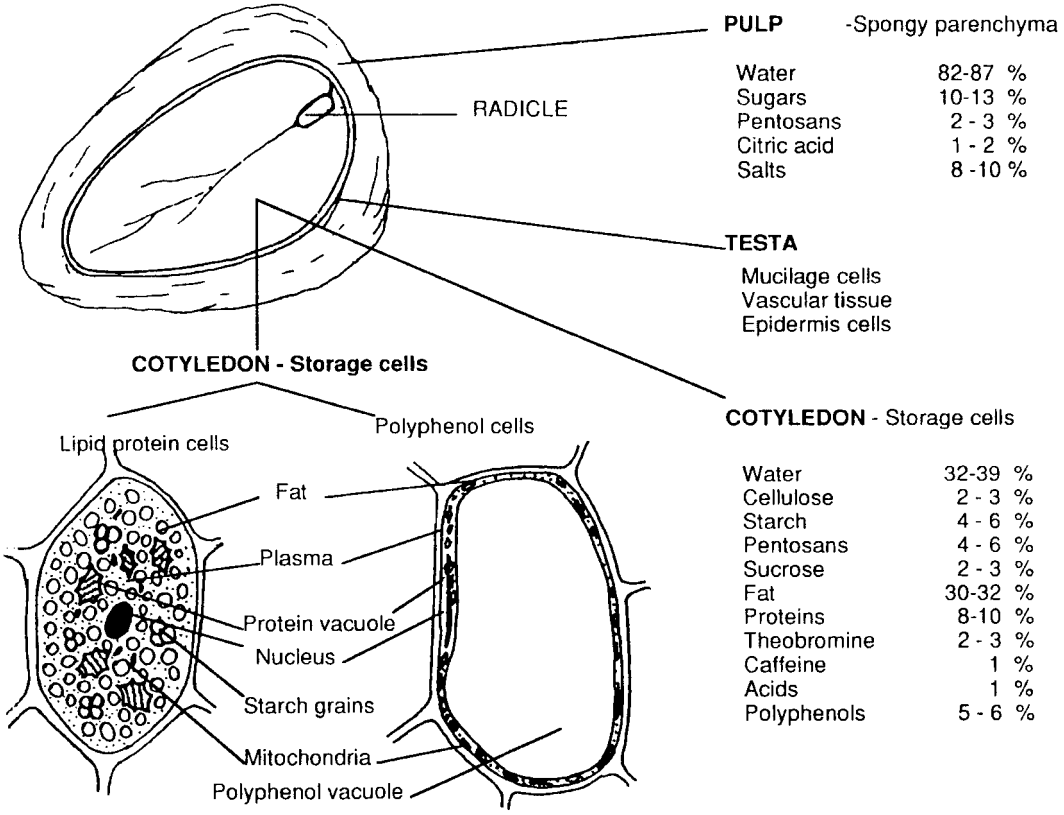

Figure: 2.Anatomy of the cocoa seed

Source:Lopez and Dimick(1995)

Fresh Forastero beans are violet in cross-section but upon proper fermentation attain the deep brown colour, which give chocolate its colour and produces a strong cocoa flavour uponproper processing (Lopez and Dimick1995; Nielsen, 2006; Afoakwa, 2010).Thecotyledons are basically made up of different types of storage cells, polyphenolic cells contain a single large vacuole filled with polyphenols and alkaloids and on the other hand, cells tightly packed with multiple small protein and lipidvacuoles and other components such as starch granules (Figure 2). All these components serve as a harbinger for cocoa flavour and aroma characters (Nazaruddin *et al*., 2001; Afoakwa, 2010).

2.3.3 Chemical composition of unfermented cocoa beans

2.3.3.1 Fat

Cocoa butter or fat constitutes about half the weight of the cocoa bean and its quantity and quality are critical to chocolate manufacture. Ranging from 45 – 55% the majority of the lipid profile exists as triacylglycerides (95%) with a minute amount of approximately 5% existing as mono- and di-glycerides, glycolipids, sterols and phospholipids (Belitz *et al.*, 2009). From previous researches it has been established that the general fat content ranges from 50.40 – 53.35%and 52.27 – 55.21% respectively for the pulp preconditionedfermented and unfermented beans (Afoakwa *et al.*, 2011a).

2.3.3.2 Proteins

Researchers over the decades have ascribed cocoa flavour during roasting to proteins as a flavour precursor. Proteins are very important for quality chocolate products and also for flavour development, with about 1.5 – 2% of the dried beans (Belitz *et al.*, 2009; Afoakwa, 2010). The storage proteins found in the cotyledon of the cocoa bean are globulins and albumins.

During fermentation, the degradation of proteins occurs partly by proteolysis to peptides andamino acids and partly by conversion to insoluble forms by the actions of polyphenols. The globulins are degraded in the fermentation process whiles the albumin is unaffected (Spencer and Hodge, 1992; Voigt *et al.*, 1993). Free amino acids in fermented beans originate from those present natively in the unfermented bean and from proteolysis of proteins during fermentation (Kirchhoff*et al.* 1989).

Table 3: Typical composition of the unfermented Forastero cocoa beans

Constituents	Dried beans (%)	Fat-free material
Cotyledons	89.60	-

Shell	9.63	-
Germ	0.77	-
Fat	53.05	-
Water	3.65	-
Ash (Total)	2.63	6.07
Nitrogen		
Total nitrogen	2.28	5.27
Protein nitrogen	1.50	3.46
Theobromine	1.71	3.95
Caffeine	0.085	0.196
Carbohydrate		
Glucose	0.30	0.69
Sucrose	1.58	3.86
Starch	6.10	14.09
Pectins	2.25	5.20
Fibre	2.09	4.83
Pentosans	1.27	2.93
Mucilage and gums	0.38	0.88
Polyphenols	7.54	17.43
Acids		
Acetic (free)	0.014	0.032
Oxalic	0.29	0.67

Sourcedfrom: Rohan (1963); Reineccius *et al*. (1972) and Afoakwa 2010

2.3.3.3 Sugars

Earlier research by Reineccius *et al*. (1972) revealed that fresh unfermented cocoa beans contained15.8 mg/g sucrose and trace amounts of fructose, sorbose, mannitol and inositol. During fermentation, most or all sucrose are hydrolysed by invertase, which is native to the cocoa bean to glucose and fructose. Afoakwa *et al.* 2011b noted that during fermentation, both the non-reducing andtotal sugars decreased significantly to 2.03 and 5.02mg/g (89 and 75%) reduction respectively. The decreases are an indication of the productionof reducing sugars however, therate of decrease in both the total and non-reducingsugars slowed down towards the end of day six (6) of fermentation.The reducing

sugars, fructose and glucose form about 0.9 and 0.7 mg/g, respectively and others (including mannitol and inositol) at less than 0.50 mg/g.Differences have been attributed to method and time of harvesting, type and origin of cocoabeans (Afoakwa and Paterson, 2010).

2.3.3.4 Polyphenols

Depending on its anthocyanincontent, the colour of the cocoa bean ranges from white to deep purple prior to fermentation (Belitz *et al.*, 2009; Afoakwa, 2010). Osman *et al.*(2004) ascribed that catechins (flavan-3-ols) and procyanidins are the predominant polyphenols stored inpigment cells within the cotyledon of the cocoa bean they have antioxidant properties that help protect the seed from damage and disease. Polyphenols account averagely for 14–20% of bean weight and play a pivotal role in flavour development as higher amounts may lead to astringent and bitter cocoa products (Belitz*et al.*, 2009; Afoakwa, 2010; Afoakwa and Paterson, 2010).

During fermentation, proteolysis occurs partly by conversion to insoluble forms by the actions of polyphenols. The enzyme polyphenol oxidase (PPO) promotes oxidative browning to give the characteristic chocolate brown colour, there is also the further reactions with sugar and amino acids that imparts flavour and colour while alkaloids contributes bitterness to the cocoa bean (Nazaruddin *et al.*, 2006; Afoakwa, 2010).

Constituents of the polyphenols include; catechins (flavan-3-ols) accounts for approximately 37%, anthocyanins and proanthocyanidins with approximately 4% and 58% respectively. The predominant catechin is (−) epicatechin, up to 35% of total polyphenols. Occurring in a rather less quantity is (+) catechin with traces of (+) gallocatechin and (−) epigallocatechin (Afoakwa, 2010; Afoakwa *et al.*, 2012b).Afoakwa (2010) stated that fat-soluble polyphenols in dried fat-free fresh *Forastero* cocoa form 15–20%, which falls to approximately 5% after fermentation. Contents of 10% or greater are considered a sign of poor fermentation.

2.3.3.5 Organic acids

A medley of the organic acids totaling 1.2 – 1.6% of the bean is dominated by citric, acetic,succinic and malic acid that contribute to the taste of cocoa. They are formed during fermentation and citric acid is predominant in fresh cocoa beans with 0.3 – 2.0% (Belitz *et al.,* 2009). The amount of acetic acid released bythe pulp and partly retained by the bean cotyledonsdepends on the duration of fermentation andon the drying method used. Belitz *et al.* 2009 noted that eight brands of cocoawere found to contain 1.22 – 1.64% total acids,0.79 – 1.25% volatile acids and 0.19 – 0.71% aceticacid.

2.4 Primary processing of cocoa beans

Before cocoa beans attain its commercial viability it must have gone through some major post harvest processes that have a profound impact on the quality of the cocoa beans. Harvesting, pulp pre-conditioning, fermentation and drying and the major and most critical primary processing that must be done meticulously to avert under fermented and astringent beans.

2.4.1 Harvesting

The transformation from raw cocoa beans into dried cocoa beans begins with the primary process of harvestingwhich is normally carried out over aperiod of 3 to 4 days, at intervals of 3 to 6weeks. This practice will vary according to thesize of the farm and pod yield (Lopez and Dimick, 1995). Ripe pods, which the farmer must assess via the external colour, are detached from the bark of the tree using sharp knives, secateurs and machetes. Those out of armsreach are harvested with special long-handledtools with a sharp edge. Care is taken to avoid damage to theflower cushions that would reduce futureyields.

The harvested pods are transported to the a fermentary where they are broken most often in Ghana with machetes to open and reveal theseeds for removal (Knight, 2001) In

Ghana, harvesting is done in two main batches with October to February being the main crop and from May to August which is the mid crop (Amoa-Awua *et al.*, 2006).

Ripeness of the pods is a very important factor for proper fermentation as a lack of control thus poor judgment of the maturity and ripeness of the pods will lead to the harvesting of immature and not fully ripen will cause variations in the quality of cocoa beans after fermentation and drying.

2.4.2 Pulp-preconditioning

There are three basic treatments that have been evaluated for the post harvest treatment of cocoa beans prior to fermentation. This includes pod storage, mechanical depulping and enzymatic depulping (Schwan and Wheals, 2004). Due to high acidity of beans from some producing areas such as Malaysia these treatment methods were developed in an attempt to reduce the problem of acidity in dried fermented cocoa beans. This problem of high acidity has been linked to the excessive production of lactic and acetic acid resulting from fermentation (Rohan, 1963). From earlier research conducted, the removal of a portion of the pulp thus the fermentable sugar content of the bean results in the production of less acids during fermentation and subsequently less acidic beans after drying (Rohan, 1963; Wood and Lass, 1985).

Pod storage is quite different from mechanical depulping and enzymatic depulping and the chemistry of the outcomes of the three different forms of pulp preconditioning are highly different. While stored pods for 5 – 7 days as shown by earlier researchers enhances pre-fermentation activity inside the pods during this dormant stage helps to facilitate rapid rise in temperature during fermentation, reduces acidity, and imparts stronger chocolate flavour (Schwan and Wheals, 2004; Nazaruddin, 2006; Afoakwa 2010; Afoakwa *et al.*, 2011ab; Afoakwa *et al.*, 2012ab) that cannot be said for mechanical depulping.

The principle for mechanical depulping is to separate pulp from fresh beans via the action of a depulper and this might cause bruising of the beans and its inherent cell structures leading to activation of enzymes that might influence the biochemical processes during fermentation. Apart from reducing acidity, depulping also shortens fermentation time and makes available excess pulp in the manufacture of jams and cocoa liquor (Buamah *et al.*, 1997; Dias *et al.*, 2007).

2.4.3 Pod-breaking

Pods are usually split open with a sharp machete to reveal and remove the seeds. In Ghana most of the farmers crack open the pods with the machete by making a sharp incision on the longitudinal section of the pod is made then a second on the opposite side of the pod and a third incision made at the base for easy removal of the husk (Quao, 2010). The beans are then scooped out with fingers or the cutlass and this is an important process as there is the transfer of microorganisms from the farmer's hands or tools to the beans. The beans are extracted without placenta and fermentation follows immediately (Afoakwa, 2010). Only mature, well-developed pods contain good beans. The colour of the pulp is a good indication of suitability as damaged pods show discoloration but pods showing symptoms of damage from black pod on the surface need not be discarded if the beans inside are unaffected (Knapp, 1937;Nair, 2010).

2.4.4 Fermentation

Fermentation plays a pivotal role in the formation of flavour harbingers, colour and aroma due to biochemical and chemical changes that occur during the process. However these are also affected not just by fermentation but also by genetic variation of the cocoa varieties (Lopez and Dimick, 1995; Afoakwa and Paterson, 2010). During and after opening of the cocoa pods the cocoa beans are spontaneously inoculated with a variety of microorganisms from the farmer's hands and the tools involved (Nielsen, 2006; Thompson *et*

al., 2001). The usual recommendation for fermentation is to start immediately following pod breaking. It is the norm but in exceptional cases such as to reduce acidity, seeds may bespread out for several hours before being fermented (Biehl *et al.,* 1990); another method also recommends partial removal of theseed mucilage prior to fermentation (Lopez and Dimick, 1995).

Typically fermentation involves the confinement of freshly scooped out beans be it in a heap or any of the other fermentation systems for a duration of 3 to 6 day with a maximum of 8 days depending on the variety of the cocoa (Lopez and Dimick, 1995). As fermentation continues, various yeasts, lactic acid bacteria, acetic acid bacteria and other microorganisms such as *Bacillus* spp. (Nielsen, 2005) develop in a form of succession which is not entirely distinct but rather overlap with other microorganisms occurring throughout the fermentation process (Thompson *et al.*, 2001; Ardhana and Fleet, 2003; Schwan and Wheals,2004).

The fermentation process involves microorganisms catabolizing fermentable pulp sugars to alcohols (ethanol) and through exothermal reactions some of the alcohols are oxidized to acetic acid resulting in the liberation of pulp juices known as sweatings (Thompson *et al.,* 2001). The penetration of the ethanol and acetic acid into the core of the beans and its associated heat emanating fromthe reaction causes the beans to swell up and destroy the embryo or the germ. The heat generated can reach a high of 45 – 50˚C from an initial of 25˚C and is very typical of heap fermentations (Senanayake *et al.,* 1995). This leads to a loss of germinating ability of the seed. This process also brings about the destruction of cellular integrity and break down of the cell walls within the bean allowing the interactions of enzymes and substrates freely. This continued process leading to well fermented beans (Roelofsen, 1958;Thompson *et al.,* 2001; Beckett, 2009; Afoakwa, 2010). The production of acids and alcohols by various yeasts, lactic acid bacteria, acetic acid bacteria and other

microorganisms causes the pH of the beans to decrease from about 7.0 to 5.0 – 5.5 (Beihl *et al.,* 1985; Thompson *et al.,* 2001).

Other metabolic processes occurring simultaneously within the bean will cause the increase in flavour precursors such as amino acids and reducing sugars as well as the reduction on total polyphenols leading to a less bitter and astringent beans and other complex chemical processes will also take place. The fermentation process produces typicalalcoholic, lactic and acetic acids in the external pulp enveloping the seed. These changes take place within the tissue,resulting in the formation of flavorprecursors(Lopez and Dimick, 1995).

2.4.5 Fermentation systems

Various cocoa fermentation systems have been developed. These methods of fermentation vary considerably from producing region to region,and in some instances even different farmers practice different fermentation techniques depending on the quantity of beans and what system is available to them (Lopez and Dimick, 1995; Afoakwa *et al.,* 2010). The generalmethods involve the seeds being placed in some kind of receptacle, confined and weighed down. Most of the world's cocoa is fermented on banana leaves covered heaps, in boxes, trays, and baskets and on dry platforms (Lopez and Dimick, 1995; Beckett, 2009; Afoakwa, 2010).

2.4.5.1 Heap fermentation

In this system, beans are piled or heaped on and coveredwith plantain or banana leaves. Cocoa seeds varying inquantities from 25 to 1000 kg are heaped ona floor covered with plantain leaves and perforated for easy drainage of sweatings. The heap is subsequently covered with the leaves, which are weighed down, by branches or other materials (Lopez and Dimick, 1995). This is done not only to protect the fermentingmass against insects and conserve heat but also to prevent the entry of rainwater, dust and other foreign matter (Wood and Lass, 1985). The heap ideally must be turned every 72 hours during the fermentation to

ensurea uniform fermentation (Baker *et al.,* 1994), enhance growth of beneficial microorganisms and limit the growth ofunwanted microorganisms (Nielsen, 2006). According to Baker *et al*. (1994) stated that about 57 % of the Ghanaian farmers do not turn the heaps. This is due to its laborious nature. The duration for this type of fermentation ranges between 4 and 7 days. The heap fermentation systemdominates in Ghana and other West African countries (Wood and Lass, 1985; Baker *et al.*,1994).

2.4.5.2 Tray fermentation

Due to the laborious nature of turning heap fermentations the tray method was developed based on the early observation that when the beans are heaped to ferment, a change in color of the beans occurs up to a depth of about 10 cm when beans are not mixed (Nair, 2010). Based on this, in trays fermentation, reasonable quantities of beans of 10 cm heightholding are held in trays stacked one over the other for sufficient development and conservation of heat and there is no mixing (Thompson *et al.*, 2001).

A typical size of wooden trays is 90 cm x 60 cm x 13 cm. Battens or reapers are fixed at the bottom of the trays with small gaps in between to avert a situation where beans might falling through and also allow for free flow of sweatings (Nair, 2010).

When filled, the trays are stacked one over the other with the last being empty allow for drainage of the sweatings. With the minimum number of trays required for a stack being six the beans of the topmost tray are covered with banana leaves but after 24 hours, the stack of trays is covered with gunnysacks to conserve the heat that develops. Tray fermentation will normally be completed in day 4 and dried on day 5 (Nair, 2010).

2.4.5.3 Box fermentation

This type fermentation requires relatively large, fixed volume of cocoa and it is usually used on larger farms. A wooden box is subdivided by eitherfixed or movable internal partitions intocompartments measuring approximately1 x 1 x I m. The containersvary in size

from country to countryandhave a holding capacity of 600 to 700 kg of fresh cocoa beans (Lopez and Dimick, 1995). The boxes are arranged in a series of steps raised above ground levelto take advantageof gravity to facilitate turning, which is effectedby simply removing a movable walland shoveling the seeds into the box below. The boxes are raised over a drain that holds sweatings. The floor of the box is usually solid and contains holes for drainage and aeration (Lehrian and Patterson, 1983).The beans are covered with banana leaves or jutesacking to maintain the heat and prevent thesurface seeds from drying and turning is done every 48 hours.

2.4.5.4 Other fermentation types

2.4.5.5 Drying platform fermentationand Basket fermentation

Fresh cocoa seeds are spread directly on drying platforms to allow for mild aerobicfermentation while drying and an anaerobicfermentation when the seeds are heaped intopiles each night. This type of fermentation is practicedin Ecuador and parts of Central America where the Criollo cocoa is usually grown (Lopez and Dimick, 1995). Though this type of fermentation is adequate for Criollo varieties it is insufficient for Forastero varieties that require longer fermentation times (Rohan, 1963).

Baskets fermentation is mainly practiced in Nigeria, smallholder farmers in the Philippinesand some parts of Ghana (Lopez and Dimick, 1995; Afoakwa, 2010). Small quantities of fresh cocoa beans are placed in baskets lined with plantainleaves with tiny perforations to allow for seepage of sweatings and the surface is covered with plantainleaves and the mass is weighed down. Fermentation usually spans 4 to 6 days and the beans are mixed regularly (Rohan, 1963).

2.4.6 Drying

Drying is an all-important process after fermentation. After fermenting the beans for the required duration, the beans are removed from their respective types of fermentation systems employed for drying. Drying can be done in two ways thus natural or sun drying and artificial drying. Drying is done to ensure to removal of moisture and also some major biochemical processes such as flavour development, reduction in bitterness and astringency occur and also develop the chocolate brown colour of well fermented cocoa beans (Fowler, 2009; Afoakwa 2010). During drying a considerable amount of moisture is lost and has been estimated that moisture is reduced from approximately 50% to about 7 – 8%. This is important as moisture content above 8% might yield mould growth during prolonged storage and moisture of below 5% is very brittle (Lopez and Dimick, 1995). Regions or countries where the weather is hot and dry the fermented beans are spread on flat surfaces dried in the sun. In countries where the weather is humid and makes natural drying difficult, mechanical drying is employed (McDonald*et al.*,1981; Lopez and Dimick, 1995; Nair, 2010).

2.4.6.1 Natural drying

Sun drying is the most widely used and accepted method for several reasons: First is that sun dryingis cheap and it allows for slower and uniform migration of moisture. This method over the years yields quality beans in traditional areas of cocoa production such as Ghana where the weather is sufficiently sunny. In Ghana, the beans are spread in a thin layer on bamboo mats in the sun raised off the ground (Afoakwa *et al.*, 2011ab) and the mats can be rolled upto protect the beans when it rains. The beans are stirred regularly and added advantage is that foreign materials are easily identified and hand picked and there is also a lesser risk of contamination. Some attempts to improve the efficiency of sun drying have been made by using solar drying cabinets (Nair, 2010).

Sun drying of cocoa beans takes about a 6 – 7 days to dry but under cloudy or rainy conditions drying may take up to about 4 weeks which might increase the risk of mouldgrowth and spoilage (Wood and Lass, 1985; Thompson *et al.*, 2001).

2.4.6.2 Artificial Drying

Artificial drying is achieved via mechanical hot air dryers fuelled by wood or any petroleum source. Many designs have been developed, but usually indirect heating using heatexchangers are preferred. However, when employed, the temperaturesshould be such that the inside of thebean remains below 60°C so that enzymes arenot deactivated before browning occurs to prevent off-flavours. Rapid drying alsotends to make the beans retain excessive amounts of acetic acid, which is deleterious toflavour (Lopez and Dimick, 1995). When wood is used, as a source ofheating fuel, there is the danger that the smoke produced if not well controlled will cause smoky beansleading to off-flavours severely affecting the value of the beans (Wood and Lass, 1985; Thompson *et al.*, 2001).

2.5 Microbiology of cocoa fermentation

Fermentation of cocoa is a spontaneous microbiological process. The undamaged and healthy beans are sterile butsoon as they are removed,they become inoculated with a variety of microorganismsfrom the pod walls, the laborer'shands, the containers used fortransportingthe beans to the plant, the dried mucilageof the previous fermentation that coats thesweat-boxes, and by insects especially fruit fly (*Drosophila melanogaster*)(Lopez and Dimick, 1995; Thompson *et al.*, 2001; Nair, 2010; Nielsen, 2005; Nazaruddin, 2006; Nielsen, 2007; Afoakwa, 2010).

Fresh cocoa pulpcontains sugars (mainly sucrose) and citric acid, which makes itan excellent medium for the growth of microorganisms.During the initial phase of fermentation the low pulp pH of about 3.4 – 4.0 (due to the relatively high content of citric acid), high sugar content(8 – 24%) and the low oxygen tension are most (Lopez and Dimick,

1995)suitable for the growth of anaerobic yeasts, which initially dominate the fermentationduring the first 24 to 36 hours (Thompson *et al.,* 2001) and it has been recorded that yeast population increases rapidly to 107-108 CFU/g and it is followed by a steady decline throughout the rest of the fermentation (Ardhana and Fleet, 2003; Nielsen, 2006).

Previous research conducted by Nielsen, (2006) shown that Ghanaian cocoa pods have wide range of yeasts and it includes:*Hanseniaspora guilliermondii* (53%),*Pichia guilliermondii* (22%), *Candia intermedia* (7%), *Candida parapsilosis* (6%),*Cryptococcus laurentii* (4%), *Candida silvicola* (2%), *P. membranifaciens* (2%),*Rhodotorula glutinis* (2%) and *Cryptococcus humicola* (2%). Some strains of yeasts produce pectinaseswhich break down the pulp cells so that the juices drains carrying away flakes of pulp however, primarily yeasts are known to produce ethanol from sugars thus sucrose Carbon dioxide and metabolizing citric acid. This leads to a sharp increase in the ethanol concentration and a decrease in the concentrationof fermentable sugars (Lopez and Dimick, 1995; Thompson *et al*, 2001; Ardhana and Fleet,2003; Nielsen, 2006).

Fermentations investigated by Nielsen (2006) revealed that approximately 80% of the sugars(glucose, fructose and sucrose) were metabolized within the first 24 hours but the researcher noted an exception wherethe centre of the large heap fermentation were slowerand this corroborated similar observations reported by Carr *et al.* (1979). The conversion of glucose and fructose to ethanol causes a moderate rise in temperature at the initial phases of fermentation due to its exothermic nature.

Thompson *et al.* (2001) noted that the decline in yeast population could be attributed to rapidly metabolizing sugars in the pulp to form carbon dioxide and ethanol. Secondly,the production of ethanol creates a toxic environment that represses yeast growth and lastly aceticacid, which is produced from ethanol by the acetic acidbacteria, is also toxic to yeasts (Thompson *et al.,* 2001; Nielsen, 2006). Coupled with the above mentioned processes, the

breakdown of pulp parenchymacells culminates into pockets of gaps which encourages air flow this is corroborated by the research findings of Ardhana and Fleet (2003) that highly pectinolytic filamentous fungi flourished during first 36 hours of fermentation and loss of citric acid by drainage andthrough microbial metabolism causes a rise in pH these now favoursthe proliferation of lactic acid bacteria (Lopez and Dimick, 1995; Thompson *et al.*, 2001; Gálvez, 2007; Afoakwa, 2010).

Counts of lactic acid bacteria increases rapidly, but may be present for only a brief period, because they prefer a low oxygen concentration or high concentration of carbon dioxide (Thompson *et al.*, 2001)their population has been observed to reach 106 to 107 CFU/g in a typical fermentation. The lacticacid bacteria population is usually at its peak for about 3 days then decreased to less than 10% of the totalmicro flora (Thompson *et al.*, 2001). Both homofermentative and heterofermentative lacticacid bacteria are present in cocoa fermentations with the former being the majority. Some of the homofermentative species included *Lactobacillus plantarum,Lactobacillus casei, Lactobacillus delbrueckii,Lactobacillus acidophilus, Pediococcus cerevisiae, Pediococcusacidilactici*, and The heterofermentative species included *Leuconostocmesenteroides* and *Lactobacillus brevis* (Thompson *et al.*, 2001). In a recent study by Nielson *et al.* (2007), the following lactic acid bacteria were isolated in Ghanaian cocoa beans;*Lactobacillus plantarum, Leuconostocpseudomesenteroides, Leuconostoc pseudoficulneum* and *Pediococcoccus acidilactici*.With increase in aeration the lactic acid bacteria decrease, which causes a rise in acetic acid bacteria.

Acetic acid bacteria reach around 108 CFU/g during Ghanaian heap fermentations according to Nielsen (2006). Aeration of the fermenting mass by turningclearly influenced acetic acid bacteria growth in the large heap fermentation (Carr *et al.*, 1979). According to Nielson (2006), following turning of the heap the acetic acid bacteria counts decreased in the

outer portions the heap followed by renewed acetic acid bacteria growth while the opposite was observed in the centre ofthe fermenting mass and this was corroborated by Carr *et al.*(1979) as it was observed that the growth of acetic acid bacteria in the centre of the fermenting beans are positively correlated with turning. *Acetobacter syzygii, Acetobacter pasteurianus and Acetobacter tropicalis* were the predominant acetic acid bacteria isolated by Nielsen *et al.* (2007). The aerobic conditions within the fermenting mass make conditions favourable for acetic acid and other aerophilic sporeformingbacteria and fungi (Thompson *et al.,* 2001). This results in exothermicoxidation of alcohol culminating in an increasein temperature to about 45 to 50°C.The rise in temperature makes it unfavorable for the survival of acetic acid bacteria (Lopez and Dimick, 1995; Thompson *et al.,* 2001).

These high temperaturescoupled with the ethanol and acetic present serves as the final phase of fermentation as this phase is characterised by a high stressfactor limiting the growth of many microorganisms (Lehrian and Patterson, 1983; Thompson *et al.,* 2001; Nielsen, 2006; Gálvez, 2007; Afoakwa, 2010; Afoakwa *et al.,* 2011a). Also occurring simultaneously in the later phases offermentation increasing pH and aeration becomes favourable for growth of filamentous fungi which are responsible for off flavours and increased free fatty acids levels and sporeforming *Bacillus* spp. often reaching about 108 CFU/g in the later stages of thefermentation (Carr *et al*., 1979; Schwan *et al*., 1995; Ardhana and Fleet, 2003). However, investigations by Nielsen (2006)showed that small heap and tray fermentations had no or limited growth of*Bacillus* spp. and these observations were because fermentations were completed after 4 rather than 6 days. *Bacillus* spp. has been shown to have high enzymatic activity (Schwan *et al.,* 1986). They are also responsible for the production of short chain fatty acids (< C_{14}) such as butyric acid and others that produces off flavours, pyrazinesand 2,3-butanediol (Schieberle, 2000; Thompson *et al.*, 2001; Nielsen, 2006; Quao, 2010).

2.6 Biochemical and chemical changes during fermentation

The development of chocolate flavour begins with the chemical and biochemical changes occurring within the bean during fermentation and drying. From previous researches, it is imperative to note that pulp pre-conditioning (pod storage, mechanical and enzymatic depulping and bean spreading), mode of fermentation and its microbial conditions and drying provide the necessary conditionsfor complex biochemical reactions to occur (Hodge, 1953; Lopez and Dimick, 1995; Thompson *et al.*, 2001; Nazaruddin, 2006; Nielsen, 2007; Afoakwa *et al.*, 2012ab). However similar cannot be mentioned of immature and unfermented beans as they tend to develop little or nochocolateflavour when roasted while excessive fermentation yields hammy and putridflavours which are not ideal (Zaibunnisa *et al*., 2000; Reineccius, 2006; Afoakwa, 2010).

Thompson *et al.* (2001) noted that although lactic and acetic acids produced externally by microbial activity affect flavour,chocolate flavour development is predominantly dependent on enzymatic formation of flavour harbingers withinthe cotyledon and it is highly peculiar to cocoa. These compounds include free amino acids, peptides,reducing sugars, and polyphenols (Kirchhoff*et al.* 1989; Hansen *et al*., 2000; Lee*et al.*, 2003; Hii*et al.*, 2009).The structure of testahas semi permeable and acts as a natural barrier between microbialfermentation activities outside the bean and chemicalreactions within the bean. But with a progression in fermentation there is a migrationof ethanol, acetic acid, and water which are metabolites from fermentation from the outside to the inside of the bean (Thompson *et al.*, 2001).

Death of the cocoa bean is a very important process as there is breakdown of cellular integrity which leads to the free interaction of enzymes and substrates also, soluble bean components are usually lost by leaching via the testa and lost by drainage (Hansen *et al*., 1998; Thompson *et al.*, 2001; Afoakwa, 2010). These biochemical reactions responsiblefor

flavour development occur within the cocoabean on the onset of bean death. Its long been known that rising temperatures and increasing aceticacid concentrations during fermentation cause seed death but Lehrian (1989) stipulated that the ethanol produced via anaerobic yeast growth phase correlates more withdeath of the seed. This usually occurs 24 hour after maximum concentrationsof ethanol are attained within the cotyledon (Thompson *et al.*, 2001). This averts problems associated with germination, which utilizes a chunk of cocoa butter and other stored nutrients leading to a more stable and desirable product.

Afoakwa (2010) explained that enzymes exhibit differentstabilities during fermentation and may be inactivated by heat, acids, polyphenols and proteases. The enzymes associated with cocoa fermentation includes; amino-peptidase, cotyledon invertase, pulp invertase and polyphenol oxidase aresignificantly inactivated, carboxypeptidase is partly inactivated, whereas endoprotease andglycosidases remain active during fermentation (Hansen *et al*., 2000; Thompson *et al.*, 2002; Afoakwa, 2010).

Research by Kirchhoff*et al.*, (1989) revealed that there is significant difference in composition of the free amino acids releasedduring fermentation as compared with the composition in total seedhydrolysates (reflecting predominantly the protein-bound amino acids) is astriking feature of cocoa fermentation and this is in agreement with findings from other researchers (Forsyth and Quesnel, 1957; Forsyth and Quesnel, 1963; Biehl*et al.*, 1985; Thompson *et al.*, 2001; Afoakwa, 2010).This is suggestive that there is a fine balance between fermentation times, microbialactivity that influences enzyme activity within thecotyledon.

2.6.1 Enzymology of cocoa beans

The immediate surroundings of the fermenting mass forms a biocenosis which is affected by pH and temperature, in the fermenting mass thatinfluence cocoa bean enzyme reactions. Enzymes are proteins with powerful catalyticactivity, which work over a range of

pH and have an optimum pH at which it is most active. Increasing temperatures also accelerates their activation energy (Belitz*et al.*, 2009).

Additionally, enzymes have high specificity for both thecompound to be converted (substrate specificity)and for the type of reaction to be catalysed (reaction specificity such as hydrolytic and enzymatic reactions in cocoa). Apart from the aforementioned specific factors; moisture is also necessary to allow for enzyme and substrate interaction (Belitz *et al.*, 2009), but with the gradual loss of water during drying, enzyme activity is reduced and finally ceased at about6 to 8% (Thompson *et al.*, 2001). Temperatureof the fermenting mass rises froman ambient of 25°C at the initial stages of fermentation to about 50°C by the 3rd day (Lopez and Dimick, 1995). Increasein temperature of more than 20°C may impact negative or positively on enzyme activityresulting in fewer flavour precursors and poorchocolate flavour (Thompson *et al.*, 2001). Finally, if appropriate amounts of organicacids are not produced during fermentation, thepH of the cotyledon will not be suitable for optimal enzymeactivity, and the flavour profile of the resulting cocoa will be affected but excessive acid will lead to heightened sourness that can mask the chocolate flavour.

2.6.2.1 Hydrolytic enzyme reactions

During the anaerobic phase of fermentation hydrolytic enzymes namely invertase, glycosidases, and proteases have highest activity (Lopez and Dimick, 1995; Thompson *et al.*, 2001; Afoakwa, 2010). The activity of invertase yields reducing monosaccharides (glucose and fructose) from sucrose that natively cannot partake in non-enzymatic browning reactions that occurduring roasting to contribute to chocolate flavour.These reducingsugars represent more than 95% of the total reducing monosaccharides in cocoa beans (Forsyth and Quesnel, 1957; Hansen *et al*., 2000).

In addition to the minuscule amounts of amino acids existing in the unfermented bean, proteases (endo- and exoproteases)account for the hydrolysis of proteins to amino acids

and peptides and their activity is dependent on pHand temperature. Proteins in cocoa are vicilin-like globular in nature and are the main target of the proteases. According to Kirchhoff*et al.* (1989) the types and ratio of free amino acids and peptides sequences are unique to cocoa. These amino acids and peptides participatein non-enzymatic browning reactions by forming complexes with reducing sugars during roasting toform important chocolate flavour precursors and also colour formation (Kirchhoff*et al.* 1989; Afoakwa *et al.*, 2011a)

Glycosidase is a unique enzyme that hydrolyses anthocyanins to cyanidins and sugars (galactose and arabinose)and has more impact on colour developmentand some minor flavour components (Lee, 1975; Biehl*et al.*, 1985; Lopez and Dimick, 1995; Thompson *et al.*, 2001; Afoakwa, 2010). Anthocyanins locatedin specialized vacuoles within the cotyledon andare responsible for the characteristic deep purple colorof the unfermented bean. These compounds are highly affected by the pH of the medium. The colours range from purple in a neutral state, violet in weak alkaline solutions and pink in acidic conditions (Lee, 1975; Konczak and Zhang, 2004).

2.6.2.2 Oxidative enzyme reactions

Polyphenoloxidase (PPO) is the major oxidase occurring in the aerobic phase of fermentationbut continuing well into the drying of cocoa beans and is responsiblefor some flavour modifications (Thompson *et al.*, 2001). Oxygen facilitates the activity of PPO however; rising temperatures and insufficientmoisture become inhibiting factors for the polyphenol oxidase enzyme during drying.Catechins of which epicatechin makes up more than 90% and leucocyanidins are the major classes ofpolyphenols that is subject to oxidation in cocoa beans.Oxidation of epicatechin during the aerobicphase of fermentation and drying is largely responsiblefor the characteristic brown color of fermented cocoabeans.

The dihydroxy configuration of polyphenols areoxidized to form quinones which in turn can polymerizewith other polyphenols or complex with amino acidsand proteins to yield characteristic colored compoundsand high-molecular-weight insoluble material (Thompson *et al.,* 2001) that result in the reduction in astringency and bitterness.

2.7 Quality indices of fermented and dried cocoa beans

Generally to develop good quality cocoa products such as chocolates, cocoa butter, cocoa powder and other confectionaries cocoa must be free from mould, well fermented, must be low in free fatty acids and less bitterness and astringency and also less acidic. Quality appraisals also rely heavily on the experience of farmers and may lead to a good deal of variation in quality. Since fermentation is a critical step in the development of flavor, it is essential that farmers be trained on the principles of fermentation. The quality of cocoa can be assessed via a number of quantitative and qualitative tests. The most important and easy to assess include cut test to ascertain to some extent the fermentative quality, free fatty acids to assess the level of lipolysis(Thompson *et al.,* 2001).

2.7.1 Cut-test

The cut test is simple, and still the most widely used method is to assess the quality of a random sample of beans from a batch by visual evaluation of the cut surfaces of cocoa beans. Although to some extent the cut testreveals certain defects that are likely to cause off-flavours and degree of fermentation it is extremely subjective and at best is limited to the measurement of bean defects (Wood and Lass, 1985; Lopez and Dimick, 1995). Results from a cut test assumes that beans showing the least amount of the prescribed defects are in good standing and can produce good chocolate flavour. A standard of 300 beans sampled randomly longitudinally sectioned to expose the surface of the cotyledons, observations are usually made during the day with the aid of the sunlight and sometimes under bright visible light.

The defects most commonly looked for are:

a. Slaty beans –occurrence is due to dried beans without fermentation and it is characterised by a greyish to ash colour;
b. Purple beans - a combination of the intensity and occurrence gives an indication of the degree of under fermentation;
c. Mouldy beans - indicator of poor quality of the product usually;
d. Germinated beans – presence suggest under fermentation and high moisture content;
e. Other physical defects such as flat beans, insect infestation and broken beans relate more to the yield rather than flavor quality (Wood and Lass, 1985; Lopez and Dimick, 1995; Thompson *et al.*, 2001).

The cut test is not a guarantee of good flavour, and attempts have been made to development a more precise means of flavour or quality determinations.

2.7.2 Free fatty acid levels

During fermentation, drying and storage of cocoa beans some free fatty acidare produced. The extent of lipolysis is determined bythe free fatty acid content (FFA). FFA level affects the fatstructure and thus the shelf life of the cocoa butter and other products manufacture from such liquor. If severe,it will cause rancidity of the cocoa butter (EEC, 1990; Belitz*et al.*, 2009).

Oils with FFA content exceeding 1% are commonlydesignated as crude oils and cocoa butter is no exception.Recommendations of the Codex Alimentarius Commission stipulate that FFA should not exceed 1.75% in cocoa butter. There is a relationship between the sensoryperception of quality deterioration and the levelsof FFA in fats, which contain thesensory-relevant compounds thus (C number <14)low-molecular free fatty acids (Shieberle, 2000; Thompson *et al.*, 2001; Stark *et al.*, 2006; Belitz*et al.*, 2009).

2.8 Storage and Transportation of cocoa beans

2.8.1 Storage of cocoa beans

Traditionally cocoa beans are packedinto jute bags of 60 to 65 kg and subject to sanitary certification in warehouses prior to shipment. During longer storage periods, the quality of the cocoa beans can seriously be affected if proper care is not taken. Some bagged cocoa beans undergo a brief periodof storage in warehouses on the farms, at the buying agencies and on the dockyardsbefore and after shipping and at the factories before processing into cocoa products (Jonfia-Essien, 2004). In Ghana the Produce Buying Company (PBC) is the sole agent licensed to buy, store and sell cocoa (Mohammed*et al.*, 2012).

During prolonged storage relative humidity of 65 – 70% will generally maintain the moisture content of the beans about 7 – 8% this prevents insect and mold attacks and also averts possibly the problem of excessive fumigation. According to earlier literature by Thompson *et al.* (2001), during the initial storage period slow oxidation and loss of volatile acids may improve flavour but prolonged periods will eventually lead to stalingof the beans. The quality of cocoa beans can change during storage depending on temperature, relative humidity and ventilation (Lopez and Dimick, 1995; Thompson *et al.*, 2001; Jonfia-Essien, 2004).

2.9 Summary

As aforementioned mechanical depulping is a method for pulp pre-conditioning cocoa beans prior to fermentation. This method separates a portion of the pulp of fresh beans to reduce the amounts available as fermentation substrate. This is geared towards reduction of cocoa bean acidity. However, the extent to which the technique of mechanical depulping influences the chemical, biochemical, physicochemical and polyphenolic constituents of Ghanaian cocoa beansduring fermentationto date, has not been investigated. Hence this study seeks to bring to light the effects of mechanical depulping as a method of pulp pre-

conditioning on fermented and dried Ghanaian cocoa beans to add to existing knowledge and also to enhance the productivity in the cocoa production sector and also to the chocolate and confectionary producers at large.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Ripe cocoa pods (*Theobroma cacao*mixed hybridsvarieties)were harvested from the plantations of Cocoa Research Institute of Ghana (CRIG), Tafo in the Eastern Region of Ghana.

3.2 Sample Preparation

3.2.1 Mechanical depulping (pulp preconditioning)

3.2.1.1 Description of the mechanical depulper

The stainless steel mechanical depulper used in this research comprisesof a perforated (approximately 2–7mm) static cylindrical screen that is positioned horizontally on its longitudinal axis. Inside the screen are two (2) paddles or scraper system, which is adapted to rotate. When the paddles are in motionthe mass of cocoa beans are rotated and the friction removes the pulp.The depulper is equipped with a hopper (inlet), a means for feeding the fresh cocoa beans, a means for removing the depulped cocoa beans at the front end of the cylindrical screen (outlet) and also beneath the cylindrical screen a means to discharge pulp (Figure 4).

3.2.1.2 Pulp pre-conditioning process

The cocoa pods were transported immediatelyafter harvest and open arrival at the fermentary they were split and the cocoa beans were scooped out of the pods to be subjected to fermentation. Cocoa beans allotted for depulping weredepulped (pulp preconditioned) using the stainless steel mechanical depulper (Figure 3)at CRIG.

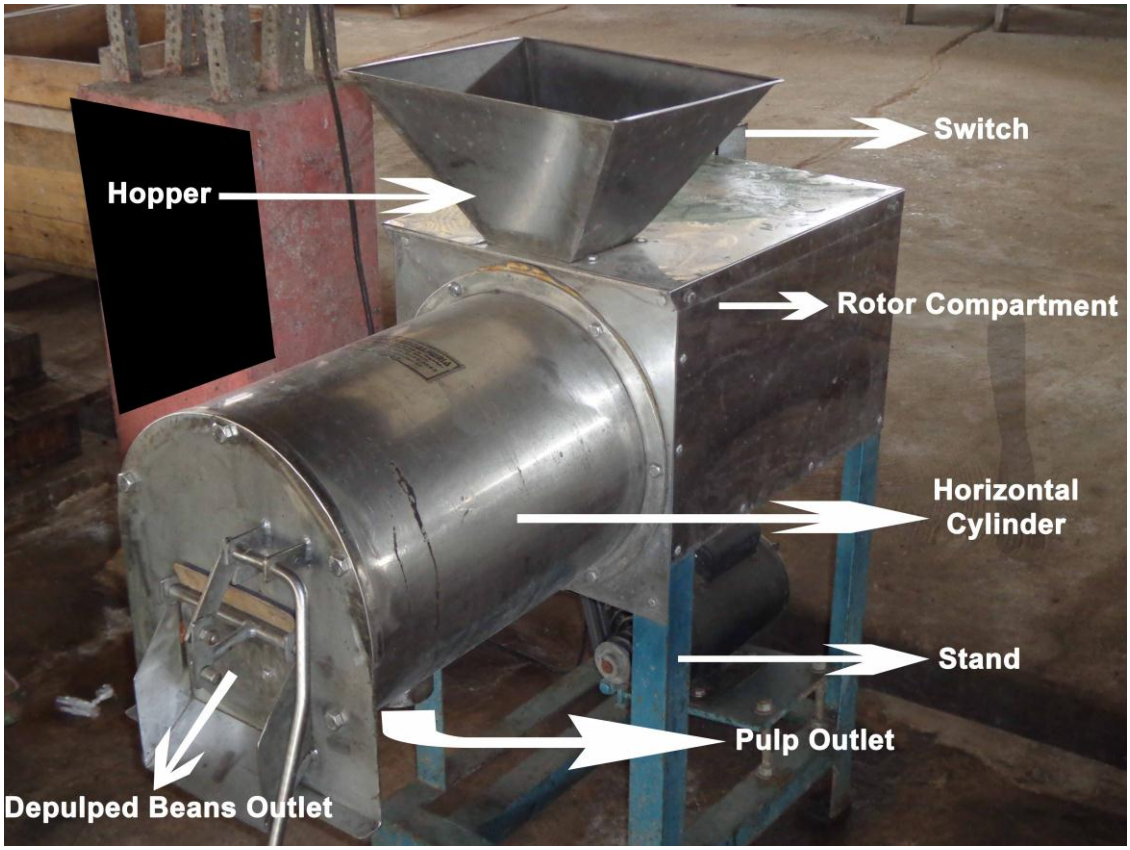

Figure 3: Mechanical depulper

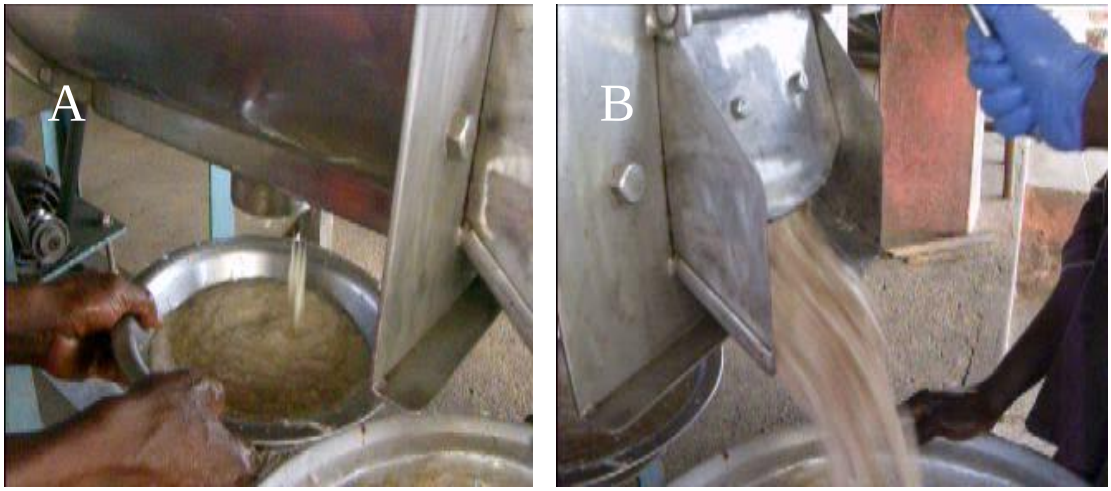

Figure 4: (A) Pulp extract and (B) Depulped cocoa beans

The concentration of depulped beans were mixed with the undepulped beans at different levels of 0, 25, 50, 75 and 100% of the total weight of fresh cocoa beans (50kg) to be used for the heap fermentation. The different ratio of depulped and undepulped cocoa beans,0:100, 25:75, 50:50, 75:25 and 100:0 respectively was formed into a 50kg heap,fermented and dried independently for subsequent analysis.

3.2.2 Fermentation and drying

The heap fermentation technique was employed for this research. The fermentation heap was made according to a ratio of mechanically depulped beans to whole beans as follows (0:100, 25:75, 50:50, 75:25 and 100:0%) respectively. Fermentation heap of about 50 kg was made on and covered with plantain leaves (Figure 5). Fermentation was allowed to proceed with a regular interval (2 days) of turnings for six (6) days.

3.2.3 Samples for analysis

Approximately one (1) kg were taken at predetermined fermentation times (0, 2, 4 and 6 days) and placed into sterile polythene bags and taken to the laboratory for drying.

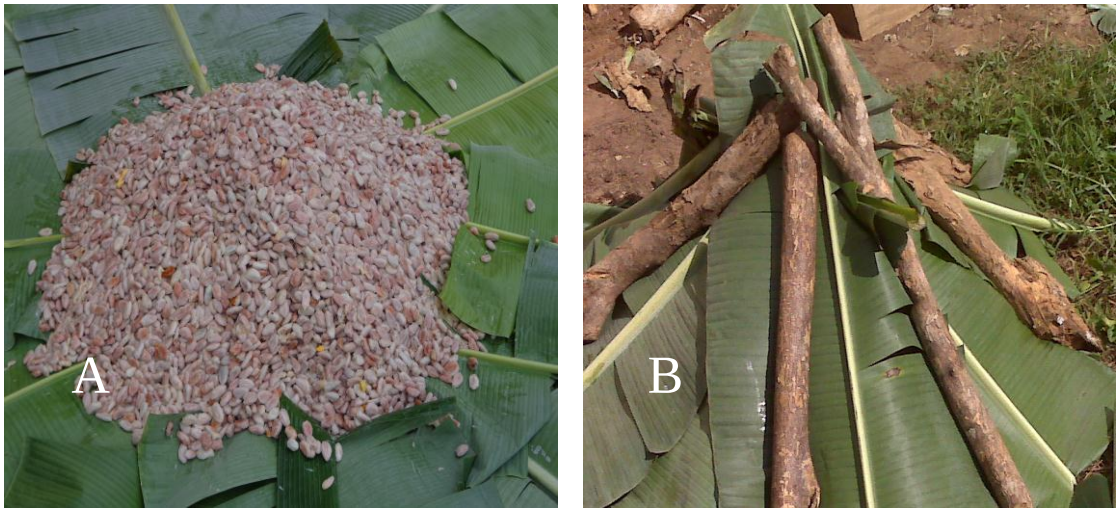

Figure 5: (A) Uncovered heap mixture of depulped and undepulped cocoa beans and (B) Covered heap mixture of depulped and undepulped cocoa beans

The sampled cocoa beans were placed on drying trays, well spaced and dried in an air oven at a temperature of 45 – 50 °C until moisture content wasbetween 7 – 8%. The dried beans were bagged and sealed airtight and stored in a dark room free from strong odour until ready for use. After the 6th day of fermentation (end of fermentation) samples to be used for the drying study were sundried immediately on raised bamboo mats for six (6) days. Samples (1kg) of the fermented cocoa beans were taken at regular intervals of 0, 2, 4 and 6 days of drying and kept in sterile polythene bags in a cool and dark area.

Samples used for biochemical, physicochemical and polyphenolic analyses for both fermentation and drying studies were milled into fine liquor of average particle size of about 0.45 mm using a Warring Blender (Christison Particles technologies – Albany, UK.).The resultant cocoa liquor was stored in an airtight polythene sample bag and well labeled.

3.3 Experimental Design

Specific objectives 1 – 3 were conducted using a 5 x 4 full factorial design.Theprincipal factors investigated were:

i. Mechanically depulped beans (0, 25, 50, 75 and 100%)
ii. Fermentation time (0, 2, 4 and 6 days).

For specific objective 4, the experiment was conducted using a 5 x 4 full factorial design. The principal factors were:

i. Mechanically depulped beans (0, 25, 50, 75 and 100%)
ii. Drying time (0, 2, 4 and 6 days).

Specific objective 1: To evaluate the effect of mechanical depulping on the chemicalcomposition of fermented Ghanaian cocoa beans

The following parameters were measured:

i. Proximate analysis – moisture, ash, protein, fat and carbohydrate (AOAC, 2005).
ii. Mineral analysis – mineral content was analyzed using the Atomic Absorption Spectrophotometer (AAS).

Specific objective 2: To determine the effect of mechanical depulping on the biochemical and physicochemical constituents of Ghanaian cocoa beans

The following indices weremeasured:

i. pH (AOAC, 2005)
ii. Titratable acidity (AOAC, 2005)
iii. Total sugars(Brummer and Cui, 2005)
iv. Reducing sugars using Luff Schoorl method (Egan *et al.* 1990)
v. Non-reducing sugars by Luff Schoorl method
vi. Free fatty acids (IOCCC, 1996)
vii. Percent pulp volume (Depulping).

Specific objective 3: To establish the effect of mechanical depulping on the polyphenol concentration, degree of fermentation and appearance properties of Ghanaian cocoa beans

The following indices were measured:

i. *O*-diphenols
ii. Total polyphenols(EEC, 1990).
iii. Cut test (Guehi *et al.* 2008)
iv. Fermentation index (Gourieva and Tserrevitinov, 1979)
v. Colour (Hunter Lab, Minolta)

Specific objective 4: To determine the influence of mechanical depulping on the acidification, browning index, anthocyanin content, colourand free fatty acids (FFAs) during drying of fermented Ghanaian cocoa beans

The samples were evaluated for the following:

i. Acidification (pH and Titratable acidity)
ii. Browning index (Gourieva and Tserrevitinov (1979)
iii. Anthocyanin content (Misnawi *et al*., 2002)
iv. Colour
v. Free Fatty Acids(IOCCC, 1996).

3.4 Analytical methods

3.4.1 Chemical analyses

3.4.1.1 Proximate analysis

The proximate composition of the dried fermented samples was evaluated for moisture, ash, protein, fat and carbohydrate using AOAC (2005) methods 931.04, 972.15, 970.22 and 963.15 respectively and carbohydrate was calculated by difference. All analyses were conducted in duplicate and the mean values reported.

3.4.2 Mineral analysis

3.4.2.1 Wet digestion

About 0.5 g of thesample was weighed into a round bottom digestion vessel. Digestion was done with Aqua Regia 3:1 HCL + HNO_3 (15 ml of concentrated HCl and 5 ml of concentrated nitric acid HNO_3). The samples were digested with great care in a block digester at 150˚Cfor 2 to 3 hours in a fume chamberuntil the solution was pale yellow. Thedigestion was continued until the solution was colourlessor nearly so (the evolution of

dense white fumes wasregarded to be indicative of the removal of nitric acid).After digestion was completed, the solution was cooled and distilled water added. The mixturewas filtered hot into a 200 ml volumetric flask using a Whatman No. 4 filterpaper. The solution was then made to the mark withdistilled water.

3.4.2.2 Determination of Ca, Mg, Zn, Fe, Cu and K

One (1)ml aliquots of the digest was used to determine the concentration of Ca, Mg, Zn, Fe, Cu and K using Atomic Absorption Spectrophotometer (AAS) (SpectrAA 220FS, Varian Co., Mulgrave, Australia) with flame from acetylene and nitrous oxide gases. The following formula was used to compute the total concentration of the elements in the sample:

$$\text{Conc (mg/g)} = \frac{\text{Conc read from AAS x Final volume after digestion (ml)}}{\text{Weight of sample (g)}}$$

3.4.2.3 Phosphorus determination

Two (2) ml aliquot of the digest was reacted with 5.0 mlmolybdic acid. The molybdic acid was prepared bydissolving 25 ml of ammonium molybdate in 300 mldistilled water; with 75 ml of concentrated sulphuric acidin125 ml of water to get 0.5 L of molybdic acid. One (1) mleach of 1% hydroquinone and 20% sodium sulphite wasadded in that sequence, and the solution was made up to100 ml and allowed to stand for 30 min in order to developcolour after which the absorption was measured at 882 nm with a Cecil Spectrophotometer (CE 7400).A standard curve of colorimetric readings versus concentrationof phosphorus using portions of standard phosphorussolutions (1 ml, 2 ml and 3 ml) subjected to reactionswith molybdic acid, hydroquinone and sodium sulphatesolutions were drawn. All readings were corrected using ablank to eliminate the effect of any colour produced by thereagents.The total concentrationof phosphorus content in the sample was estimated from the standard phosphorus graph. Analyses were conducted in duplicate and the mean reported.

3.4.3 Biochemical and physicochemical analyses

3.4.3.1 pH and titratable acidity

The pH and titratable acidity of the cocoa beans were determined using the method described by AOAC (2005). For the determination of pH, 10 gof powdered cocoa beans were homogenized for 30 s in 100ml of hotdistilled water and vacuum filtered through Whatmanfilter paper No. 4. A 25 ml aliquot was pipetted into abeaker and the pH measured using a pH meter (Model MP230 Mettler Toledo, Mettler Company Limited, Geneva, Switzerland).Titratable acidity wasdetermined by titrating juice with 0.1M NaOH and expressed as the percentage of acetic acid. Twenty five(25) ml aliquot was titrated to an end point pH of 8.02 with 0.1M NaOH and the values reported as molesof sodium hydroxide per 100 g dry nibs. The analysis was conducted in duplicates and the mean values are reported.

3.4.3.2 Total sugars

Total sugars weredetermined using phenol sulphuric acid method(Brummer and Cui, 2005) with slight modifications. The sample (powdered cocoa beans) was refluxed in 80% ethanol for 30 mins and the supernatant was separated from residue. Reflux continued until the solution was clear. This was followed by the addition of 25ml of 1.5N H_2SO_4 to the residue remaining after evaporation of 80% ethanol and refluxed for 1 hour. It was then filtered through Whatman No. 41 filterpaper and wash residue several times with water. The acidic filtrate was neutralized by theaddition of $BaCO_3$powder and confirmed by a litmus paper. The resultant filtrate was labeled as acid extract.

To the soluble extract, ethanol was evaporated under reducedpressure in a rotary evaporator. Some water was addedto make sure that the sugar was present in solution. After evaporation of alcohol, alcohol-soluble and waterinsolublesubstances were precipitated. The extract was clarified by adding 5ml of $ZnSO_4$solution to the extract followed by 4.94 ml of $Ba(OH)_2$solution. The solution was allowed to stand for about5 min and filtered through

Whatman No. 41 filterpaper; the filter paper was washed with distilled waterto remove any sugar still trapped in the precipitatethis was labelled as soluble extract.

To acid and soluble extracts, a mixture of Amberjet 1200 (H^+), a cation exchange resin and Amberlite IRA-402 (Cl^-), an anion exchangeresin were added in stepwise, agitated for 1–2 mins to allow for reaction then filtered. To 1ml of each extract,1ml phenol reagent and 5ml H_2SO_4 were added with caution andallowed to stand for an hour to develop colour (orange to red) and to cool. Theabsorbance of the samples was read at 490nm and theresults were reported. The analysis was conducted in duplicates and the mean values were reported.

3.4.3.3 Reducing sugars

Estimation of reducing sugars was determined using the Luff-Schoorlf method(Egan *et al*. 1990). Three grams of fine powderedsample were weighed into a wide-necked 250-ml volumetric flask. Water-soluble matterwas extracted by agitating the sample with 150 ml hotdistilled water(approximately 30 minutes). The solution was clarified by theaddition of 5ml Carrez I solution followed by 5mlCarrez II solution. The volume was made up to 200 ml with distilled water and allowed to stand for about 5 minutes, then filtered.Twenty five (25) ml of copper reagent (Copper II pentahydrate, citric acid and sodium carbonate) was measured into each of the two 250 ml Quickfit round bottom flasks. To another flask, a blank was also prepared by the addition of 25 ml of distilled water to make up to 250 ml. A fewanti-bumping granules were added and transferred to heating mantleand refluxed gently for 10mins and then transferred with tongs to acooling bath for 5 mins. About 3.0 g of potassiumiodide was added to the cooled flask, with constantswirling; 20 ml of 6N HCl was added through ameasuring cylinder.

The content of the flask wastitrated with 0.1N sodium thiosulphate until the iodine colour turnedbeige or very light brown. To the resultant solution, 1.0 ml starch indicatorwas added, and titration continued until the bluecolour changed to give a white precipitate of

cuprousiodide with no trace of blue colour. Sample titrationwas subtracted from the blank to obtain the quantity ofsodium thiosulphate used, and the results were calculated and the mean reported. Analyses were conducted in duplicate.

3.4.3.4 Non-reducing sugars

The non-reducingsugars were determined using the Luff-Schoorlfmethod (Egan *et al.* 1990). Non-reducing sugarswere completely hydrolyzed to reducing sugars byrefluxing with 1N HCl. Six (6) ml of clarified sample was measured into a 250 ml quickfit round bottom flask and 2ml of 1N HCl was added andmade up to 20ml using distilled water. Refluxing wasdone for 10mins on a hot plate. The flask and itscontent were allowed to cool and 2ml of NaOH and3ml of water added. The protocol for reducing sugars was then followed. The analysis was conducted in duplicate and the mean values reported.

3.4.3.5 Free fatty acids (FFAs)

Fat from the samples wasextracted with petroleum ether (40–60^{o}C) in a Soxhletapparatus using the AOAC (2005) method 963.15.FFA of the oils extracted was determined using theIOCCC (1996) method 42-1993. Five grams of the oil was weighed into a dry 250 ml stoppered conicalflask and 25ml of 95% ethanol/diethyl ether (1:1) and few drops of phenolphthalein indicator were added. The solutionwas titrated with 0.1N NaOH by swelling constantlyuntil pink colour persisted for 30 seconds. The percentageFFA was determined. The analysis was conducted in duplicates and the mean values reported.

3.4.3.6 Colour determination

The colour of all the different sample treatments wasanalyzed with aMinolta CR-310 Tristimulus Colorimeter (MinoltaCamera Co. Ltd, Tokyo, Japan). The colour meter is based on the L* a* b* colour system. The colour parameters are; L* (L_s-L_0) for lightness thus (100 = brightness to 0 = blackness); a* (a_s-a_0) for the extent of green colour (-a =green to +a =

redness); b* (b_s-b_o) quantifies the colour blue in the range of (–b = blue to +b = yellow).A reference white porcelain tile with the followingreadings: L_0 = 98.01, a_0 = +4.98 and b_0 = -3.77 was used to calibrate the colour meter.

3.4.4 Polyphenolic constituents

3.4.4.1 Total polyphenols

The Soxhlet apparatus was used to defat the dried cocoa nibs by extracting the fat with petroleum ether (40-60°C). The Folin Ciocalteau procedure (EEC, 1990) was followed to determine the total polyphenolic constituents of the defatted cocoa beans. About 0.2 g of dry defatted cocoa powder was extracted with Methanolic: HCl (80% MeOH containing 1% HCl) on an orbital shaker for 2 hours at room temperature. The mixture was allowed to settle and decanted.Five (5.0) ml Folin-Ciocalteau reagentwas added to about 1.0 ml of supernatant for colour development. Four (4.0) ml of Na_2CO_3 was subsequently added to the supernatant and allowed to stand for 1 hour at room temperature (30˚C), then another 1 hour at 0°C in a refrigerator. The absorbance was read at a wavelength of 760 nm using a spectrophotometer (Beckman CoulterDU 730 Life Science, UV/VIS Spectrophotometer).The mean value for the duplicate measurements was calculated and reported. Standard cathechin concentrations of blank (0), 0.2, 0.4, 0.6, 0.8 and 1 µg/ml was prepared. After the colour development, the absorbance was read at 760 nm and a standard curve drawn from plotted points. The total polyphenolic content in the sample was estimated from the standard cathechin graph.

3.4.4.2 Anthocyanins

The determination of anthocyanin content was conducted using the method described by Misnawi *et al.*(2002). The extract obtained from total polyphenol analysis was filtered using Whatman No.4 filter paper, and the resultant filtrate was read with the spectrophotometer (Beckman CoulterDU 730 Life Science, UV/VIS Spectrophotometer) for

total absorbance (TOD) at 535nm. Total anthocyanin was calculated with the following formula:

$$\text{Total Anthocyanin (mg/kg)} = \frac{\text{TOD x 1000}}{(AvE530)1cm/10}$$

Where TOD is the total optical density (absorbance) and (AvE530)1cm is the average extinction coefficient for total anthocyanin when a 1 cm cuvette and 1% (10 mg/ml) standards are used; the value is 982. The analysis was conducted in duplicates and the mean values reported.

3.4.4.3 *O*-diphenols

O-diphenols content was determined with Arnow's reagent (10 g $NaNO_2$, 10g Na_2MoO_4 in 100 ml H_2O). To 1 ml of extract (ethanol or methanol extract), 1 ml 0.5N HCl, 1 ml Arnow's reagent, 10 ml H_2O and 2 ml 1N NaOH was added. The solution was mixed and the absorbance was read at 520 nm after 30 sec. The analysis was conducted in duplicates and the mean values reported. A working standard cathechol solution of blank (0), 0.2, 0.4, 0.6, 0.8 and 1 µg/ml was prepared. The colour was developed, the absorbance read at 520nm with a spectrophotometer (Beckman CoulterDU 730 Life Science, UV/VIS Spectrophotometer) and a standard curve drawn. From the standard graph, the amount of *o*-diphenol present in the sample preparation was calculated. Means of duplicates were reported.

3.4.5 Fermentative quality

3.4.5.1 Fermentation index (FI)

The fermentation index (FI) was determined according to the methoddescribed by Gourieva and Tserrevitinov (1979). About 0.1g of comminuted cocoa nibs was extractedwith 10 ml of a 97:3 mixture of methanol and HCl respectively. The homogenate was allowed to stand in a cold room at 5°C for 20 hours and then vacuum filtered. The absorbance of the

filtrate was read with a Spectrophotometer(Beckman CoulterDU 730 Life Science, UV/VIS Spectrophotometer)at 460 nm and 530 nm respectively. The fermentation index was derived by calculating the ratio of absorbance at460 nm to the absorbance at 530 nm. The mean value of the duplicates was reported.

3.4.5.2 Cut test

The International method described by Guehi *et al*.(2008) was used. By the use of a scalpel, a total of 300 beans were cut lengthwise via the mid-section to expose the maximumcut surface of the cotyledons. The exposed cotyledons were examined under ultra violet (UV) light of the sun during the day at approximately 12 noon when sunlight is presumed to be at its peak and placed in one of thefollowing categories: purple, partly brown or partly purple, brown, slaty, germinated and mouldy.

3.6 Statistical analyses

The data were analyzed using STATGRAPHICS Centurion XV version 15.2.14 (STSC, Inc., Rockville, MD, USA) foranalysis of variance (ANOVA). Least significantdifference (LSD) was used to separate and comparethe means, and significance was accepted at 5% level ($P<0.05$). Furthermore, analyses were conducted toevaluate the combined effect of mechanically depulped beans and fermentation time on the studied parameters using the responsesurface methodology. Minitab version 16.0.0.1 was used to conduct a stepwise multiple regression procedures.Models were developed to relate mechanical depulping and fermentation time on the chemical, biochemical, polyphenolic and degree of fermentation indices of the cocoa beans. Thecoefficients of the variables in the models and theircontribution to the model's variation were reported. The R^2 values were used to judge the adequacy of the models. The R^2 of a model refers to the proportion ofvariation in the response attributed to the model ratherthan random error. For a good fit of a model, an R^2

ofat least 60% was used. All treatments and analyses were conducted in duplicates and the mean computed and reported.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Effect of mechanical depulping on the chemical composition of fermented Ghanaian cocoa beans

4.1.1 Proximate composition

4.1.1.1 Moisture

During fermentationof cocoa beans there was an increase in the moisture contentof samples containing 50, 75 and 100% depulped beans (Table 4). For the treatment containing 50% depulped beans moisture increasedfrom 4.97 to 9.55%. Similar trends of increases were observed for treatments containing 75 and 100% depulped beans. However, the moisture content of the undepulped cocoa beans and the 25% depulped beans decreased from 5.60% and 6.24% at the start of fermentation to 5.09% and 5.29% at the end of fermentationrespectively (Table 4). With the exception of cocoa beans depulped at 50% which recorded high moisture content of 9.55% at the end of fermentation, all the depulped samples however, recorded moisture content within acceptable limits (6 to 7 %) for safe keeping during storage (Wood and Lass, 1985; Dand, 1997).

Depulping caused a marginal increase in the moisture content of freshly harvested unfermented cocoa beans. It increased from 5.60% for the undepulped beans to 5.90% for the 100% depulped beans.All levels of depulped beans (0, 25, 50, 75 and 100%) recorded moisture content within acceptable limits (7 to 8 %) for safe keeping during storage. The moisture content of 7 to 8% is necessary to avert possible spoilage when beans are stored for a protracted period (Wood and Lass, 1985; Dand, 1997).Findings from this work suggest that increasing depulping and fermentation had an increasing effect on moisture content.Analysisof the data revealed that both depulping and fermentation time

significantly($p<0.05$) affectedthe moisture content. However, there was no statistical significance at $p>0.05$ between samples containing 25 - 0% and 100 –75% depulped beans (Table 5).

Moisture content increase at the initial stages of fermentation is due to the break down of the cell wall which increases permeability and facilitates enzymatic processes however, the apparent increases in moisture with respect to increasing depulping might have been due to the absorption of moistureby semi dried beans from the humid fermenting mass as by the end of fermentation 50, 75 and 100% depulped beans werealmost dried.

4.1.1.2 Ash

Depulping of cocoa beans caused a slight increase in the ash content of the cocoa beans.With the exception of samples containing 50% depulped beans, which decreased in ash, depulping generally, increased the ash content.It increased from 2.60% in samples containing 0% depulped beans to 2.92% in samples containing 75% depulped beans by the sixth (6^{th}) day of fermentation.Fermentation time had a decreasing effect on the ash content. Samples containing 0% depulped beans had their ash content decrease from 3.10% at the start of fermentation to 2.60% at the end of fermentation. Similar decreases occurred in samples containing 50% and 100% depulped beans. The decrease in the ash content was consistent for all levels of depulped beans at the end of fermentation (Table 4).

Both fermentation time and depulping had significant($p<0.05$)effecton the ash content (Table 5). ANOVA showedsignificant differences between the 0% depulped beans and all the other levels of depulped beans. However, there was no statistical significance ($p>0.05$) between samples containing 25, 50 and 100% depulped beans.

The ash content shows that depulping does not adversely affect the mineral content and it is comparable to that of the other pulp pre-conditioning process. It is important to note that the minerals contained in chocolates contribute to good cardiac health.

4.1.1.3 Protein

Protein content decreased consistently with fermentation time. The protein content decreased drastically from 16.23% at the beginning of fermentation to 13.64% by the sixth (6th) day of fermentation for the 0% depulped beans. Protein reduction was consistent with increasing fermentation time in samples containing 25 to 100% depulped beans (Table 4). Depulping of cocoa beans causes a slight increase in protein content. Protein content for 0% depulped beans was 13.64% while samples containing 25, 50, 75 and 100% had 13.79, 15.08, 14.11 and 15.04% respectively at the end of fermentation (Table 4). The observed decrease in protein content with depulping and fermentation time might be as a result of protein hydrolysis or deamination by native proteases to amino acids and peptides and partly by conversion to insoluble forms by the action of polyphenols (Minifie, 1970; Afoakwa and Paterson 2010).Biochemically, the formation of melanoidins via maillard reactions could also have caused a reduction in the protein content. According to Lopez and Dimick (1995), although proteolysis generates peptides and amino acids, usually there is a net decrease intotal nitrogen due to the loss of soluble nitrogencompounds by exudation through thetesta (Spencer and Hodge, 1992; Voigt *et al*., 1993) and this could have been exacerbated via the agitation of the seeds through the action of the mechanical depulper hence the lower protein content reported.

Fisher's LSD conducted showed that there were significant differences between samples containing 0 – 25% and 0 – 100% depulped beans. There was also a significant ($p<0.05$) interaction between fermentation time and the depulping on the protein content (Table 5). This means that the combined effect ofdepulping and fermentation had a greater effect on protein break down than depulping alone.

Table 4: Proximate composition of whole and mechanically depulped beans

Depulped Beans (%)	Fermentation Time (Days)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
0	0	5.60 ± 0.13	3.10 ± 0.07	53.63 ± 1.46	16.23 ± 0.16	27.03 ± 1.23
	2	6.73 ± 0.24	2.34 ± 0.31	40.75 ± 2.53	16.43 ± 0.19	40.48 ± 2.65
	4	6.66 ± 0.17	1.86 ± 0.36	42.74 ± 0.91	15.77 ± 0.33	39.64 ± 0.88
	6	5.09 ± 0.02	2.60 ± 0.02	48.86 ± 3.67	13.64 ± 0.19	34.91 ± 3.84
25	0	6.24 ± 0.12	2.85 ± 0.39	45.39 ± 0.99	16.21 ± 0.27	35.56 ± 0.33
	2	6.55± 0.01	3.92 ± 0.45	41.35 ± 1.97	16.12 ± 0.21	38.61 ± 2.63
	4	5.99 ± 0.33	2.63 ± 0.00	40.03 ± 3.79	14.44 ± 0.18	42.90 ± 3.61
	6	5.29 ± 0.03	2.88 ± 0.38	47.16 ± 0.92	13.79 ± 0.18	36.17 ± 1.11
50	0	4.97 ± 0.40	4.18 ± 0.03	32.72 ± 1.03	15.85 ± 0.26	47.25 ± 0.80
	2	7.79 ± 0.06	3.75 ± 0.02	36.30 ± 2.23	15.99 ± 0.10	43.95 ± 2.31
	4	6.85 ± 0.06	2.93 ± 0.37	39.64 ± 0.97	15.23 ± 0.42	42.20 ± 0.93
	6	9.55 ± 0.57	2.48 ± 0.42	36.19 ± 5.71	15.08 ± 0.08	46.26 ± 5.37
75	0	5.97 ± 0.16	2.92 ± 0.39	49.09 ± 1.15	15.97 ± 0.20	32.02 ± 0.56
	2	7.82 ± 0.01	3.78 ± 0.00	29.31 ± 0.53	16.10 ± 0.01	50.81 ± 0.52
	4	6.68 ± 0.14	2.93 ± 0.39	51.49 ± 2.93	15.53 ± 0.52	30.05 ± 2.02
	6	6.65 ± 0.11	2.92 ± 0.38	37.07 ± 0.74	14.11 ± 0.32	45.89 ± 1.44
100	0	5.90 ± 0.15	3.42 ± 0.37	41.90 ± 0.99	15.90 ± 0.07	38.78 ± 0.69
	2	6.32 ± 0.20	3.46 ± 0.37	30.82 ± 3.31	15.45 ± 0.00	50.27 ± 3.68
	4	6.42 ± 0.16	2.67 ± 0.01	55.13 ± 1.40	14.90 ± 0.02	27.31 ± 1.39
	6	8.17 ± 0.22	2.70 ± 0.00	33.01 ± 3.28	15.04 ± 0.27	49.26 ± 3.55

**Protein (N x 6.25) and **Carbohydrate was calculated by difference.*

The results presented above are on dry matter basis and mean values of duplicate analyses ± standard deviations

4.1.1.4 Fat

The fat content of the cocoa beans showed varied composition with both increasing fermentation time and depulping. It decreased from 53.63% at the start of fermentation to 48.86% at the end of fermentation for the undepulped beans (Table 4). Similar trends of decreases were observed for treatments depulped by 75% and 100%. Fat content however increased slightly at the start of fermentation from 45.39–47.16% and 32.72–36.19% at the end of fermentation for 25% and 50% depulped samples respectively (Table 4).

Increasing depulping caused a reduction in the fat content of the unfermented cocoa beans. It decreased from 53.63% (freshly harvested undepulped beans) to 41.90% in the 100% depulped beans. The fat content also decreased from 48.86% in 0% depulped beans to 33.01% for samples containing 100% depulped beans at the end of fermentation (Table 4). Presumably,there was someinterplay existing between fat and carbohydrate as the two chemical variants are inversely related. Most microorganisms especially bacteria convert fat/lipids to carbohydrate via gluconeogenesis (Nelson and Cox, 2004).It has also been reported by earlier researchers that pod stored beans with smaller bean sizes and weights usually have lower fat composition (Wood and Las, 1985; Dand, 1997).

Analysis of the data showed that both fermentation time and level of depulping as well as the interaction between fermentation time and depulping significantly affected ($p<0.05$) the fat content of the cocoa beans (Table 5). Further analysis using Fisher's LSD revealed that samples containing 25 – 75% and 75 – 100% depulped beans had no significant difference.

Table 5: ANOVA summary table showing F-ratios for variations in proximate composition of depulped and fermented cocoa beans

Variables	Protein	Fat	Carbohydrate	Ash	Moisture
Main Effects					
A: Depulped Beans	2.65	22.30*	15.43*	8.90*	52.56*
B: Fermentation Time	121.63*	37.86*	30.28*	20.40*	78.80*
Interaction					
A x B	5.00*	15.65*	15.82*	4.36*	48.78*

** Significant at P<0.05*

4.1.1.5 Carbohydrate

Depulping of cocoa beans caused an increase in total carbohydrates. Samples containing higher levels of depulped beans recordedhigher carbohydrate content. At the end of the fermentation period, samples containing 0 to 100% depulped beans increased from 34.91 to 49.26% respectively and this was consistent for all other levels of depulping. Increasing fermentation timealso increased the carbohydrate content of the cocoa beans. Carbohydrate content ranged from 27.03% at the start of fermentation to 34.91% by the sixth (6^{th}) day of fermentation with 0% depulped beans. Similar trends were observed with samples containing 25 to 100% depulped beans.

Theapparentinverse relationship between the levels offat and total carbohydrate in fermenting cocoa might be as a result of conversion of fat to carbohydrate via gluconeogenesis. Gluconeogenesis is an aerobicprocess with starting points of amino acids, Krebs cycle intermediates, or fattyacids (Nelson and Cox, 2004; Afoakwa *et al*., 2011a). Depulping of cocoa beans acts to reduce pulp content

and thus leads to the creation of easy airflow, which might have increased the rate of gluconeogenesis during the later part of fermentation (i.e. days 4 and 6). This pathway normally operatesin microorganisms especially in bacteria (Lee, 1975; Nelson and Cox, 2004).

ANOVA results showed that fermentation time and depulping caused significant ($p<0.05$) increasein carbohydrate content of the beans (Table 5). Fisher's LSD revealed thatsamples containing 25 – 75% and 75 – 100% depulped beans were not statistically significant at $p>0.05$ (Table 4).

Overall the chemical changes due to mechanical depulping might have a positive impact on cocoa bean quality as the moisture, proteins and ash were within acceptable limits however the fat content were much lower and might affect the quantity and quality of cocoa liquor hence chocolate production.

4.1.2 Mineral content of depulped and fermented cocoa beans

The effects of mechanical depulping as a means of pulp preconditioning and fermentation on the mineral composition of cocoa beans are shown in Tables 6 and 8. Generally, there were decreases in some of the micronutrients with depulping and fermentation time but there were slight increases in others.

4.1.2.1 Potassium (K)

Fermentation time caused decreases in the potassium (K) content of the depulped cocoa beans.Potassium decreased from 1000.43 mg/100g at the start of fermentation to 718.18 mg/100g at the end of fermentation of the 0% depulped beans. Similar decreasing trends were observed for samples containing 25, 50, 75 and 100% depulped beans (Table 6). Depulping hadonly marginal effect on the potassium content. Potassium remained fairly constant with increasing levels of depulping. Samples containing 50, 75 and 100% depulped beans recorded 765.77, 761.94 and

763.33 mg/100g of potassium at the end of fermentation (Table 6). This suggests that the mineral content for that matter potassium is located within the cotyledon of the cocoa beans hence depulping had marginal effect.

These high values suggest that the cocoa might have been planted in soils with high potassium that translated into the cocoa beans and this corroborates findings by Afoakwa *et al.* (2011a) where potassium was the highest mineral in pod stored and fermented cocoa beans. Potassium plays a vital role in human nutrition. It helps to maintain electrolyte balance and also regulate heart rhythms, stabilize blood pressure and reduce the occurrence of strokes (Nelson and Cox, 2004).

Analysis of variance on the data showedthat fermentation time decreased the potassium content significantly($p<0.05$) (Table 8).Fishers LSD showed that sample containing 0 – 25%, 25 – 50%, 25 – 75% and 25 – 100% depulped beans were statistically different.Enzymatic activity within the cotyledon of the cocoa beans must have accounted for the reduction in potassium content with the progression of fermentation.

4.1.2.2 Magnesium (Mg)

Magnesium content decreased with increasing fermentation time. Magnesium decreased from 387.33 and 391.28 mg/100g from day 0 to 281.86 and 292.04 mg/100g in samples containing 0% and 100% depulped beans respectively at the end of fermentation (Table 6).

Depulping had marginal effect on the magnesium content. Magnesium remained fairly constant with increasing levels of depulping. Samples containing 0, 50 and 100% depulped beans had 281.86, 296.80 and 292.04 mg/100g respectively at the end of fermentation. Magnesium content showed no increase or decrease with increasing depulping.

It is generally known in medical and nutritional literature that magnesium is a vital anti-stress mineral, which also promotes cardio-protective properties, bone and tooth integrity (Nelson and Cox, 2004; Belitz*et al.*, 2009). Magnesium and potassium are known to have synergistic effect on human health. Its depletion causes a decrease in both cellular and extracellular potassium(Belitz*et al.*, 2009).

ANOVA revealed that fermentation time caused a significant ($p<0.05$) decrease in magnesium content (Table 8). Post hoc ANOVA analyses showed that samples containing 0 – 25%, 25 – 50%, 25 – 75% and 25 – 100% depulped beans were statistically similar.

4.1.2.3 Calcium (Ca)

Calcium increased with the progression of fermentation (Table 6). As fermentation timeincreased, calcium increased drastically from 640 mg/100g to 1657.76 mg/100g in samples containing 25% depulped beans at the end of fermentation. Similar to the other minerals the enzymatic activities with the cotyledon might have contributed to the increment. Similar trends of increment were observed in calcium for samples containing 50 to 75% depulped beans. Calcium increased from 1704.40 mg/100g at the beginning of fermentation to 1839.53 mg/100g in samples containing 50% depulped beans. Similar increases from 1903.86 mg/100g to 2145.34 mg/100g were observed in samples containing 75% depulped beans.

Depulping generally caused increases in calcium composition. There were general increases in calcium content for samples containing 0 to 100% depulped beans.Increasing levels of depulped beans from 0% to 25% caused increases in calcium from 412.38 mg/100g to 1657.76 mg/gby the end of fermentation.Agitation of the depulper might have facilitated the disruption of the cellular integrity that caused enzyme substrate interaction therefore leading to the increase in calcium

content. Calcium is essential in bone formation and in maintaining the structure of the osseous tissues.

ANOVA showed that fermentation time and depulping significantly ($p<0.05$) increased the calcium content of the beans (Table 8). LSD post hoc analysis showed that only 0 and 100% depulped beans were similar but the others were different.

4.1.2.4 Phosphorus (P)

Phosphorus content of the cocoa beans decreased marginally with depulping. Phosphorus levels decreased from 4.20 mg/100g to 3.41mg/100g in samples containing 0% and 50% depulped beans respectively by the end of fermentation (Table 6).

Fermentation time caused marginal decreases in phosphorus content. Increasing fermentation time resulted in decreases in phosphorus content. Phosphorus decreased from 4.61 mg/100g at the beginning of fermentation to 4.20 mg/100g at the end of fermentation for 0% depulping. Similar marginal decreases were observed for samples containing 25, 50 and 100% depulped beans. Phosphorus decreased from 3.58 mg/100g at the start of fermentation to 3.24 mg/100g by the end of fermentation in samples containing 25% depulped beans.

Phosphorus is one of the abundant macro-minerals in our bodies. Nutritionally phosphorus and calcium have a synergistic effect on human health. Calcium is essential in bone formation and in maintaining the structure of the osseous tissues in combination with phosphorus. It is also imperative to note that the mineral content of the cocoa beans might vary according to that of the soils in which the cocoa was grown(Belitz *et al.,* 2009).

ANOVA analysis shows revealed that both fermentation time and depulping significantly ($p<0.05$) decreased the phosphorus content (Table 8). However LSD was

used to separate the means and 25–50% and 100–75% depulped beans were statistically similar.

4.1.2.5 Copper (Cu)

Depulping caused slight increasesin copper content (Table 6).Samples containing 0% depulped beans had relatively lower copper content of 8.23mg/100g. However, those with 25% depulped beans had higher copper content of 16.90 mg/100g by the sixth day of fermentation. Similarly, samples containing 75% depulped beans had comparatively lower copper content of 13.75 mg/100g than in samples containing 100% depulped beanswhich had 20.95 mg/100g in samples.

Fermentation time caused both increases and decreases in copper content. Copper increased from 9.83 mg/100g to 16.90 mg/g in samples containing 25% depulped beans. Similar increment with fermentation time was observed for samples containing 75 and 100% depulped beans. However, copper content decreased with fermentation time for samples containing 0 and 50% depulped beans. At the end of fermentation copper decreased from 18.02 mg/100g in samples containing 50% depulped beans.

Copper is a component of a number of oxidoreductaseenzymes (tyrosinase, phenolase, uricase, amine oxidase) of which polyphenol oxidase (PPO) is a part. Copper ionsas a prosthetic group of PPO participate in several redox reactions (Chazarra *et al*., 1999). Polyphenoloxidase of *Neurospora crassa* containstwo copper atoms, each of which is ligandedto three histidine residues. Copper acts as an activator for the polyphenoloxidase activity and is requiredin oxygen binding during polyphenol oxidation. Reduction in polyphenols due to enzymatic degradation of polyphenols during fermentation might have cause a slight increase in copper content with increasing depulping and fermentation time. Copper is also known to be less

desirable during food processing and storage since it catalyzesmany unwanted reactions (Belitz*et al.*, 2009).

ANOVA analysis showed that both depulped beans and fermentation time at had significant ($p<0.05$) increasing or decreasing effect on copper content (Table 8). However LSD analysis revealed that 25–75 – 50% depulped beans were the same.

4.1.2.6 Zinc (Zn)

Fermentation caused marginal decreases in zinc content (Table 6) as duration of fermentation period inceased. Zinc reduced from 7.97 mg/100g at the start of fermentation to 7.18 mg/100g at the end of fermentation in samples containing 25% depulped beans. Similar trends in reduction were observed for samples containing 50 to 100% depulped beans with increasing fermentation time.

Depulping caused both marginal increases and decreases zinc content. Zinc increased slightly from 7.06 mg/100g in 0% depulped beans to 7.18 mg/100g in samples containing 25 % depulped beans (Table 6). There was a reduction in zinc from 8.02 mg/100g in samples containing 75% depulped beans to 6.98 mg/g in samples containing 100% depulped beans. Since zinc is also a common component of a number of enzymes the low values obtained might have been as a result of the utilization of zinc by numerous enzymes.Depulping of cocoa beans caused both significant ($p<0.05$) increase and decrease in zinc content (Table 8). LSD analysis revealed that there were statistical differences between samples containing 0 – 75%, 25 – 75% and 50 – 75% depulped beans.

Table: 6. Mineral content of depulped fermented cocoa beans (mg/100g)

Depulped beans (%)	Fermentation time (Days)	K	Ca	Mg	P	Cu	Zn	Fe
0	0	1000.43 ± 53.03	310.58 ± 84.42	387.33 ± 1.86	4.61 ± 0.23	9.52 ± 0.34	7.03 ± 0.09	5.12 ± 0.82
	2	956.19 ± 15.57	414.04 ± 104.47	388.39 ± 5.38	4.72 ± 0.08	5.44 ± 1.72	6.57 ± 0.11	5.32 ± 0.31
	4	784.66 ± 61.96	365.61 ± 149.53	325.55 ± 31.98	4.82 ± 0.20	2.07 ± 1.66	8.03 ± 0.92	6.18 ± 2.05
	6	718.18 ± 10.75	412.38 ± 111.22	281.86 ± 1.14	4.20 ± 0.06	8.23 ± 1.01	7.06 ± 0.66	4.99 ± 0.33
25	0	943.09 ± 1.95	640.30 ± 35.32	365.81 ± 9.02	3.58 ± 0.08	9.83 ± 0.18	7.97 ± 0.41	7.47 ± 2.97
	2	920.34 ± 29.81	759.72 ± 63.86	339.82 ± 1.78	4.31 ± 0.13	7.83 ± 6.22	6.89 ± 0.54	6.78 ± 2.50
	4	717.67 ± 44.98	933.64 ± 153.00	286.29 ± 19.78	3.40 ± 0.12	7.91 ± 2.59	6.82 ± 0.25	6.34 ± 0.87
	6	631.71 ± 3.99	1657.76 ± 29.09	262.23 ± 1.74	3.24 ± 0.02	16.90 ± 0.43	7.18 ± 0.35	8.25 ± 0.55
50	0	1013.27 ± 14.62	1704.40 ± 125.97	356.66 ± 9.83	3.74 ± 0.31	18.02 ± 0.91	7.85 ± 0.06	8.66 ± 1.20
	2	947.66 ± 10.61	1247.59 ± 85.13	360.73 ± 8.84	4.16 ± 0.32	9.74 ± 3.13	7.92 ± 0.56	7.17 ± 2.75
	4	805.94 ± 77.08	1642.21 ± 102.68	328.08 ± 25.91	3.75 ± 0.13	6.12 ± 0.16	7.27 ± 0.67	7.49 ± 0.59
	6	765.77 ± 46.07	1839.53 ± 119.00	296.80 ± 15.25	3.41 ± 0.23	13.02 ± 1.18	6.86 ± 0.20	5.54 ± 1.24
75	0	971.97 ± 6.02	1903.86 ± 146.60	363.31 ± 0.63	3.49 ± 0.03	13.64 ± 7.63	10.70 ± 1.64	5.10 ± 0.55
	2	975.68 ± 21.65	2223.55 ± 155.27	369.52 ± 6.90	4.07 ± 0.46	10.06 ± 4.19	9.08 ± 0.05	10.20 ± 5.63
	4	839.96 ± 12.93	1952.50 ± 55.06	319 66 ± 3.59	4.23 ± 0.04	8.58 ± 2.67	7.67 ± 0.03	5.39 ± 0.39
	6	761.94 ± 1.87	2145.34 ± 56.95	288.05 ± 0.08	4.65 ± 0.18	13.75 ± 7.57	8.02 ± 1.17	5.98 ± 1.71
100	0	978.34 ± 3.47	146.55 ± 4.88	391.28 ± 23.21	4.36 ± 0.10	16.86 ± 3.66	7.77 ± 0.39	9.10 ± 0.01
	2	908.93 ± 28.16	227.58 ± 79.08	370.40 ± 7.99	4.12 ± 0.01	17.04 ± 2.67	11.06 ± 5.00	10.83 ± 0.71
	4	773.29 ± 76.62	402.25 ± 1.94	311.52 ± 13.88	4.07 ± 0.48	15.35 ± 0.91	7.73 ± 0.25	9.03 ± 1.30
	6	763.33 ± 29.14	495.08 ± 102.25	292.04 ± 7.02	3.85 ± 0.08	20.95 ± 0.61	6.98 ± 0.12	10.02 ± 1.18

4.1.2.7 Iron (Fe)

Irongenerally increasedwith depulping of cocoa beans. At the end of fermentation, iron increased from 4.99 mg/100g in 0% depulped beans to 8.25 mg/100g in samples containing 25% depulped beans (Table 6). A similar increasing trend occurred in samples containing 50, 75 and 100% depulped beans.

Fermentation time caused slight fluctuations iron content. Increasing fermentation time caused an increase in iron content from 7.47 mg/100g at the beginning of fermentation to 8.25 mg/100g at the end of fermentation for samples containing 25% depulped beans. There were also increases in iron content with increasing fermentation time occurring in samples containing 75 and 100% depulped beans (Table 6).

Theiron contentsby day 6 of 0, 25, 50, 50, 75 and 100% were 4.99, 8.25, 5.54, 5.98 and 10.02 mg/100g respectively (Table 6).Iron metals are also present in a number of enzymes (peroxidase, catalase and hydroxylases).

ANOVA revealed that depulping caused significant ($p<0.05$) increase in the iron content. Fishers LSD showed that there were statistical differences between samples containing 0 – 100%, 25 – 100%, 50 – 100% and 75 – 100% depulped beans.

Table: 7. ANOVA summary table showing F-ratios for variations in mineral composition of depulped and fermented cocoa beans

Variables	K	Ca	Mg	P	Cu	Zn	Fe
Main Effects							
A: Depulped Beans	6.85*	465.99*	6.98*	24.63*	11.36*	2.90*	5.65*
B: Fermentation Time	111.89*	27.99*	102.14*	6.77*	8.28*	1.85	0.85
Interaction							
A x B	1.13	9.29*	1.52	5.73*	0.90	1.43	1.07

**Significant at P<0.05*

4.2 Effect of mechanical depulping on Physico-chemical and biochemical composition of Ghanaian cocoa beans during fermentation

4.2.1 Pulp volume

The cocoa pulpis a substrate rich in nutrients, which apart from serving as substrate for microbial activity, can be used inindustrial processes for by-product manufacture (Schwan and Wheals, 2004; Afoakwa, 2010).Microorganisms can readily ferment cocoa pulp within 4 to 6 days of fermentation and thus results in a successive reduction in pulp volume. Pulp volume decreased from 44.89% at the start of fermentation to 20.82% at the end of fermentation in undepulped beans (figure 6). The decrease in pulp volume was consistent for samples containing 25, 50, 75 and 100% depulped beans.With increasing fermentation time, pulp volume decreased in samples containing 25% depulped beans from 39.60% at the beginning of fermentation to 17.89% at the end of fermentation.

Depulping of cocoa beans caused drastic reduction in pulp volume (Figure 6). Pulp volume decreased from 20.82% in undepulped beans to 8.35% in samples containing 100%depulped beans at the end of fermentation.The reduction in pulp volume during fermentation can be attributed to the higher level of pulp reduction by the action of the depulper hence the remainder of the pulp might have been depleted at a faster rate due to the proliferation of microorganisms.

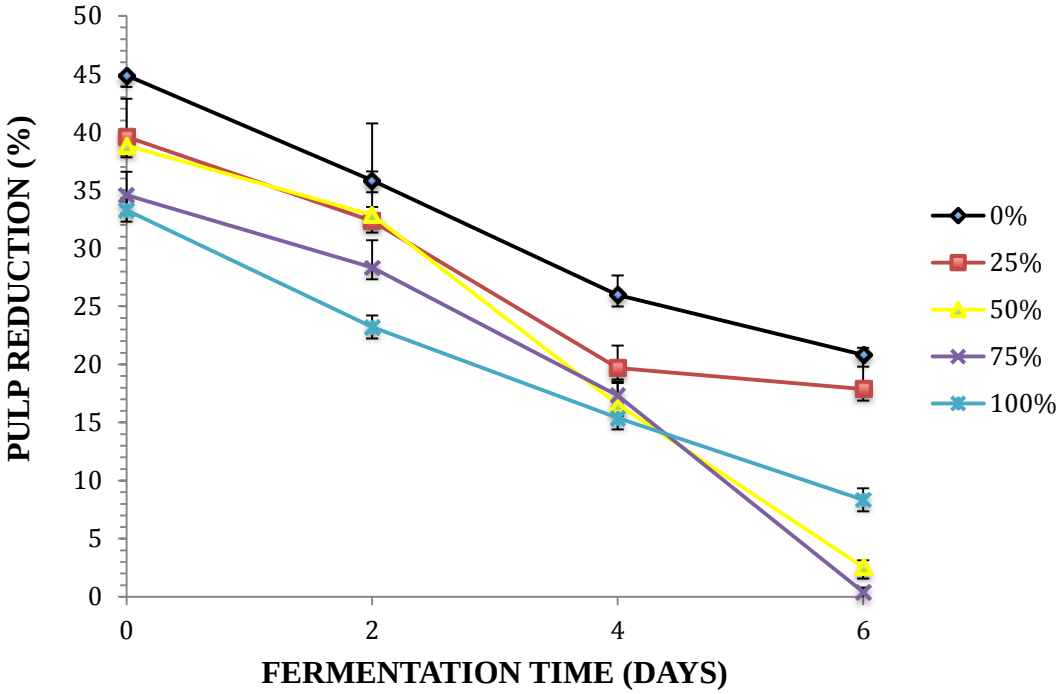

Figure 6:Percentage (%) pulp reduction of cocoa beans

Regression analysis on the data showedsignificant ($p<0.05$) influence of the linear factors of depulped beans and fermentation time on the pulp volume. Also the quadratic effect of depulping had a significant effect on the pulp volume. The model developed could explain approximately 92% of the variations in pulp volume (Table 9).

4.2.2 pH

Depulping of cocoa beans causes marginal decrease in pH as showed in the response plot (Figure 7). The pH decreased from 6.5 to 6.3 for samples containing 0% to 100% respectively at the beginning of fermentation.Depulping caused a slight increase in pH from 5.7 at the end of fermentation in 0% depulped beans to 6.02 at the

end of fermentation in samples containing 100% depulped beans. This explains thatincreasing depulping ratios reduces the levels of acidity in the beans.

As fermentation progressed the pH of the fermenting cocoa beans decreased. The pH decreased from 6.5 the start of fermentation to 5.7 at the end of fermentation in samples containing 100% depulped beans (Figure 7). The pH also decreased from 6.4 at the beginning of fermentation to 5.4 at the end of fermentation in samples containing 50% depulped beans. Decreases in pH were consistent for the samples containing 25, 75 and 100% depulped beans.

These observations are inagreement with previous report, which considered pod storage as a means of pulp pre-conditioning by Biehl *et al.* (1985). Henoted that there was diffusion of acids (predominantlyacetic acid) in the fermenting bean leading to decreasein the pH of the cotyledon from 6.5 to 4.6 within thefirst 3 days of fermentation and then increased to5.2 by the sixth day of fermentation.

Several authors have reported the reductions in pH during cocoa fermentation. Volatileacids (acetic, propionic, butyric, isobutyric andisovaleric) and non-volatile acids (citric, lactic, malic,succinic, oxalic and tartaric) develop in the pulp by microorganisms, which diffuse into the cotyledon, andresult in the reduction in the pH of the beans (Schwan and Wheals 2004; Afoakwa 2010; Afoakwa 2011b).The problem of acid beans might be effectively resolved by influencing microbial activity through mechanical depulpingof pulp prior to fermentation.

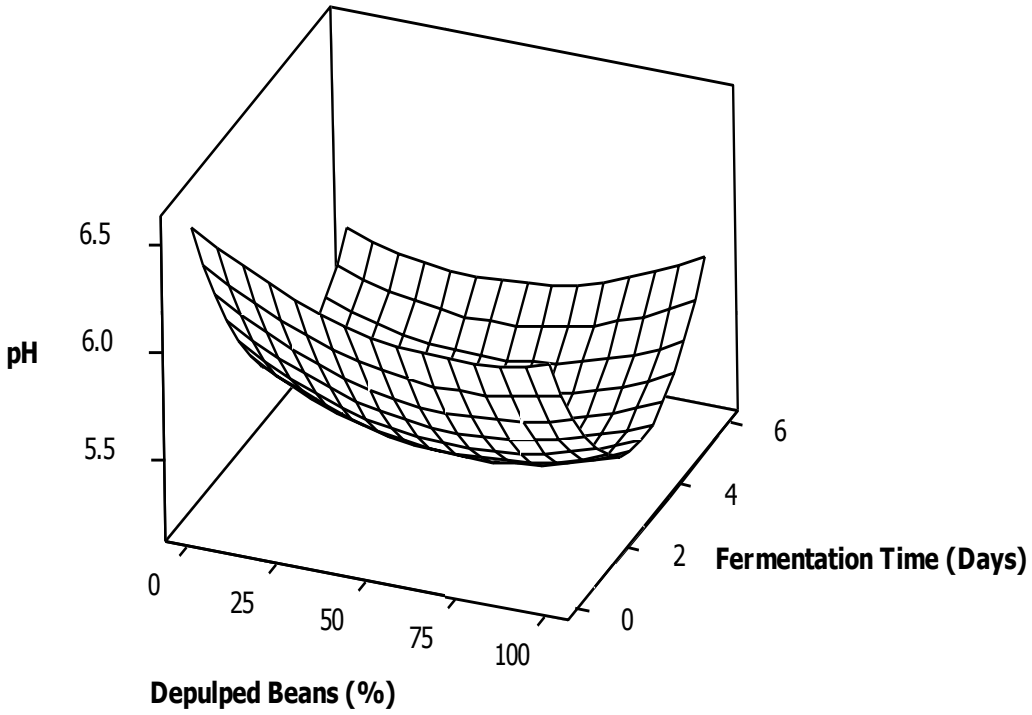

Figure 7: Response surface plot showing the effects of depulping and fermentation time on the pH of cocoa beans

Regression analysis showedthat the linear and quadratic factors of depulped beans and fermentation time caused significant ($p<0.05$) reductionin pH (Table 9). The model developed could explain over 85% of the variations in pH (Table 9).

4.2.3 Titratable acidity

Organic acids have a pronounced impact on food flavourand quality. Usually, in acetic acid fermentations such as cocoa fermentation systems, it is important to know how much acidity comes from the acetic acidand how much is contributed naturally by other acids in the system (Nielsen, 2010).

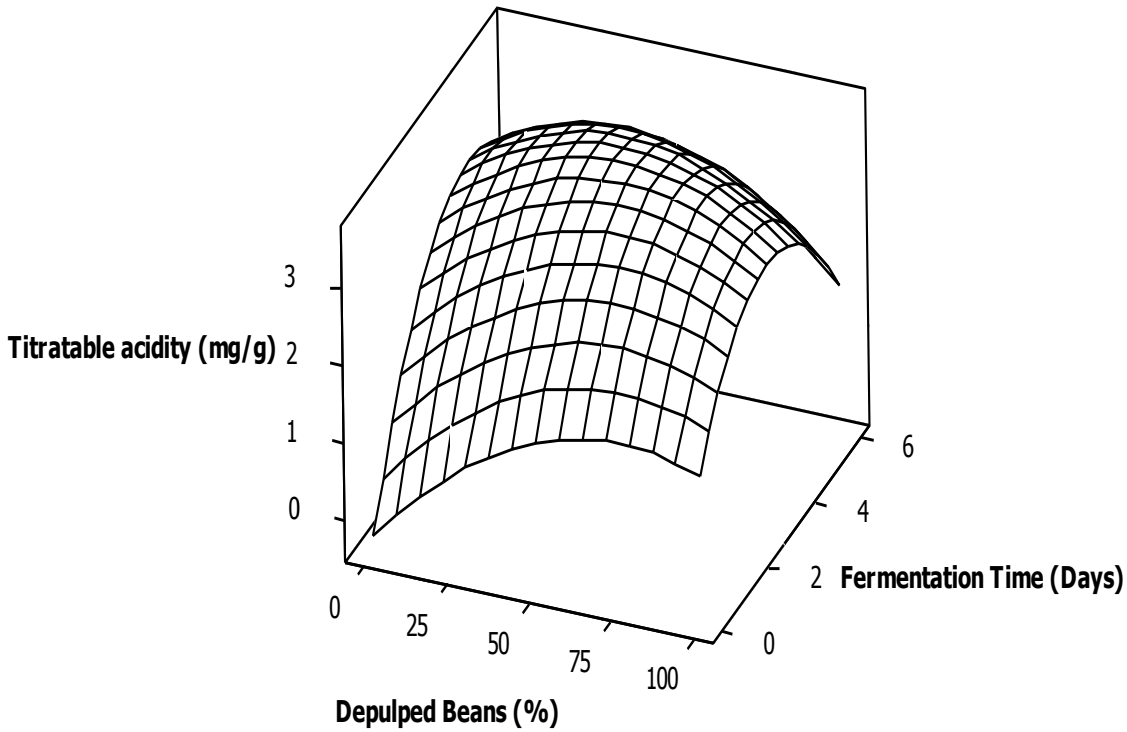

Figure 8: Response surface plot showing the effects of depulping and fermentation time on the titratable acidity (mg/g) of cocoa beans

Fermentation caused increases in titratable levels.The Titratable acidity increased from 0.37 mg/g at the start of fermentation to 2.98 mg/g by the fourth (4^{th}) day of fermentation in samples containing 0% depulped beans. Titratable acidity also increasedfrom 1.79 mg/g from the beginning of fermentation to 3.49 mg/g by the fourth (4^{th})day of fermentation in samples containing 75% depulped beans. The increases in titratable acidity were consistent with all samples containing 0 to 100% depulped beans as shown in the response surface plot (Figure 8).

However therewere slight decreases in titratable acidity from the forth day (2.98 mg/g) to the end of fermentation to(2.59 mg/g) in undepulped beans. Titratable acidity also reduced from 2.88 mg/g on the fourth day of fermentation to 1.06 mg/g at the end of fermentation in samples containing 100% depulped beans.

Table: 8. Regression coefficients and their R^2 values in the models for pH, Titratable acidity, pulp volume, free fatty acids, total, non-reducing and reducing sugars of cocoa beans

Variables	pH	Titratable acidity	Pulp volume	FFA	Total sugars	Non-reducing sugars	Reducing sugars
Constant	6.55998*	-0.30184	45.1738*	1.92991*	1071.86*	881.143*	190.722*
X_1	-0.01092*	0.05021*	-0.2259*	-0.06619*	6.37*	5.284*	1.088*
X_2	-0.58348*	1.44344*	-3.9627*	-0.96403*	119.74*	90.352*	29.392*
X_1^2	0.00008*	-0.00033*	0.0012*	0.00053*	-0.06*	-0.057*	-0.004
X_2^2	0.07269*	-0.16868*	-0.0798	0.12035*	-26.75*	-24.554*	-2.192*
X_1 x X_2	0.00087*	-0.00437*	-0.0074	0.01641*	-0.28	-0.074	-0.208*
R^2	0.8533	0.8469	0.9216	0.8686	0.7317	0.712	0.6083

X_1: Depulped Beans and X_2: Fermentation Time

*Significant at $P<0.05$.

The surface plot also revealed that depulping of cocoa beans causes increases in titratable acidity up to the fourth day of fermentation then a decrease till the end of fermentation (Figure 8).Several authors have reported that during cocoa fermentation, total acidity (volatile and non-volatile acids) develops as a result of microbial activity (Schwan and Wheals 2004; Afoakwa 2010; Afoakwa 2011b).

Titratable acidity (total acidity) provides an estimate ofthe total acid content of a food because food systems often contain many acidsthat cannot be differentiated through titration (Nielsen, 2010).The regression analysis showed that both the quadratic effects of depulped beans and fermentation time hadsignificant ($p<0.05$) influence on titratable acidity (Table 9). The regression model explained about 85% of the variations in pH.

4.2.4 Changes in total sugars

The response surface plot revealed that fermentation caused a reduction in total sugar content. Total sugar content decreased with increasing fermentation time (Figure 9). At the start of fermentation total sugars increased from 1139.37mg/g at the beginning of fermentation in 0% depulped beans to 1268.94 mg/g by the fourth day of fermentation. However there was a decrease from 1268.94 mg/g by the fourth day to 715.57mg/g in 0% depulped beans at the end of fermentation (Figure 9). Similar trends were observed for all samples containing 25%, 50%, 75% and 100% depulped beans.

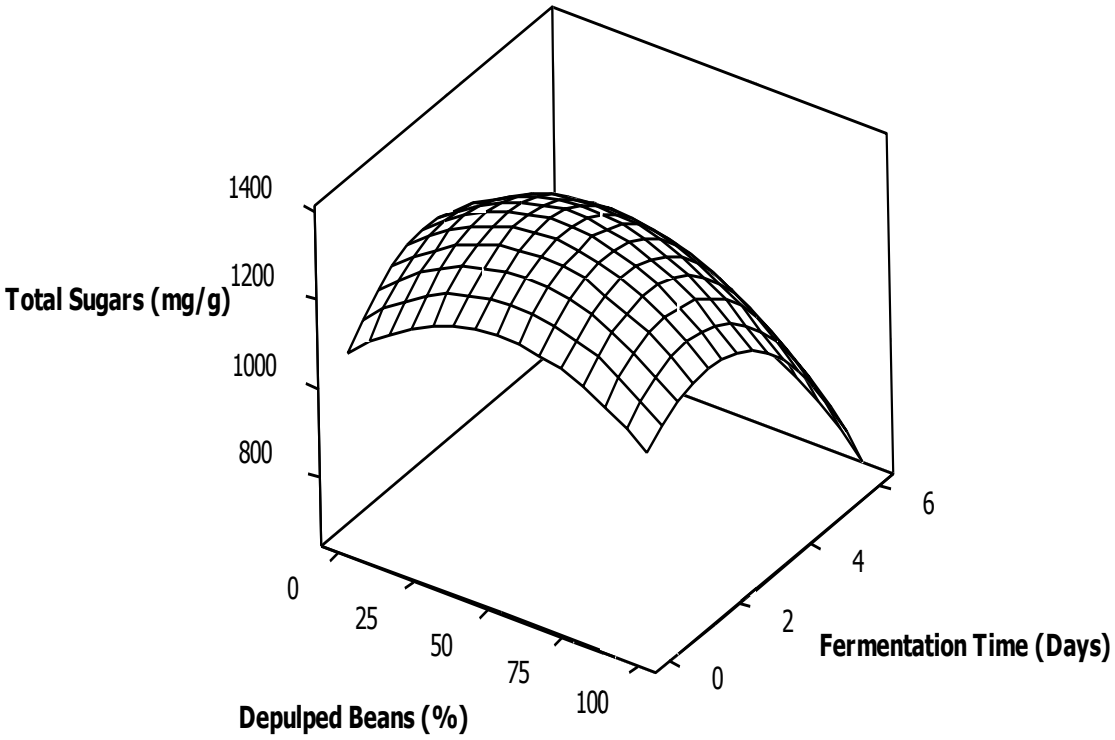

Figure 9: Response surface plot showing the effects of depulping and fermentation time on the total sugar (mg/g) content of cocoa beans

Depulping of cocoa beans led to an increase in total sugar content during fermentation. The decreasing effect of fermentation of total sugars was as a result ofreduction in non-reducing sugars by theaction of native cocoa seed invertase, which hydrolyzessucrose into glucose and fructose during fermentation (Lopez and Dimick, 1995; Hansen *et al.*, 2000; Thompson *et al.*, 2001).

Samples containing 0% to 75% depulped beans had an increase in total sugar content at the end of fermentation. Total sugar increased from 715.57 mg/g in 0% depulped beans to 929.96 mg/g in samples containing 25% depulped beans at the end of fermentation. The increasing trend was similar to samples containing 50 and 75% depulped beans, which increased from 965.97 mg/g to 1020.50 mg/g at the end of

fermentation respectively. However samples containing 100% depulped beans decreased at the end of fermentation (Figure 9).

The trends observed are similar to previous findings by Afoakwa *et al.* (2011b), howevertheir investigations were based on pod storage as pulp pre-conditioning.The sucrose concentrationin the unfermented beans generally comprisedabout 90% of the total sugars, whereas both fructose and glucose made up about 6% (Thompson *et al.,* 2001). Total sugar content apart from being produced from carbohydrate,glucose, arabinose and galactose in the fermentingseed, they may also arise by hydrolysisof the anthocyanins and polymeric phenolicglycosides (Lee, 1975; Thompson *et al.,* 2001; Afoakwa *et al.,* 2011b). Form the plot it is evident that the rate of total sugars degradation in the cocoa beans is largely affected by fermentation than depulping.

The regression analysis showed that the linear factors of depulped beans and fermentation time had significant ($p<0.05$) influence on thetotal sugar content (Table 9). Both the quadratic effects of depulped beans andfermentation time had significant influence on total sugars. The regression model explained 73% of the variations in the total sugar content caused by depulping and fermentation time (Table 9).

4.2.5 Changes in non-reducing sugars

Fermentation caused a drastic decrease in non-reducing sugar content of the cocoa beans. At the start of fermentation non-reducing sugars increased from 982.04 mg/g at the beginning of fermentation in 0% depulped beans to 1000.30 mg/g by the fourth day of fermentation. However there was a decreased from 1000.30 mg/g by the fourth day to 410.62 mg/g in 0% depulped beans at the end of fermentation (Figure 10). Similar trends were observed for all samples containing 25%, 50%, 75% and 100% depulped beans (Figure 10).

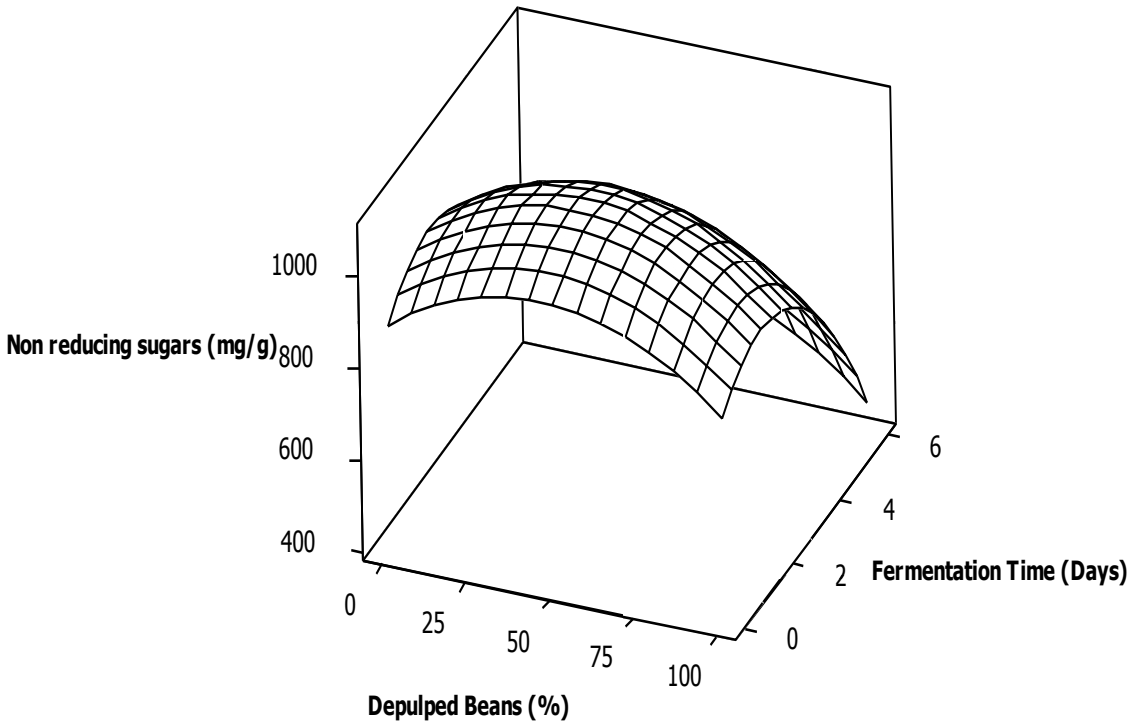

Figure 10: Response surface plot showing the effects of depulping and fermentation time on non-reducing sugars (mg/g) of cocoa beans

Depulping of cocoa beans caused an increase in non-reducing sugar content. Samples containing 0% to 75% depulped beans had increases in non-reducing sugar content at the end of fermentation. The non-reducingsugarcontent increased from 410.62 mg/g in 0% depulped beans to 756.25 mg/g in samples containing 75% depulped beans at the end of fermentation.However there was a decrease in non-reducing sugar from 756.25 mg/g in samples containing 75% depulped beans to 162.39 mg/g in samples containing 100% depulped beans at the end of fermentation (Figure 10).

Theresponse surface plot shows the combined effects of depulping and fermentation on the non-reducing sugar content in cocoa beans. Increase in DB resulted in marginal increase intotal sugar concentrations form 0 to 100%. It was also

observed that within the first 2 days of fermentation however there was a sharp increase in non-reducing sugars but followed with a consistent decrease from day 4 to day 6 of fermentation. This decrease in non-reducing sugars with increasing fermentation time can be associated with hydrolyses of non-reducing sugars (sucrose) by the enzyme invertase (Thompson *et al.*, 2001).

Regression analysis of the data showed that both thelinear effects of depulped beans and fermentation time significantly($p<0.05$) influenced the non-reducing sugars. The quadratic effect of fermentation time also had a significant effect on the non-reducing sugars (Table 9). The R^2 obtained was 71%, indicating that depulped beansand fermentation could have contributed to 71% of the changes in non-reducingsugars and 29% of the variation was due to other factors not included in the model.

4.2.6 Reducing sugars

In contrast to total and non-reducing sugars, reducing sugars usually increase after the death of the beans and attains its maximum concentrationsafter 4 days of fermentation, and remains fairly constant until the end of thefermentation (Thompson *et al.*, 2001). The response surface plot (Figure 11) showed that fermentation generally caused an increase in reducing sugars. The reducing sugars increasedwith increasing fermentation time. Reducing sugar increased from 157.33 mg/g at the start of fermentation to 304.95 mg/gbysixth day of fermentation in 0% depulped beans while there was also an increase from 225.89 mg/g at the beginning of fermentation to 274.11 mg/g at the end of fermentation in samples containing 25% depulped beans.

Depulping caused a slight decrease in reducing sugar content as shown in the response plot (Figure 11). The reducing sugars decreased from 304.95 mg/g in the 0% depulped beans to 274.11 mg/g in samples containing 25% depulped beans. There

was a similar decrease in reducing sugar from 264.25 mg/g in samples containing 75% depulped beans to 250.22 mg/g in samples containing 100% depulped beans.

This is however the normal trend with cocoa fermentation systems. During fermentation of thecocoa beans there is the hydrolysis of non-reducing sugars to yield reducing sugars, which are flavour precursors (Lopez and Dimick, 1995; Afoakwa, 2010).

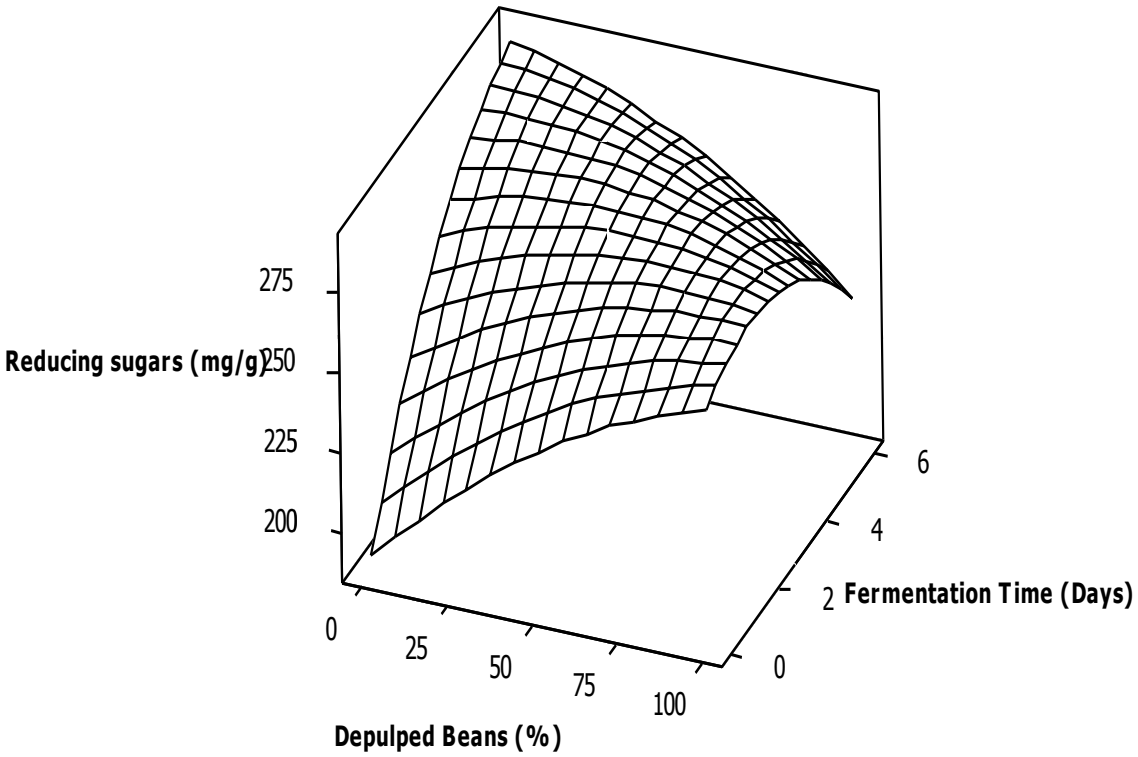

Figure 11: Response surface plot showing the effects of depulping and fermentation time on the reducing sugars (mg/g) of cocoa beans

Regression analysis of the data showed that thelinear effects of depulped beans and fermentation time were significant atp<0.05 (Table 9). The model could explain 61% (R^2) of the variations in reducing sugars.

4.2.7 Free fatty acid

Free fatty acids (FFA) are significant forthe quality of the oil because they increase the oils susceptibility to oxidation, contribute bitter and soapy off-flavours in chocolate and cocoa butter (Dand, 1997; Wrolstad*et al.*, 2005).

The response surface plot (Figure 12) showed that fermentation caused an increase in FFA content. As fermentation increased there was an increase in FFA. FFA content increased from 0.26% at start of fermentation to 0.89% at the end of fermentation in 0% depulped beans. Similar increasing trends were observed for all samples containing 25 to 100% depulped beans. The highest FFA increased from 0.56% at the beginning of fermentation to 10.66% at the end of fermentation in samples containing 100% depulped beans.

Depulping also caused an increase in FFA content. The increase in FFA with increasing depulping might be as a result of the cellular disruption that allowed free enzyme substrate interaction during the agitation of the cocoa beans via the depulper.

The FFA content increased from 0.89% in 0% depulping to 0.94% in samples containing 25% depulped beans. FFA also increased from 1.55% in samples containing 50% to 10.66% in samples containing 100% depulped beans.Free fatty acids indicate the breakage of theester bond between the fatty acid and the glycerolbackbone, releasing the fatty acid. The increase of FFA observedcan be attributed to the presence of lipases during fermentation. Lipases are not only native to the cocoa bean but also introduced by microorganisms.

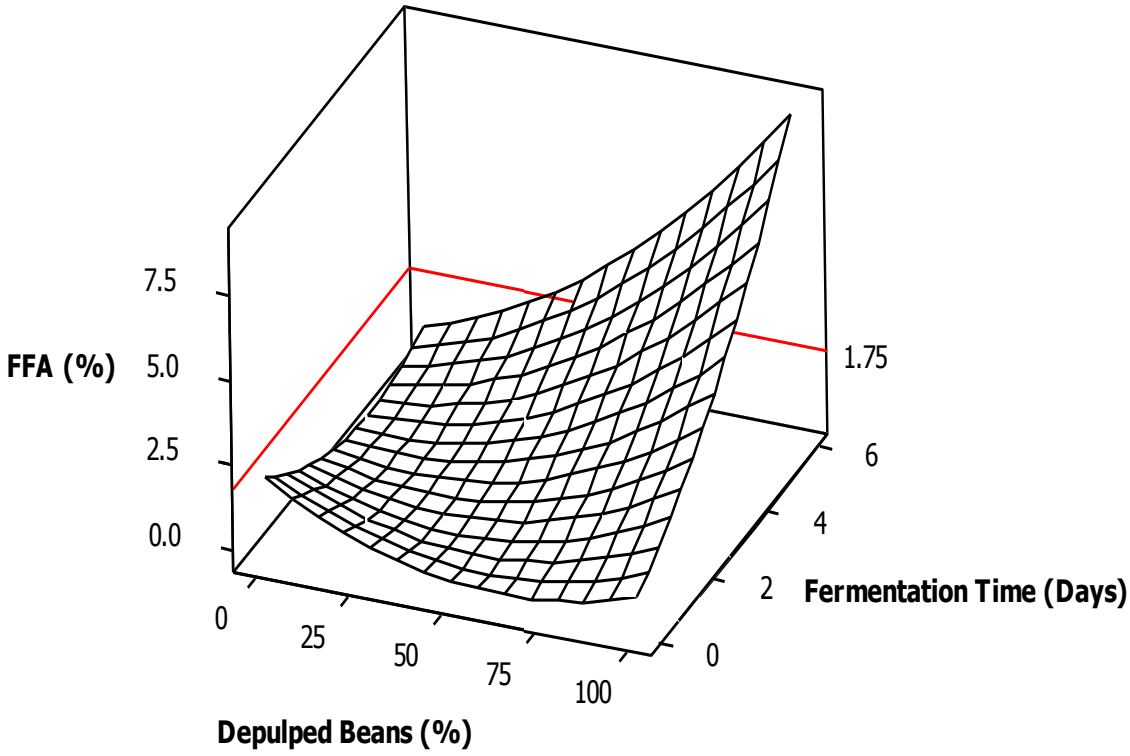

Figure 12: Response surface plot showing the effects of depulping and fermentation time on the free fatty acid (%) content of cocoa beans

Increasing trends of FFA observed for samples containing 50%, 75% and 100% depulped beans from the second (2^{nd}) day through to the end of fermentation, could also be associated with the development of *Bacillus* species that accounts for 80% of the microbial population during the later stages of fermentation (Thompson *et al.*, 2001) is favouredby an increased aeration (largely due to the break down and liquefaction of pulp by microorganisms and also the facilitation of the process by mechanical depulping), increased pH (3.5 to 5.0)of the pulp, and an increase in temperature to 45 to 50°C all of which are very characteristic of the fermenting mass from the 2^{nd} to the 6^{th} day of fermentation. According to Thompson *et al.* (2001)short chain (C3, C4, and C5) free fatty acids, which are predominant in the fermenting mass

during the aerobic phase, may contribute to the development of some of the off-flavours.

Regression analysis also showed thatthe linear and quadratic factors of depulped bean and fermentation time had significant ($p<0.05$) influence on the FFA content (Table 9). The R^2 could explain 87% of the variations in FFA content while the remaining 13% were not included in the model.

4.3 Effect of Mechanical Depulping on the Polyphenolic Compounds Concentration, Degree of Fermentation and Appearance Properties of Cocoa Beans

4.3.1 Total polyphenols

Total polyphenols decreased with increasing fermentation for all levels of depulping as shown in the response surface plot (Figure 13). Total polyphenolic content decreased from 31.29 mg/gat the start of fermentation to 22.23 mg/gat the end of the fermentation process in undepulped beans.The polyphenolic content also decreased from 28.98 mg/g to 19. 9 mg/g in samples containing 50% depulped beans. There was consistent decrease from 33.02 mg/gto 21.47 mg/g in samples containing 100% depulped beans at the end of fermentation.Results from this study suggested that fermentation of cocoa beans leads to loss of total polyphenols in fermented cocoa beans.Due to the bitter and astringency of cocoa beans, fermentation is necessary to reduce it to appreciable contents.

Depulping on the other hand caused a marginal reduction in total polyphenols. It reduced from 22.23 mg/g in 0% depulped beans to 19.9 mg/g in samples containing 25% depulped beans at the end of fermentation. There was also a decrease from 15.47 mg/g in samples containing 50% depulped beans to 8.76 mg/g in samples containing 75% depulped beans. But there was a sharp increase from 8.76 mg/g in samples containing 75% depulping to 21.47 mg/g in samples containing 100% depulped beans at the end of fermentation.

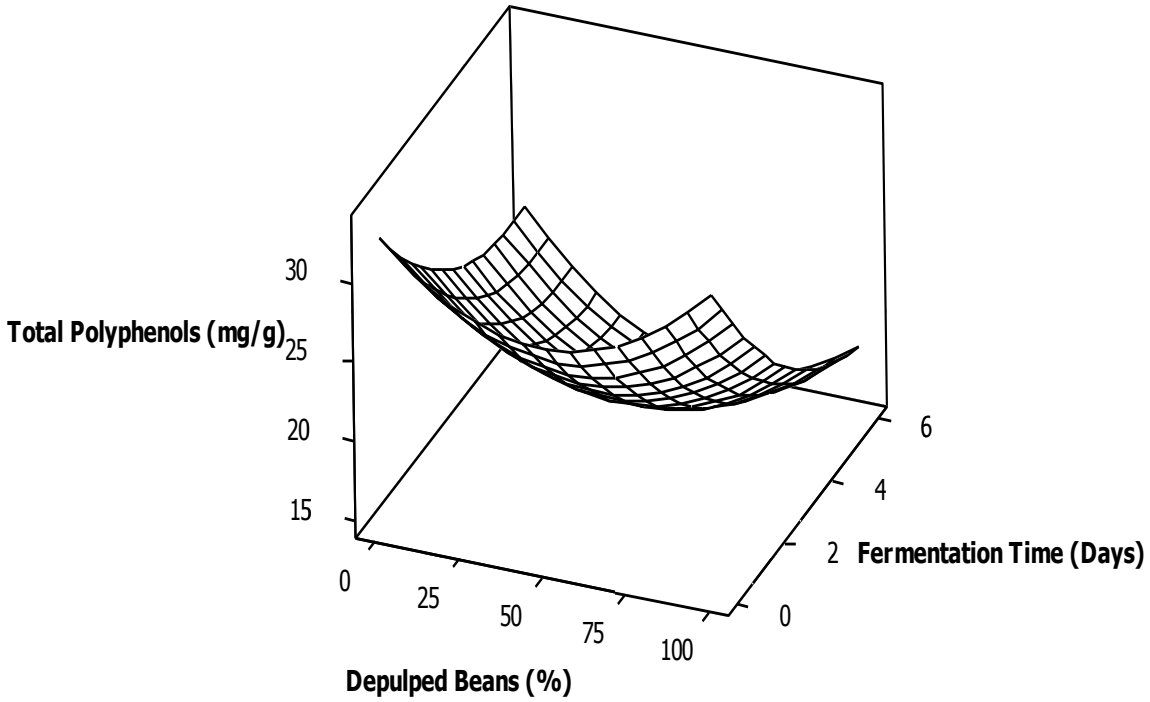

Figure 13: Response surface plot showing the effects of depulping and fermentation time on total polyphenolic (mg/g) contents of cocoa beans

Hydrolysis of polyphenols is important during fermentation since there is an inverse relationshipbetween the flavour development andthe purple color retained after fermentation (Lopez and Dimick, 1995; Nazaruddin, 2006; Afoakwa *et al.*, 2012b). Polyphenol oxidase (PPO) becomes active during the aerobic phase of fermentationas a result of oxygen permeating the beans.

This cannotonly be attributed to the oxidation process but also caused by diffusingof polyphenols into fermentation sweatings (Kimand Keeney, 1984). The reduction will drastically lower the level ofastringency in the cocoa bean. The changes in total polyphenols during fermentation as shown in Figure 13, the reduction in pulp volumemight have facilitated the oxidation and polymerization of polyphenols to high molecular compounds such as insoluble tannins.

Regression analysis also showed thatthe linear and quadratic factors of depulped beans and fermentation time had significant ($p<0.05$) influence on the polyphenolic content of the cocoa beans (Table 10). The R^2 could explain 77% of the variations in total polyphenol content.This study indicates that samples containing 25%, 50% and possibly 75% depulped beans(pulp pre-conditioning) could create optimum conditions for the degradation of total polyphenols.

4.3.2 *O*-diphenols

O-diphenols decreased with increasing fermentation for all levels of depulping as shown in the response surface plot (Figure 14). *O*-diphenols content decreased from 59.92 mg/gat the start of fermentation to 47.95 mg/gat the end of the fermentation process in 0% depulped beans.*O*-diphenols content also decreased from 49.94 mg/g to 32.68 mg/g in samples containing 50% depulped beans; there was consistent decrease from 51.55 mg/g to 28.64 mg/g in samples containing 100% depulped beans at the end of fermentation.Results from this study suggested that fermentation of cocoa beans leads to loss in *O*-diphenols in fermented cocoa beans.

Depulping on the other hand caused a slight reduction in *O*-diphenols. *O*-diphenols reduced from47.95 mg/g in 0% depulped beans to 44.46 mg/g in samples containing 25% depulped beans at the end of fermentation. There were also decreases from 32.68 mg/g in samples containing 50% depulped beans to 28.64 mg/g in samples containing 100% depulped beans

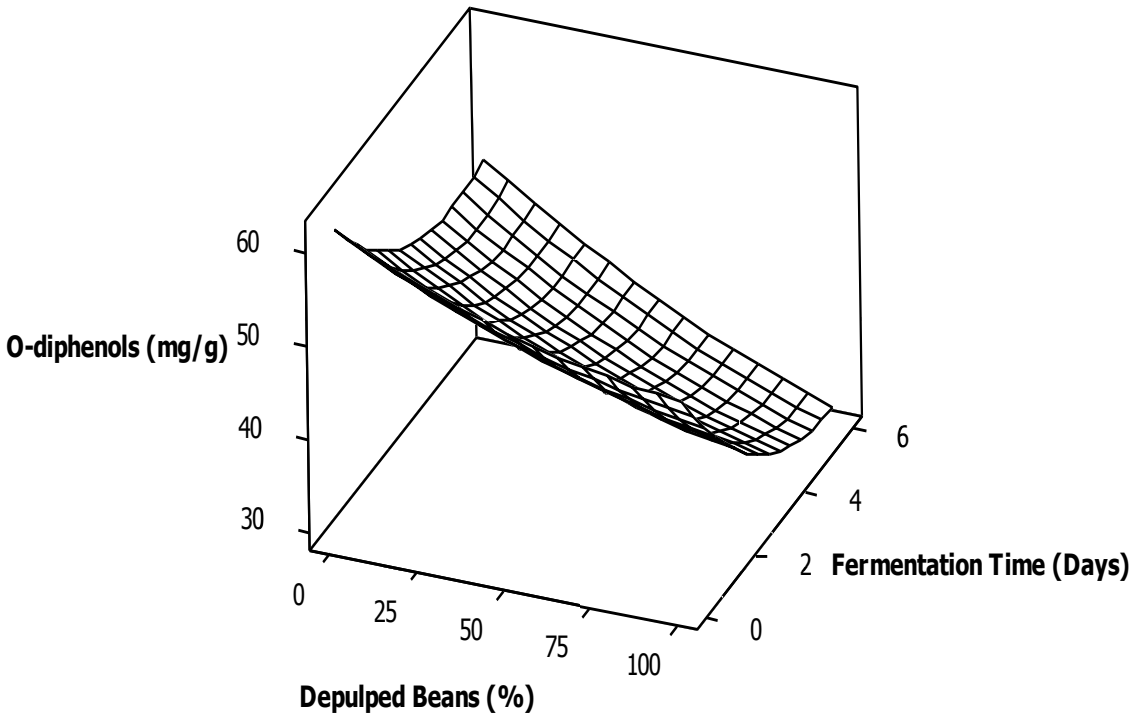

Figure 14: Response surface plot showing the effects of depulping and fermentation time on the *o*-diphenol (mg/g) content of cocoa beans

Due to the anaerobic nature of the polyphenol oxidase (PPO) reaction, oxygen requiredfor the catalytic processes that lead to hydroxylationof monophenol to *o*-diphenoland oxidation of*o*-diphenol to highly reactive *o*-quinone is highly favoured among the depulped beans due to the reduction of pulp volume via the action of the depulper and the oxidation is further exacerbated by the liquefaction of pulp by the action of microorganisms with the progression of fermentation. This allows more air current flow within the fermenting mass, which aids the reaction of PPO, which in turn non-enzymatically polymerise into brown pigments. The PPO is also affected by the pH of the medium, with an optimum pH of 6 at about 31.5 – 34.5˚C (Chazarra *et al.*, 1999), the pH of the fermenting bean was between 5.7 at the end of fermentation in undepulped beans to 6.02 at the end of fermentation in samples containing 100% depulped beans.

Regression analysis also showed that only the linear and quadratic factors of fermentation time significantly ($p<0.05$) influenced the *O*-diphenols (Table 10). The R^2 could explain 75% of the variations in *O*-diphenols contents.This study indicates that samples containing 25%, 50% and possibly 75% depulped beans(pulp pre-conditioning)could create optimum conditions for the degradation of *O*-diphenols in cocoa beans.

Table: 9. Regression coefficients and their R^2 values in the models for total polyphenols, *o*-diphenols and fermentation index of cocoa beans

Variables	Total Polyphenols	*O*-diphenols	Fermentation Index
Constant	32.3477*	61.6522*	0.413181*
X_1	-0.2006*	-0.1825	0.002770
X_2	-3.5991*	-5.6970*	0.200625*
X_1^2	0.0021*	0.0007	-0.000025
X_2^2	0.3306*	0.5987*	-0.014437*
X_1 x X_2	-0.0088	-0.0139	-0.000020
R^2	0.7675	0.7455	0.9029

X_1: Depulped Beans and X_2: Fermentation Time

*Significant at $P<0.05$.

4.3.3 Fermentation index

Well-fermentedcocoa beans should attain a fermentation index (FI) value of 1 and above (marked in red in Figure 15) after the stipulated fermentation period (Gourieva and Tserrevitinov, 1979).

The response graph shows that fermentation caused consistent increases in fermentation index. The FI increased from 0.41 at the start of fermentation to 1.09 at the end of the sixth day of fermentation in 0% depulped beans. FI also increased from

0.46 at the beginning of fermentation to 1.22 at the end of fermentation in samples containing 25% depulped beans. These increases in FI were consistent for all levels of depulped beans (Figure 15).

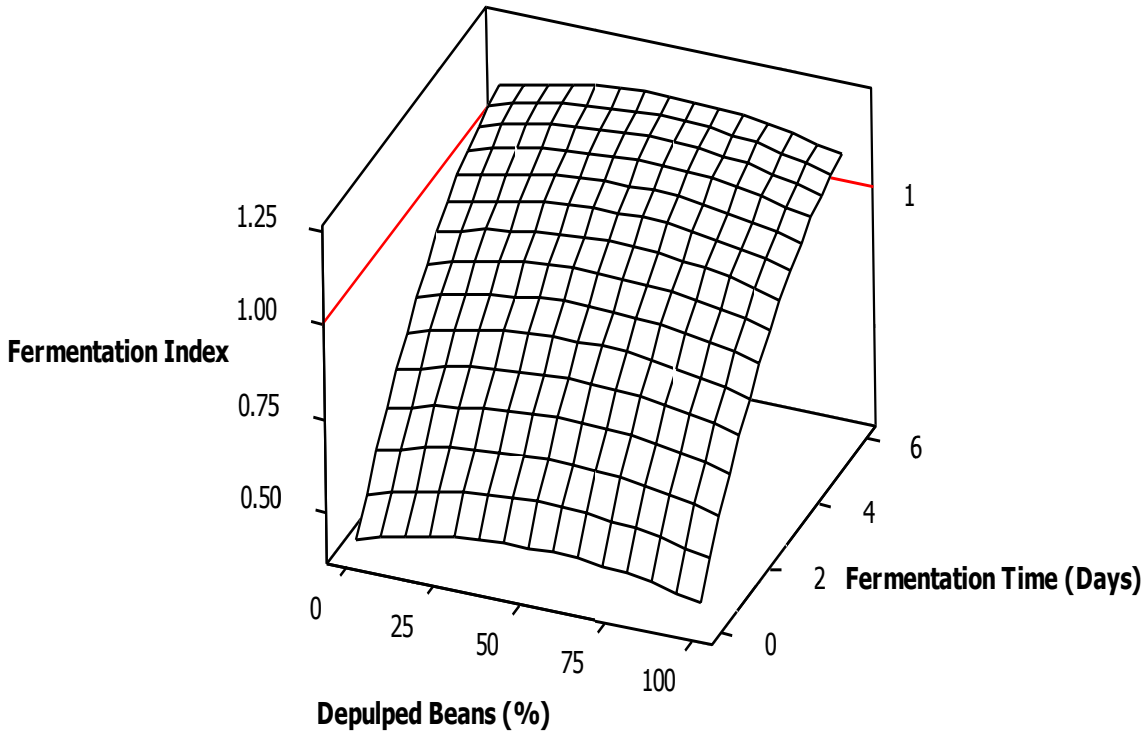

Figure 15: Response surface plot showing the effect of depulping and fermentation time on the fermentation index of cocoa beans

Depulping of cocoa beans had marginal effect on fermentation index. FI was fairly constant with an increase from 1.09 in 0% depulped beans to 1.22 in samples containing 25% depulped beans at the end of fermentation. The FI also reduced from 1.24 in samples containing 50% depulped beans to 0.98 in samples containing 75% of depulped beans. To a large extent all depulped beans but especially 0%, 25%, 50% and 100% depulped beansobtained FI of one (1) by the forth (4^{th}) day of fermentation (Table 11).This could be attributed to the rapid degradation of polyphenols during the

initial stages of fermentation leading to the leaching of coloured compounds and during the later stages the development of insoluble complex compound leading to the formation of brown colour of well-fermented cocoa beans (Nazaruddin, 2006; Afoakwa, *et al.*, 2012b).

Regression analysis showed that the linear and quadratic factors of fermentation time significantly($p<0.05$) influenced the FI (Table 10). The R^2 could explain 90% of the variations in fermentation index. The unexplained 10% variation was not accounted for by the model.

Table: 10. Effect of mechanical depulping and fermentation time on the colour fractions absorbance and fermentation index

Depulped Beans (%)	Fermentation Time (Days)	Colour Fractions Absorbance		Fermentation Index (460nm/530nm)
		Fractions I 460nm	Fraction II 530nm	
0	0	0.813	1.983	0.41 ± 0.02
	2	0.534	0.711	0.75 ± 0.00
	4	0.489	0.478	1.03 ± 0.05
	6	0.545	0.499	1.09 ± 0.00
25	0	0.609	1.326	0.46 ± 0.00
	2	0.433	0.510	0.85 ± 0.02
	4	0.509	0.600	0.85 ± 0.04
	6	0.525	0.432	1.22 ± 0.01
50	0	0.616	1.479	0.42 ± 0.01
	2	0.424	0.476	0.89 ± 0.02
	4	0.561	0.510	1.10 ± 0.00
	6	0.456	0.369	1.24 ± 0.03
75	0	0.528	0.948	0.56 ± 0.01
	2	0.447	0.464	0.96 ± 0.02
	4	0.435	0.465	0.94 ± 0.01
	6	0.333	0.338	0.98 ± 0.01
100	0	0.642	1.726	0.37 ± 0.02
	2	0.512	0.693	0.74 ± 0.03
	4	0.517	0.512	1.01 ± 0.01
	6	0.456	0.378	1.21 ± 0.00

4.3.4 Colour

Increasing fermentation caused a decrease in lightness (L).The lightness decreased from 24.00 at the beginning of fermentation to 21.71 at the end of fermentation in 0% depulped (undepulped) beans. There was also a decrease from 23.81 at the start of fermentation to 22.22 after fermentation in samples containing 50% depulped beans (Table 12). Generally, the cocoa samples became darker with increasing fermentation time.

Depulping also caused slight decreasein the lightness (L) of the cocoa beans. The L reduced from 24.05 in samples containing 25% depulped beans to 23.81 in samples containing 50% depulped beans at the start of fermentation (Table 12).

Fermentation caused slight increase in the a-value of the cocoa beans (Table 12) with the exception of sixth day of fermentation which decreased. The a-value increased from 7.37 at the start of fermentation to 8.12 by the fourth day of fermentation in 0% depulped beans. The increment was consistent for all levels of depulped beans.

Depulping had a varied influence on the a-value of cocoa beans. The a-value remained fairly constant for all levels of depulped beans. Depulping caused an increase in redness from 7.68 in 0% depulped beans (undepulped) to 8.10 in samples containing 25% depulped beans at the end of fermentation. It also decreased from 8.47 in samples containing 75% depulped beans to 7.07 in samples containing 100% depulped beans at the end of fermentation (Table 12).

This might be due to the hydrolysis of anthocyanins by Glycosidases (β-gaiactosidase) to 3-β-D galactosidyl cyanidin and 3-α-L-arabinosidyl cyaniding causing a reduction in purplish colour.

Table: 11. Colour of cocoa beans with respect to depulping and fermentation time

Depulped Beans (%)	Fermentation Time (Days)	L*	a*	b*
0	0	24.00 ± 0.05	7.37 ± 0.06	2.56 ± 0.11
	2	21.90 ± 0.02	8.67 ± 0.08	2.55 ± 0.09
	4	21.56 ± 0.02	8.12 ± 0.14	3.28 ± 0.14
	6	21.71 ± 0.02	7.68 ± 0.06	2.59 ± 0.14
25	0	24.05 ± 0.05	7.85 ± 0.18	2.23 ± 0.13
	2	21.94 ± 0.02	9.45 ± 0.12	2.51 ± 0.14
	4	21.18 ± 0.02	8.01 ± 0.02	2.06 ± 0.09
	6	21.32 ± 0.00	8.10 ± 0.04	2.88 ±0.06
50	0	23.81 ± 0.06	7.64 ± 0.04	2.28 ± 0.12
	2	23.11 ± 0.02	9.63 ± 0.07	3.61 ± 0.09
	4	23.05 ± 0.03	9.58 ± 0.08	3.41 ± 0.13
	6	22.22 ± 0.04	7.62 ± 0.07	3.66 ± 0.07
75	0	21.37 ± 0.08	8.86 ± 0.05	2.59 ± 0.11
	2	23.34 ± 0.00	9.08 ± 0.14	3.96 ± 0.16
	4	21.06 ± 0.05	7.87 ± 0.09	2.27 ± 0.20
	6	23.49 ± 0.02	8.47 ± 0.09	3.76 ± 0.11
100	0	23.91 ± 0.02	7.30 ± 0.07	1.68 ± 0.09
	2	22.47 ± 0.04	8.72 ± 0.30	2.77 ± 0.17
	4	22.44 ± 0.02	8.73 ± 0.07	3.11 ± 0.15
	6	22.44 ± 0.03	7.07 ± 0.14	3.90 ± 0.18

Increasing fermentation caused an increase in the b-value of the cocoa beans. The b-value increased from 2.56 at the start of fermentation to 2.59 at the end of fermentation in 0% depulping (undepuled). Similar increasing b-value with fermentation time was observed for all samples containing 25%, 50%, 75% and 100% depulped beans.

This can be attributed to excessive loss of polyphenols via diffusing via exudates and also enzymatic oxidation by polyphenoloxidase in the beans. It is evident that at the end of fermentation, cocoa beans became darker, less reddish and less yellowish.

Colour of the cocoa beans come as result of complex chemical reactions which can only take place at the end of a carefully and well-conductedfermentation process. The different precursor molecules in the nib of the cocoa bean oxidize and condense to form insoluble compounds called tannins that provide the brown colour of a well-fermented bean.

Bleaching andsubsequent browning of the beans has also been reported by earlier researches (Lopez and Dimick, 1995; Thompson *et al.*, 2001; Nazaruddin, 2006; Afoakwa *et al*., 2012a). However, the values obtained were far lower than that of pod stored beans as pulp pre-conditioning as illustrated by Afoakwa (2012a). The breakdown of the flavonoids (anthocyanins), which normally imparts the purple colour to under-fermented cocoa beans leads to a rather darker colour since anthocyanins are highly affected by pH. The colours range from purple in a neutral state, violet in weak alkaline solutions and pink in acidic conditions (Lee, 1975; Konczak and Zhang, 2004).

Analysis of variance (Table 13) revealed that all factors (depulping and fermentation) significantly ($p<0.05$) influenced the lightness (L), redness (a) and yellowness (b).

Table: 12. ANOVA summary table showing F-ratios for variations in colour of depulped and fermented cocoa beans

Variables	**L***	**a***	**b***
Main Effects			
A: Depulped Beans	1601.07*	105.05*	79.40*
B: Fermentation Time	5803.57*	504.57*	199.06*
Interaction			
A x B	2214.11*	80.23*	53.26*

** Significant at P<0.05*

4.4 Influence of Mechanical Depulping on the Acidification, Browning index, Anthocyanins, Colour and FFA during Drying of Fermented Ghanaian Cocoa Beans

4.4.0 Introduction

After fermenting the depulped cocoa beans,on the sixth day, the depulped beans were transferred from the heap onto raised bamboo mats. The beans were spread thinly on the mats under sufficient sunlight for six day with regular mixing to ensure uniform drying.

4.4.1 Acidification

4.4.1.1 pH

Dryingcaused a slight increase in pH from 5.46 by the fourth (4^{th}) day of fermentation in 0% depulped beans to 5.78by the fourth (4^{th}) day of fermentation in samples containing 100% depulped beans. The pH also increasedfrom 5.46in 0% depulped beans to 5.77 in samples containing 100% depulped beans at the end of drying.This explains thatincreasing levels of depulping reduces the levels of acidity in the beans.

Drying had a varying influence on the pH of the cocoa beans. The pH decreased from 5.69 atthe start of drying to 5.46 at the end of drying in 0% depulped (undepulped) beans (Figure 16).The decrease in pH was consistent for samples containing 25 and 100% depulped beans with the exception of samples containing 75% depulped beans which remained constantat the beginning and at the end of drying with a pH of 5.59. Also there was a slight increase in pH from 5.43at the beginning of drying to 5.52 at the end of drying in samples containing 50% depulped beans.

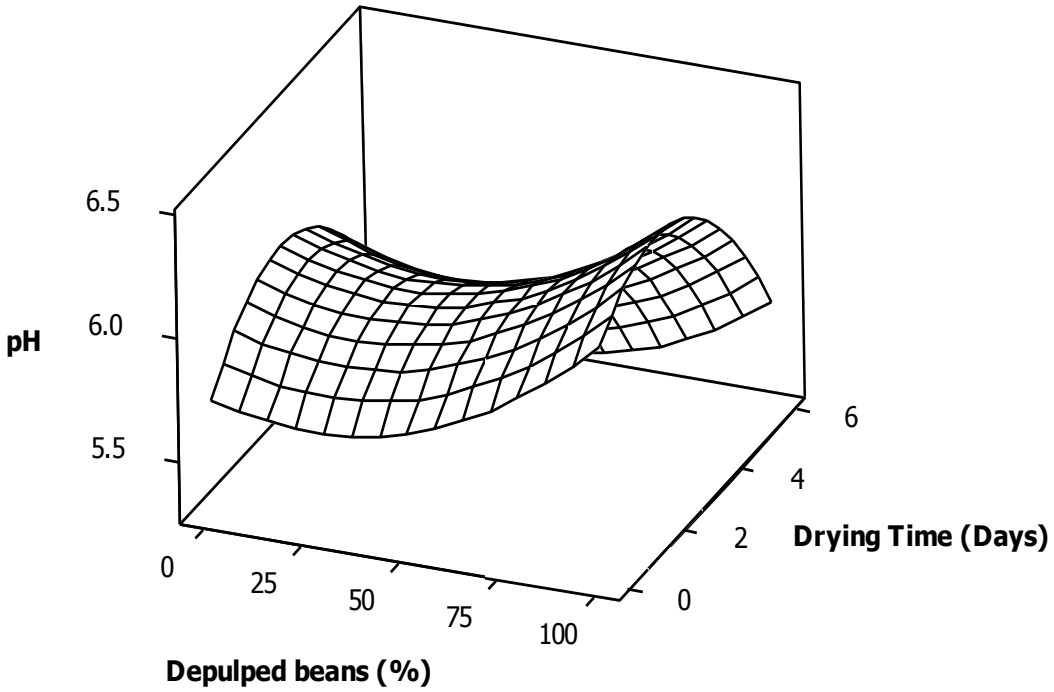

Figure 16: Response surface plot showing the effect of depulping and drying time on the pH of cocoa beans

The pH of the dried cotyledon is between 5 and 6 (Thompson *et al.*, 2001; Becket, 2009; Afoakwa, 2010). During drying of fermented cocoa beans, moisture content gradually decreases, making it increasinglydifficult for enzymes and substrates to react. Enzymatic activity ceases when amoisture content of 6to 8% is achieved (Thompson *et al.*, 2001; Belitz *et al.*, 2009). The drying processfavours aerobic microorganisms, which proliferate due to high initial moisture but decreases with moisture loss. According to Afoakwa (2010), drying reduces levels of acidity and astringencyin cocoa nibs by decreasing the volatile acids and total polyphenols.

During dryingcell liquids move across cell walls, which is aided great by the movement of water and leads to a rapidutilization of the surface acetic acid brings thepH to between *5* and 6 (Thompson *et al.*, 2001). Also due to higher air current flow

with increasing level of depulping, most volatile acids might have been lost, thus causing an increase in pH.

Regression analysis also showed thatonly the quadratic factors of drying time and depulping significantly ($p<0.05$) influenced the pH of the dried beans (Table 15). The R^2 could explain half (50%) of the variations in pH. This means that other 50% of the variation was not included in model.

4.4.1.2 Titratable acidity

Changes in titratable acidity (TA) follow anincreasing trendfor all depulped beans (Figure 17). The total acidity of the dried beans increased with drying time. The surface plot (Figure 17) showed titratable acidity increased from 0.77mg/g at the beginning of drying to 0.78 mg/g at the end of drying in 0% depulped (undepulped) beans. Also titratable acidity increased from 0.48 mg/g at the start of drying to 2.89 mg/g after drying. The increase in titratable acidity was consistent for all levels of depulped beans.

Depulping had varied influence on the titratable acidity of the dried cocoa beans. There was consistent increase in titratable acidity from 0.78 mg/g in 0% depulped beans to 2.28 mg/g in samples containing 100% depulped beans at the end of drying. But depulping increased the titratable acidity from 1.02 mg/g in 0% depulped beans to 2.52 in samples containing 25% depulped beans. Titratable acidity also increased from 2.15 mg/g in samples containing 50% depulped beans to 2.72 mg/g in samples containing 75% depulped beans.

This observed trend might be due to an increased aeration and high moisture content at the initial stages of drying growth of *Bacillus* species (over 80% of the microbial population) which proliferate at the later stages of fermentation and continue during first three days of drying due to more air flow within the bean mass

and also an increased pH (averagely 5) causes an increment of several compounds that may contribute to the total acidity of the dried cocoa.

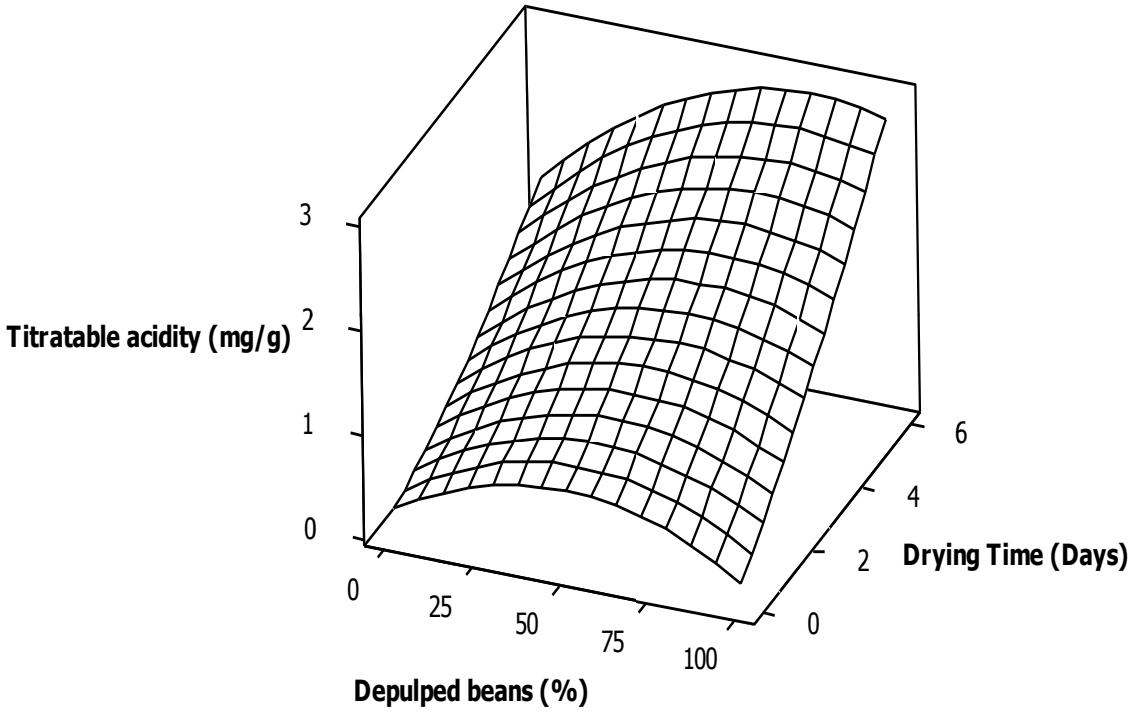

Figure 17: Response surface plot showing the effect of depulping and drying time on the titratable acidity (mg/g) of cocoa beans.

Reports by Wood and Lass (1985) revealed the rate of drying has animportant bearing on the flavour and quality of the dried beans. When drying is tooslow, there is the danger that moulds will develop and penetrate the testa whiles rapid drying may prevent the oxidative changes being completed and this might result in excessive build up of acidity.This is suggestive that drying must be done by day 4 to prevent high acidity of the depulped and fermented cocoa beans.

Regression analysis (Table 15) also showed that linear and quadratic factors for depulped beans significantly($p<0.05$) influenced the titratable acidity of the dried beans (Table 15). The R^2 could explain 72% of the variations due to depulping and fermentation time.

4.4.2 Anthocyanins

Condensationproducts of anthocyanins are usually formed during fermentation and continue during drying. The response surface plot (Figure 18) showed that the anthocyanins content of the beans varied significantly ($p<0.05$) with increasing dryingtime for all depulped beans. It decreased from 7.60 mg/gat the start of drying to 5.35 mg/gby the fourth day of drying in 0% (undepulped) beans. It also decreased from 6.35 at the start of drying to 6.16 mg /g by the fourth day of drying in samples containing 25% depulped beans. However there was a slight increase in anthocyanin content from the fourth day to the last day of drying. Anthocyanin content slightly increased from 5.35 mg/g by the fourth day of drying to 6.19 mg/g at the end of drying in 0% depulped beans. Similar increasing trends were also observed in samples containing 25, 50, 75 and 100% depulped beans.

Depulping also had a varying effect on the anthocyanin content. The anthocyanin content decreased from 6.19 mg/g in 0% depulped beans to 5.55 mg/g in samples containing 50% depulped beans at the end of the drying period (Figure 18). However, anthocyanin also increased from 6.74 mg/g in samples containing 75% depulped beans to 7.48 mg/g in samples containing 100% depulped beans at the end of the drying period.

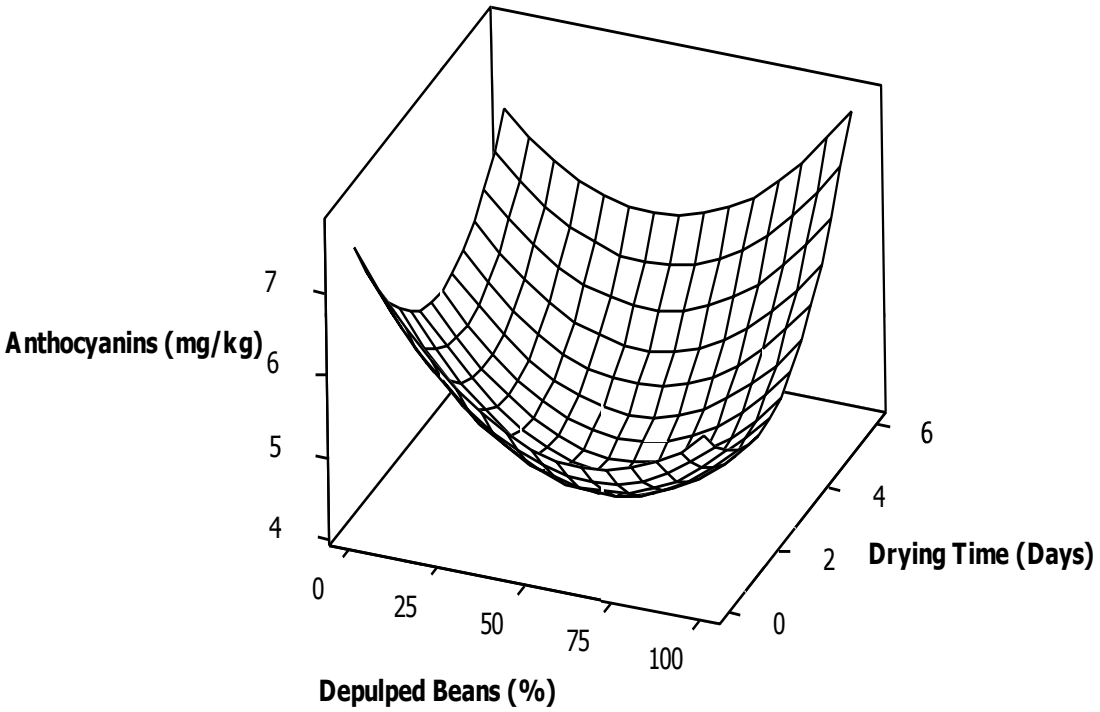

Figure 18: Response surface plot showing the effect of depulping and drying time on the anthocyanin content of cocoa beans

One of themost important aspects of drying is the browning of cocoa beans to appreciable amounts. Thompson *et al*. (2001) noted that the anthocyanin pigment themselves do not possess any marked flavour potential but its hydrolysis is important to formation of desirable brown colour and the reduction of the purple colour. It also contributes reducing sugars (galactose and arabinose) by the action of the enzyme glycosidases. Pettipher (1985) observed that drying could reduce an existinganthocyanin content of the beans by 13 – 44% depending on the time offermentation.

Regression analysis (Table 15) also showed that all the factors; linear, quadratic and interaction effects for both depulped beans and drying time significantly ($p<0.05$)influenced the anthocyanin content (Table 15). However the R^2 could explain 52% of the variations in the anthocyanin content.

4.4.3 Free fatty acid

Free fatty acid (FFA)level affects the fatstructure and thus the shelf life of cocoa butter and other products manufactured from cocoa liquor. If severe,it causes rancidity of the cocoa butter (EEC, 1990; Belitz*et al*., 2009). In dried cocoa beansFFA should not exceed 1.75% (marked red inFigure 19).

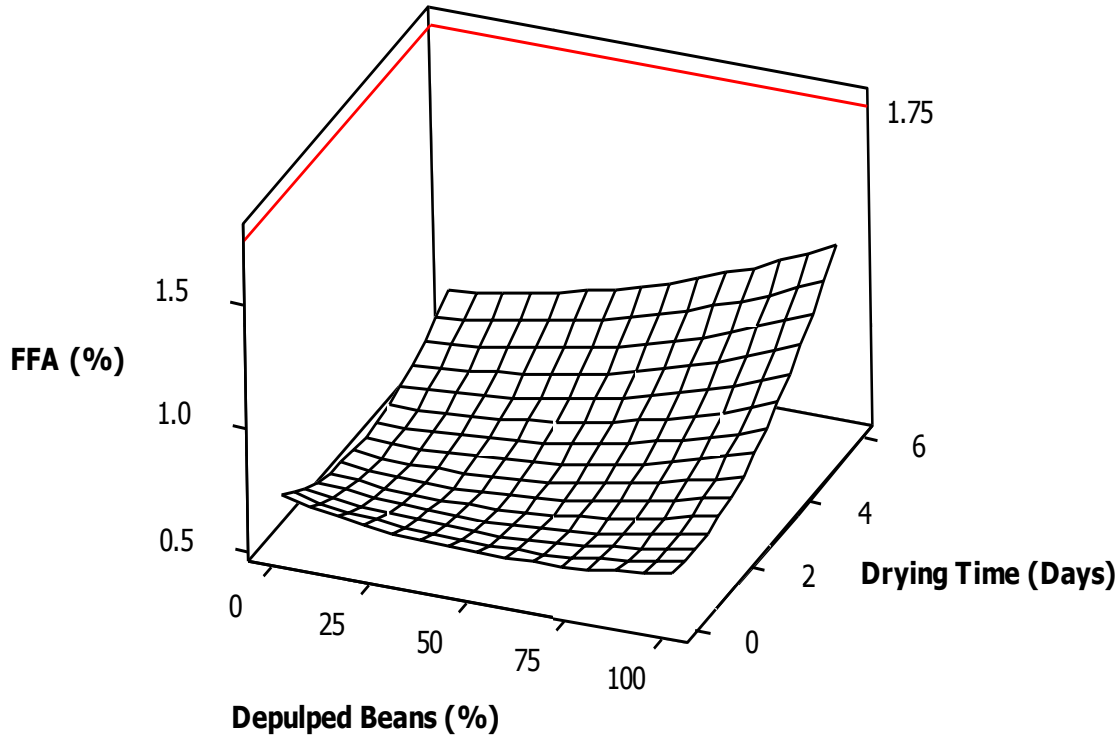

Figure 19: Response surface plot showing the effects of depulping and drying time on the free fatty acid (%) content of cocoa beans

The response surface plot (Figure 19) revealed that during drying there was a marginal increase in the FFA levels. As drying progressed, there was an increase in FFA. The FFA content increased from 0.66% at start of drying to 0.82% at the end of drying in samples containing 25% depulped beans. Similar increasing trends were observed for all samples containing 50% to 100% depulped beans. The highest FFA

increased from 0.42% at the beginning of dryingto 1.30% at the end of drying in samples containing 75% depulped beans.

Depulping also caused an increase in FFA content. Increasing depulping caused an increase in FFA. The FFA content increased from 0.70% in 0% depulping to 0.89% in samples containing 100% depulped beans at the beginning of drying. FFA also increased from 0.54% in 0% depulped beans to 1.12% in samples containing 100% depulped beans at the end of drying. This increasing trend was consistent with all the levels of depulped beans (Figure 19).

The FFA content after drying was far below the stipulated 1.75% (marked red in Figure 8) this can be attributed to short chain (C3, C4, and C5) fatty acids, which might be predominant duringthe aerobic phase of fermentation but reduced during the drying stages. The reduction of the short chain fatty acid might be due to their volatility that might have been exacerbated by the heat from drying also according to Belitz *et al.* (2009) carboxylic acids usually (C1 – C5) are polar hence can be dissolved in water suggesting that majority of theses free fatty acids produced could have been within a carbon 1 to carbon 5 (C1 – C5) range which were lost via the reduction in moisture content during drying but higher (C6 and above) are less soluble due to the hydrophobic nature of the alkyl chain. This also suggests that depulping and fermentation might not cause a surge in FFA after drying hence can be employed as an effective mechanism of pulp pre-conditioning.

Regression analysis also showed thatonly the linear and quadratic factors of drying time and the interaction effect significantly ($p<0.05$) influenced the free fatty acid content of the dried beans (Table 15) and the R^2 could explain 52% of the variations in free fatty acid content.

4.4.4 Browning index

The response plot (Figure 20) shows that drying caused a decrease in browning index (BI). The BI decreased from 1.23 at the start of drying to 1.09 at the end of the sixth day of drying in samples containing 25% depulped beans. BI also decreased from 1.09at the beginning of drying to 0.88 at the end of drying in samples containing 50% depulped beans. The increase in FI was consistent for all levels of depulped beans (Figure 20) with the exception of 0% depulping which had a small increase of BI from 1.02 at the start of drying to 1.05 at the end of drying (Table 14).

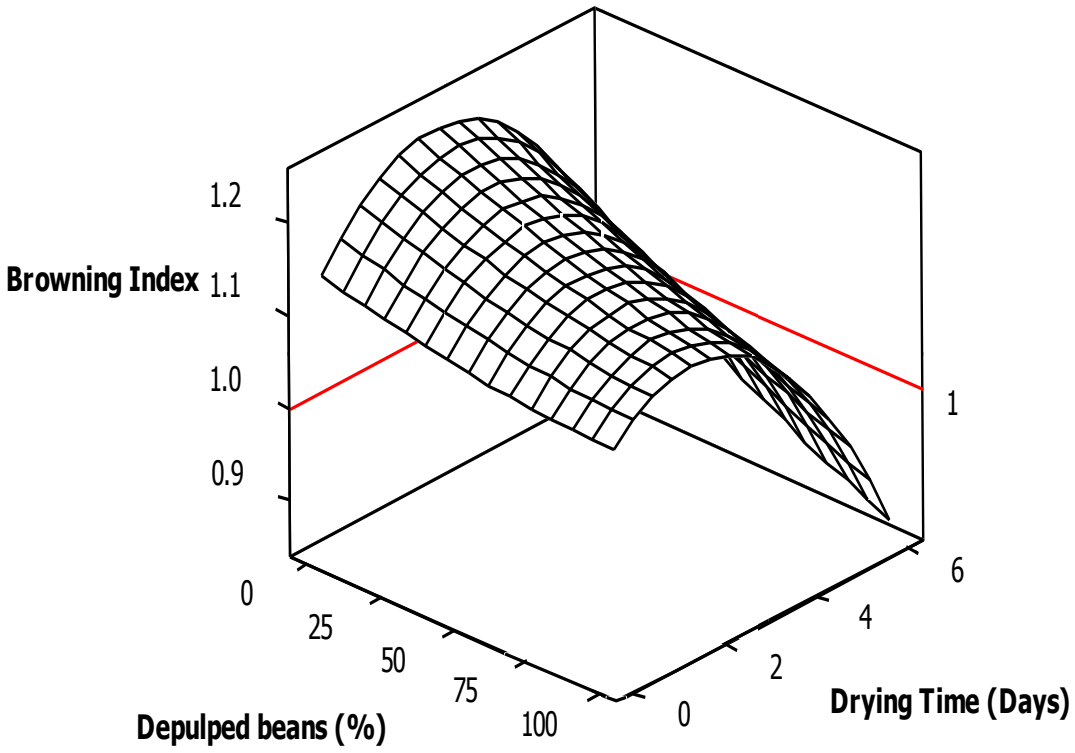

Figure 20: Response surface plot showing the effect of depulping and drying time on browning index of cocoa beans

Depulping of cocoa beans had varied effect on the browning index. BI increased from 1.05 in 0% depulped beans to 1.09 in samples containing 25% depulped beans at the end of drying.

Table: 13. Effect of fermented depulped beans and drying time on the colour fractions absorbance and browning index

Depulped Beans (%)	Drying Time (Days)	Colour Fractions Absorbance		Browning Index (460nm/530nm)
		Fractions I 460nm	Fraction II 530nm	
0	0	0.578	0.565	1.02 ± 0.00
	2	0.509	0.415	1.23 ± 0.00
	4	0.637	0.547	1.17 ± 0.00
	6	0.584	0.555	1.05 ± 0.03
25	0	0.622	0.507	1.23 ± 0.01
	2	0.495	0.368	1.35 ± 0.01
	4	0.584	0.491	1.19 ± 0.12
	6	0.541	0.497	1.09 ± 0.05
50	0	0.618	0.569	1.09 ± 0.02
	2	0.569	0.445	1.28 ± 0.02
	4	0.608	0.679	0.90 ± 0.03
	6	0.555	0.634	0.88 ± 0.02
75	0	0.549	0.542	1.01 ± 0.03
	2	0.385	0.295	1.31 ± 0.00
	4	0.523	0.580	0.90 ± 0.00
	6	0.523	0.574	0.91 ± 0.01
100	0	0.601	0.567	1.06 ± 0.01
	2	0.382	0.314	1.21 ± 0.04
	4	0.514	0.514	0.99 ± 0.00
	6	0.485	0.497	0.97 ± 0.03

The BI also increased from 0.88 in samples containing 50% depulped beans to 0.91 in samples containing 75% of depulped beans at the end of drying. Samples containing 0% and 25% had BI of one (1) by the forth (4^{th}) day of drying (Table 14).

The observed increase of BI (day 0 to 3) might have occurred as result of continued fermentation in the early stages of drying but however this was immediately curtailed by day 4 of drying with diminishing moisture content and rising heat and air current flow (Table 14 and Figure 8) which culminated in the drastic reduction of browning index. To a large extent all depulped beans were observed to have browning index (BI) of more than 1 (one) by the forth (4^{th}) day of fermentation but reduced averagely to 1 or slightly below 1 by day 4 to day 6 of

drying (Table 14). This means drying can conveniently be done for four (4) days to achieve an average browning index of 1 (one).

Regression analysis also showed thatonly the linear and quadratic factors of drying time and the interaction effect significantly ($p<0.05$) affected the browning index of the dried cocoa beans(Table 15) and the R^2 could explain only 46% of the variations of the browning index.

Table: 14. Regression coefficients and their R^2 values in the models for pH, titratable acidity, free fatty acids, anthocyanins and browning index of dried cocoa beans

Variables	pH	Titratable acidity	Free fatty acids	Anthocyanins	Browning Index
Constant	5.72389*	0.227709	0.698365*	7.47898*	1.14174*
X_1	-0.00484	0.020883*	-0.003121	-0.06690*	-0.00092
X_2	0.24455	0.123321	-0.110694*	-1.11507*	0.07505*
X_1^2	0.00010*	-0.000211*	0.000029	0.00052*	0.00000
X_2^2	-0.04788*	0.017366	0.019649*	0.16930*	-0.01514*
X_1 x X_2	-0.04788	0.002126	0.000819*	0.00381*	-0.00023*
R^2	0.4985	0.7180	0.5184	0.5239	0.4567

X_1: Depulped Beans and X_2: Fermentation Time. *Significant at $P<0.05$.

4.4.5 Colour

Most prominent amongthe flavonoids are the anthocyanins. They are universal plant colorantsresponsible for the red, purple, and blue hues evidentin many fruits, vegetables, cereal grains, and flowers (Konczak and Zhang, 2004).Drying of cocoa beans showed no distinct trendfor lightness (L), a-value and b-value for all levels of depulped beans; 0%, 25%, 50%, 75% and 100% (Table 16).

Generally, the samples became darker (Lightness)with increasing drying time and similar decrease was also recorded for increasing depulped beans. Lightness (L) decreased from 22.29 in 0% depulped beans to 21.55 in samples containing 100% depulped beans at the end of the drying period.

Table: 15. Colour of cocoa beans with respect to depulping and drying time

Depulped Beans (%)	Drying Time (Days)	L*	a*	b*
0	0	21.15± 0.01	7.74 ± 0.06	2.7 ± 0.17
	2	26.62 ± 0.01	8.15 ± 0.08	7.56 ± 0.09
	4	22.16 ± 0.04	8.02 ± 0.06	4.16 ± 0.13
	6	22.29 ± 0.03	7.46 ± 0.21	3.55 ± 0.14
25	0	20.39 ± 0.33	7.29 ± 0.06	2.41± 0.19
	2	25.32 ± 0.03	8.19 ± 0.17	6.98 ± 0.19
	4	21.98 ± 0.02	8.16 ± 0.08	3.87 ± 0.06
	6	21.73 ± 0.01	8.41 ± 0.11	3.56 ± 0.17
50	0	23.44 ± 0.01	8.68 ± 0.11	4.13 ± 0.12
	2	22.65 ± 0.02	7.09 ± 0.05	5.64 ± 2.77
	4	23.63 ± 0.02	8.66 ± 0.13	3.86 ± 0.13
	6	24.48 ± 0.01	8.22 ± 0.05	3.42 ± 0.01
75	0	23.52 ± 0.01	8.60 ± 0.11	4.11 ± 0.15
	2	21.30 ± 0.01	5.70 ± 0.10	3.68 ± 0.18
	4	22.66 ± 0.01	7.88 ± 0.10	3.82 ± 0.17
	6	23.09 ± 0.01	8.29 ± 0.08	3.60 ± 0.07
100	0	22.82 ± 0.02	6.93 ± 0.07	3.56 ± 0.06
	2	22.00 ± 0.04	6.40 ± 0.17	4.33 ± 0.06
	4	22.59 ± 0.03	7.12 ± 0.12	3.85 ± 0.10
	6	21.55 ± 0.03	7.40 ± 0.06	3.56 ± 0.01

The a-valueremained fairly constant during drying for all levels of depulped beans. A-value marginally reduced from 7.46 in 0% depulped beans to 7.40 in samples containing 100% depulped beans by the end of drying. This might be due anthocyaninsthat normally imparts the purple colour to underfermented beans.

The b-value of the cocoa beans remained fairly constant with increasing drying time and increasing depulped beans. The b-value remain constant with 3.56 in samples containing 25% and 100% depulped beans at the end of drying.

Colour may providean indication of chemical changes in a food and food materials,such as browning. The colour of cocoa beans at the end of drying translates into cocoa powder, and is one of the criteria by which cocoa powder is selected is the required colour of the endproduct. However, colour of the cocoa beans come as result of complex chemical reactions which can only take place at the end of a carefully and well-conductedfermentation and drying process.

Analysis of variance (Table 17) revealed that all factors (depulping and drying) significantly ($p<0.05$) influenced all the colour parameters; lightness (L), redness (a) and yellowness (b).

Table: 16. ANOVA summary table showing F-ratios for variations in colour of depulped and dried cocoa beans

Variables	Browning Index	L*	a*	b*
Main Effects				
A: Depulped Beans	39.75*	296.24*	229.99*	2.67*
B: Drying Time	141.68*	1015.23*	221.31*	40.51*
Interaction				
A x B	8.91*	1266.63*	125.97*	7.67*

** Significant at P<0.05*

4.4.6 Cut Test

The cut test is a qualitative standard test used to assess the suitability of cocoa beans purchase and also for processing. Different categories: purple, purple/brown, brown, slaty, germinated and mouldy are used in the assessment (Table 18).

Purple beans generally reduced with increasing fermentation time. Reduction was more prominent in 0% depulped beans which decreased from 70 at the start of fermentation to 0 at the end of fermentation. Samples containing 25%, 50% and 75% depulped beans also recorded reduction in purple colour from 50 to 15, 17 to 19 and 31 to 12 respectively (Table 18). Samples containing 100% depulped beans reduced from 42 at the start of fermentation to 25 purple beans at the end of fermentation (Table 18).

Purple/brown beans, according to Minifie (1970) should make up at least 20% of the beans with an upper limit of 50%. Samples containing 25% and 50% had no purple beans at the end of fermentation. But samples containing 0%, 75% and 100% depulped beans had values below 30% (Table 18). This gives a good indication that the beans wereadequately fermented and will not give rise to bitter and astringency since an increase in purple beans over brown beans causes an increase in astringency (Minifie, 1970).

Brown beans were noted toincrease with increasing fermentation time from the4th to the 6th day. Theincrement of brown beans from 0 to 90, 0 to 80, 41 to 76, 36 to 70 and 7 to 34for samples containing 0%, 25%, 50%, 75% and 100% depulped beans respectively from start of fermentation (day 0) to the end of fermentation (Table 18). Increase inbrown beans with fermentation time can be associated with changes in anthocyanin and oxidationproducts of the polyphenol oxidase activities which, form brown pigments during the fermentation period.The brown pigments might also be produced from non-enzymatic browning.

Table: 17. Effect of depulping and fermentation time on the dried cocoa beans cut surface colour and cut test score

Depulped Beans (%)	Fermentation Time (Days)	Purple (%)	Purple/Brown (%)	Brown (%)	Slaty (%)	Germinated (%)	Mouldy (%)
0	0	70	0	0	30	0	0
	2	79	21	0	0	0	0
	4	9	18	73	0	0	0
	6	0	5	90	5	0	0
25	0	80	10	0	10	0	0
	2	11	6	68	15	0	0
	4	11	7	76	6	0	0
	6	15	0	80	5	0	0
50	0	17	8	41	34	0	0
	2	13	0	84	3	0	0
	4	11	7	77	5	0	0
	6	19	0	76	5	0	0
75	0	31	24	36	9	0	0
	2	11	10	76	3	0	0
	4	12	18	67	1	2	0
	6	12	17	70	1	0	0
100	0	42	20	7	31	0	0
	2	33	28	21	18	0	0
	4	30	27	33	8	2	0
	6	25	32	34	6	3	0

Germination occurred in 75% depulped beans on day 4 and 100% depulped beans on day 4 and 6 of fermentation (Table 18). However germination was not recorded in any other depulped beans. This occurrence might have been to a failure of bean death in 75 and 100% depulped beans since the pulp was insufficient to have caused an increase in temperature and or to produce the required amounts of ethanol to ensure killing of the beans. This suggests that higher depulping levels could lead under-fermentation causing germination to occur.

Slaty beans decreased with both depulping and drying time. It reduced from 30 to 6, 10 to 5, 34 to 5, 9 to 1 and 31 to 6 (Table 18) from the start of fermentation to

the sixth (6^{th})day of fermentation for samples containing 0%, 25%, 50%, 75% and 100% depulped beans respectively.

Mouldy beans are the most serious defects of dried cocoa beans. However mouldy beans werenot detected in all the depulped and fermented cocoa beans (Table 18). Wood and Lass (1985) explains that moulds cause the development of off-flavours by increasing the free fatty acid (FFA) content of the beans.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Mechanical depulping and fermentation influenced all the studied parameters.Results from the proximate analysisshowed that protein, ash and fat content decreased with increasing fermentation time whilst depulping did not have any significant effects on these components. Mineral analysis showed that potassium was the dominant mineral followed by calcium, magnesium, phosphorus, copper, zinc and iron being the least. Fermentation progression caused a decrease in the potassium (K) content of the depulped cocoa beans.

Changes in biochemical composition and physicochemical properties were variable during fermentation of the depulped beans. The pH decreased from 6.5 to 6.3 for samples containing 0% to 100% respectively at the beginning of fermentation.Depulping caused slight increases in pH from 5.7 at the end of fermentation in 0% depulped beans to 6.02 at the end of fermentation in samples containing 100% depulped beans. Fermentation generally caused increases in reducing sugars. Reducing sugar increased from 157.33 mg/g at the start of fermentation to 304.95 mg/gbythe sixth day of fermentation in 0% depulped beans. Depulping caused slight decreases in reducing sugar content. FFA content increased from 0.26% at start of fermentation to 0.89% at the end of fermentation in 0% depulped beans. All the treatments (0%, 25%, 50%, 75% and 100% depulped beans)obtainedfermentation index of one (1) by the forth (4^{th}) day of fermentation.

Polyphenolic compositions including total polyphenols and *o*-diphenols reduced with respect to depulping and fermentation time.Total polyphenolic content decreased from 31.29 mg/gat the start of fermentation to 22.23 mg/gat the end of the

fermentation process in 0% depulped beans.Depulping on the other hand caused a marginal reduction in total polyphenols. It reduced from 22.23 mg/g in 0% depulped beans to 19.9 mg/gin samples containing 25% depulped beans.

Mechanical depulping and drying had variable influence onall the studied parameters. Depulping of cocoa beans caused an increase in pH in the fermented beans which reduced to 5-6 during drying. Depulping caused a slight increase in pH from 5.46 by the fourth day of fermentation in 0% depulped beans to 5.78 by the fourth day of fermentation in samples containing 100% depulped beans. Drying had a varying influence on the pH of the cocoa beans.

Depulping caused an increase in FFA content however the FFA content after drying was far below the stipulated 1.75%. The anthocyanins content of the beans varied significantly with increasing dryingtime and depulping. Drying of cocoa beans showed no distinct trendfor lightness (L), redness (a) and yellowness (b) for all levels of depulped beans; 0%, 25%, 50%, 75% and 100%.Both, the a-value and b-valueremained fairly constant during drying for all levels of depulped beans.

Cut test on the samples showed that purple beans generally reduced with increasing fermentation time. Brown beans were noted toincrease with increasing fermentation time from the fourth to the sixth day. These findings suggest that mechanical depulping influenced to a varying degree the chemical, biochemical and polyphenolic content of fermented and dried Ghanaian cocoa beans.

5.2 RECOMMENDATIONS

The following proposals are being made for further research:

i. Mechanical depulping must be considered as a way of reducing acidity of cocoa beans but must not exceed 50% level of depulping to achieve good results.

ii. Change in flavour profile resulting from the effects of mechanical depulping and fermentation time.

iii. Characterization of various enzymes associated with mechanical depulping and fermentation.

iv. Microbial succession must be studied to evaluate how depulping affects their activity during fermentation.

v. Optimization of the mechanical depulping process and its effect on chocolate flavour quality.

CHAPTER SIX

6.0 REFERENCES

Adomako, D. (1983). Cocoa. *Outlook on Agriculture, 12*, 83-9.

Afoakwa, E. O. (2010). Chocolate Science and Technology.Wiley-Blackwell Publishers, Oxford, UK. Pp 1-22.

Afoakwa, E. O. and Paterson, A. (2010).Cocoa Fermentation: Chocolate Flavor Quality.*Encyclopedia of Biotechnology in Agriculture and Food,1:1*, 171 – 173.

Afoakwa, E. O., Quao, J.,Takrama, J., Budu, A. S. and Saalia, F. K. (2011a).Chemical composition and physical quality characteristicsof Ghanaian cocoa beans as affected by pulppre-conditioning and fermentation. *Journal of Food Science Technology,47*, (1), 1 – 11.

Afoakwa, E. O., Quao, J., Budu, A. S., Takrama, J. and Saalia, F. K. (2011b).Effect of pulp preconditioning on acidification, proteolysis, sugarsand free fatty acids concentration during fermentation of cocoa(*Theobroma cacao*) beans.*International Journal of Food Sciences and Nutrition* 1-10.

Afoakwa, E. O., Quao, J., Budu, A. S., Takrama, J. and Saalia, F. K. (2012a).Influence of pulp-preconditioning and fermentation on fermentativequality and appearance of Ghanaian cocoa (*Theobroma cacao*) beans.*International Food Research Journal,19,* 59-66.

Afoakwa, E. O., Quao, J., Takrama, F. S., Budu, A. S. and Saalia, F. K. (2012b).Changes in total polyphenols, o-diphenols and anthocyanin concentrations during fermentation of pulp pre-conditioned cocoa *(Theobroma cacao)* beans.*International Food Research Journal, 19*,(3).1071-1077.

Amoa-Awua, W., Madsen, M., Takrama, J., Olaila, A. O., Ban-Koffi, L. and Jakobsen, M. (2006).Quality Manual for Production & Primary Processing of Cocoa. Pp. 2 – 18.

AOAC (2005) Official methods of analysis, 18th edn. Association ofOfficial Analytical Chemists, Washington, D.C USA

Ardhana, M. and Fleet, G. H.(2003).The microbial ecology of cocoa fermentationsin Indonesia.*International Journalof Food Microbiology,86*, 87–99.

Aremu C. Y., Agiang M. A., and Ayatse J. O. I. (1995) Nutrient and anti-nutrientprofiles of raw and fermented cocoa beans.*Plant Foods Human Nutrition*, *48*, 221–223.

Ashitey, E. (2012). Cocoa Report Annual – Accra. Grain Report Number GH1202 United States Department of Agriculture.

Baker, M. D., Tomlins, K. I. and Gay, C. (1994).Survey of Ghanaian cocoa farmer fermentationpractices and their influence on cocoa flavour.*Food Chemistry,51*, 425-431.

Beckett, S. T. (2009). Industrial Chocolate Manufacture and Use.4TH Ed. Blackwell Publishing Ltd, UK. Pp. 10 – 12, 20 – 28.

Buamah, R., Dzogbefia, V. P. and Oldham, J. H. (1997). Pure yeast culture fermentation of cocoa (*Theobroma cacao* L): effect on yield of sweatings and cocoa bean quality. *World Journal of Microbiology and Biotechnology,13*, 457 – 462.

Biehl, B., Brunner, E., Passern, D., Quesnel, V. C. and Adomako D. (1985). Acidification, proteolysisand flavor potential in fermenting cocoabeans, *Journal of the Science ofFood and Agriculture,36*,583-598.

Biehl, B., Meyer, B., Said, M. B. & Samarakoddy, R. J. (1990). Bean spreading: a method for pulp preconditioning to impair strong nib acidification during cocoa fermentation in Malaysia. *Journal of the Science ofFood and Agriculture,51*, 35-45.

Belitz, H. D., Grosch, W., and Schieberle, P. (2009).Food Chemistry.4th Ed. Springer-Verlag, Berlin. Pp. 173, 959 – 966.

Brummer, Y., & Cui, S. W. (2005).Understanding carbohydrate analysis. In: *Food carbohydrates: chemistry, physical properties and applications*. *Chapter 2*.Cui, S. W. (Ed.). Taylor & Francis Group, LLC, Boca Raton pp. 1–38.

Carr, J.G., Davies, P.A. and Dougan, J. (1979).Cocoa Fermentation in Ghana andMalaysia. Tropical Products Institute, London.

Chazarra, S., Garcia-Carmona, F. and Cabanes, J. (1999).Characterization ofmono-phenolase activity of polyphenoloxidase from Iceberg Lettuce.*Journal of Agricultural and FoodChemistry,47*, 1422-1426.

Dand, R. (1997). The international Cocoa Trade. Oxford, UK: Wiley. Pp. 25 – 51, 185 – 203.

Dias, D. R., Schwan, R. F., Freire, E. S. and dos Sandos Serodio, R. (2007).Elaboration of a fruit wine from cocoa (*Theobroma cacao* L.) pulp.*International journal of Food science &Technology,42*, 319 – 329.

EEC, (1990).Folin-Ciocalteu index.*Official Journal of European Communities*, Ch. 41. Pp. 178–179.

Egan, H., Kirk, R. S., Sawyer, R. (1990). Pearson's chemical analysis of foods. 8th ed., Oxford: Churchill Livingstone.

Forsyth, W. G. C. and Quesnel, V. C. (1957), Cocoaglycosidase and colour changes during fermentation,*Journal of the Science ofFood and Agriculture,8*,505-509.

Forsyrh, W. G. C. and Quesnel, V. C. (1963). Mechanismsof cocoa curing, *Advance. Enzymology*,*25*,457-492.

Fowler, M. S. (2009). Cocoa beans: From Tree to Factory. In: Industrial Chocolate manufacture and use.Fourth Edition.Beckett ST (eds) Wiley-Blackwell Science, Oxford, UK. Pp 137-152.

Gálvez, S. L., Loiseau, G., Paredes, J. L., Barel, M. and Guiraud, J. P. (2007).A Study on the microflora and biochemistry of cocoa fermentationin the Dominican Republic.*International Journal of Food Microbiology*, *114*,124–130.

Gourieva, K.B. and Tserrevitinov, O.B. (1979).Method ofevaluating the degree of fermentation of cocoa beans.USSR Patent no. 646254.

Guehi, T.S., Yao, N.D., Manizan, N.P., Nevry, K.R., Koffi,L.B. and Konan, Y.M. (2008). Comparison of the degreeof fermentation and fungal profiles of raw cocoa beanssourced from three Ivorian main producing regions.*African Journal of Food Science*,*2*, 112-118.

Hansen, C. E., del Olmo, M. and Burri, C. (1998). Enzyme activities in cocoa beans during fermentation.*Journal of the Science of Food and Agriculture*, *77*, 273–281.

Hansen, C. E., Manez, A., Burri, C. and Bousbaine, A. (2000).Comparison of enzyme activities involved inflavour precursor formation in unfermented beans of different cocoa genotypes.*Journal of the Science ofFood and Agriculture*, *80*, 1193–1198.

Hii, C. L., Law C. L., Suzannah, S., Misnawi and Cloke, M. (2009). Polyphenols in cocoa (*Theobroma cacao L.*).*Asian Journal of Food and Agro-Industry*,*2*, (04), 702 – 722.

Hodge, J. (1953). Chemistry of browning reactions in model systems.*Agriculture and Food Chemistry*,*1*,928-943.

Huber, M., Hintermann, G. and Lerch, K.(1985).Primary structure of tyrosinasefrom *Streptomyces glaucescens.Biochemistry*,*24*, 6038-6044.

ICCO (1997).El Niño and cocoa production: an exploration of the influence of El Niño on world cocoaproduction. A presentation by the ICCO secretariat, *Cocoa Newsletter*, London: International CocoaOrganization, No.15

ICCO (2010). ICCO document: Annual report. *International Cocoa Organization, London U.K*

ICCO (2012). ICCO document: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXVIII, No. 2, Cocoa year 2011/12 Published: 30-05-2012.*International Cocoa Organization, London U.K*

ICCO (2013). ICCO document: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXVIII, No. 3, Cocoa year 2011/12 Published: 28-08-2012. *International Cocoa Organization, London U.K*

IOCCC(1996).Determination of free fatty acids (FFA) contentof cocoa fat as a measure of cocoa nib acidity.International Office of Cocoa, Chocolate and Sugar Confectionery.*Analytical Method*, *42*,130–136.

IRI (2009).El Niño tele-connections in Africa, Latin America and Caribbean, and Asia Pacific: Overviewof current socio-economics and enhanced odds of anomalous seasonal precipitation".*International Research Institute for Climate and Society Earth Institute*, *Columbia.*

Jonfia-Essien, W. A.(2004). Cocoa storage in Ghana. In: Hodges R. J. and Farrell G. (Eds) Crop Post-harvest.Durables.Blackwell Science Ltd.*Science and Technology*,*2*.

Knapp, A. W. (1937), Cocoa Fermentation: A Critical Survey of its Scientific Aspects.Curnow,John Bale.

Knight, I. (ed). (2001). Chocolate and Cocoa: Health and Nutrition. Blackwell Science, Oxford, UK. Pp. 10 – 40.

Konczak, I. and Zhang, W. (2004). Anthocyanins - More Than Nature's Colours. *Journal of Biomedicine and Biotechnology*,*2004*, (5), 239–240.

Kim, H. and Keeney, P. G.(1984). (−)Epicatechin content in fermented andunfermented cocoa beans.*Journal of Food Science*,*49*, 1090–1092.

Kirchhoff, P. M., Biehl, B. and Crone, G. (1989).Peculiarity of the Accumulation of Free Amino Acids during Cocoa Fermentation.*Food Chemistry*,*31*, 295 – 311.

Lee, F. (1975).Basic Food Chemistry. The AVI publishing company, Inc. Connecticut. Pp. 328 – 347.

Lee, K.W., Kim, Y.J., Lee, H. J. and Lee, C. Y. (2003). Cocoa hasmore phenolic phytochemicals and a higher antioxidantcapacity than teas and red wine. *Journal of Agriculture and Food Chemistry*,*51*, 7292.

Lehrian, D. W. and Patterson G. R. (1983). Cocoa fermentation, In: Biotechnology, Comprehensive Treatise, vol 5, ed. by Reed G. Verlag Chemie, Basel, pp. 529–575.

Lehrian, D. W. (1989). Recent developments in the chemistry and technology of cocoa processing.In:Che-Man Y. B., Abdul Karim M. N. B., and Amabi, B. A. (ed.), FoodProcessing Issues and Prospects.Faculty of Food Scienceand Biotechnology, Universiti Pertanian Malaysia. Pp. 22-33.

Lopez, A.S., and Dimick, P. S. (1995). Cocoa fermentation In:Reed, G.,Nagodawithana, T.W.(Eds.), Enzymes, Biomass, Food and Feed. VCH, Weinheim, Pp. 561–577.

McDonald, C.R., Lass, R. A. and Lopez, A. S.(1981).Cocoa drying - a review.*Cocoa Growers'Bulletin*,*31*,5-39.

Minifie, W. B. (1970). Chocolate, Cocoa and Confectionary: Science and Technology. AVI publishing company, Inc. Pp. 1 – 25.

Misnawi, Jinap S., Jamilah, B. and Nazamid, S. (2002). Effectsof incubation and polyphenol oxydase, enrichmenton colour, fermentation index, procyanidins andastringency of unfermented and partly unfermentedand partly fermented cocoa beans.*International Journal of Food Science and Technology*,*38*, 285-295.

Mohammed, D., Asamoah, D. and Asiedu-Appiah, F.(2012).Cocoa Value Chain - Implication for the Smallholder Farmer in Ghana.Southwest Decision Science Institute Conference. Pp. 1041 – 1049.

Nair, K. P. P. (2010). The Agronomy and Economy of Important Tree Crops of the Developing World.Elsevier Inc., Burlington, USA. Pp. 131, 133-136.

Nazaruddin, R., Ayub, M. Y., Mamot, S. and Heng, C. H. (2001). HPLC determination of methylxanthinesand polyphenols levels in cocoa and chocolate products, *Malaysian Journal Analytical Science*,*7*, 377–386.

Nazaruddin, R., Seng, L. K., Hassan, O. and Said, M. (2006).Effect of pulp preconditioning on the content of polyphenols incocoa beans (*Theobroma Cacao*) during fermentation.*Industrial Crops and Products*,*24*, 87–94.

Nelson, D. L. and Cox, M. M. (2004).Lehninger Principles of Biochemistry.4th Ed. W. H. Freeman & Co. Pp. 521 – 530, 544.

Nielsen, D.S., Hønholt, S., Tano-Debrah, K., Jespersen, L.(2005). Yeastpopulations associated with Ghanaian cocoa fermentations investigatedusing Denaturing Gradient Gel Electrophoresis (DGGE). *Yeast*,*22*, 271.

Nielsen, D. S. (2006).The microbiology of Ghanaiancocoa fermentations.PhD Thesis. Department of Food Science, Food MicrobiologyThe Royal Veterinary and Agricultural University, Denmark. Pp. 5, 6, 7

Nielsen, D. S., Teniola, O. D., Ban-Koffi, L., Owusu, M., Andersson, T. S. and Holzapfel, W. H. (2007).The microbiology of Ghanaian cocoa fermentations analysed usingculture-dependent and culture-independent methods. *International Journal of Food Microbiology*,*114*, 168–186.

Nielsen, S. S. (2010). Food Analysis. 4th Ed. Springer, New York. Pp. 221 – 230, 233, 235.

Osman, H., Nazaruddin, R. and Lee, S. L. (2004).Extracts of cocoa (*Theobroma cacao* L.) leaves and theirantioxidation potential.*Food Chemistry*, *86*, 41–45.

Quao, J. (2010). Effect of Pulp Pre-Conditioning on the Biochemical Quality and Flavour Precursor Formation During Fermentation of Ghanaian Cocoa Beans. MPhil thesis.Department of Nutrition and Food Science.Pp. 18 – 31, 37 – 41.

Rohan, T. A. (1963). Processing of raw cocoa for the market.*FAO Agricultural Studies Report*, Number 60.Rome, Italy: FAO Press.

Roelofsen, P. A.(1958). Fermentation, drying, and storage of cacao beans.*Advancesin Food Research*,*8*, 225–296.

Reineccius, G. A., Andersen, D. A., Kavanagh, T. E. & Keeney, P. G. (1972).Identification and quantificationof the free sugars in cocoa beans.*Journal of Agriculture and Food Chemistry*, *20*, 199–202.

Reineccius, G. (2006). Flavour Chemistry and Technology, 2nd Ed. CRC Press.Boca Raton, FL.

Scharbert, S., Holzmann, N. and Hofmann, T. (2004). Identificationof astringent taste compounds by combining instrumentalanalysis and human bioresponse. *Journal of Agriculture and Food Chemistry*,*52*, 3498.

Schieberle, P. (2000). The chemistry and technology of cocoa.In: Caffeinated beverages. ACS Symposium Series754, 262.

Schwan, R.F., Rose, A.H., Board, R. G.(1995). Microbial fermentation of cocoabeans, with emphasis on enzymatic degradation of the pulp.*Journal of Applied Bacteriology,79*, 96S–107S (Suppl).

Schwan, R. F. and Wheals, A. E. (2004).The microbiology of cocoa fermentation and its role in Chocolate quality.*Critical Reviews in Food Science and Nutrition,44*, 205 – 221.

Senenayake, M., Jansz, E. R. and Buckle, K. A.(1995). Effect of variety and locationon optimum fermentation requirements of cocoa beans: an aid tofermentation on a cottage scale. *Journal Science of Food and Agriculture,69*, 461–465.

Spencer, M. E. and Hodge, R. (1992). Cloning and sequencing of a cDNA encoding the major storage proteinsof *Theobroma cacao*. Identification of the proteins as members of the vicilin class of storage proteins.*Planta,186*, 567–576.

Stark, T., Bareuther, S. and Hofmann, T. (2006).Moleculardefinition of the taste of roasted cocoa nibs (*Theobroma cocoa*) by means of quantitative studies andsensory experiments.*Journal of Agriculture and Food Chemistry,54*,5530.

Thompson, S.S., Miller, K. B.and Lopez, A. S. (2001). Cocoa and coffee In: Doyle, M.P., Beuchat, L.R. and Montville, T.J. (Eds.), FoodMicrobiology Fundamentals and Frontiers. ASM Press, Washington, DC, Pp. 837-850.

Tomlins, K. I., Baker, D. M., Daplyn, P. and Adomako, D. (1993).Effect of fermentation and drying practices on the chemical and physical profiles of Ghana cocoa.*Food Chemistry,46*,257-263.

United Nations Conference on Trade and Development (2008). Cocoa Study: Industry Structures and Competition. Prepared by Irene Musselli, associate expert, UNCTAD, under the supervision of Olivier Combe.UNCTAD/DITC/COM/2008/1 United Nations, Geneva.

Voigt, J., Biehl, B. and Kamaruddin, S. (1993). The major seed proteins of *Theobroma cacao* L. *Food Chemistry*,*47*, 145–171.

Wood, G.A.R. and Lass, R.A. 1985. Cocoa (4th ed).Longman Group, London.

Wong, D.W.S. (1995). Food Enzymes: Structure and Mechanism.Chapman&Hall, New York.

Wrolstad, R. E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Smith, D. and Sporns, P. (2005). Handbook of Food Analytical Chemistry: Water, Proteins, Enzymes, Lipids, and Carbohydrates. John Wiley & Sons, Inc. Pp. 475, 477 – 482.

www.goldentreeghana.com/thecompany.htm

www.cargill.com

www.adm.com/en-US/worldwide/ghana/Page/default.aspx

www.worldcocoafoundation.com

Zaibunnisa, A. H., Russly, A. R., Jinap, S., Jamilah, B. and Wahyudi, T. (2000). Quality characteristics ofcocoa beans from mechanical fermentation.*Abstracts of 13th International cocoa Research Conference*,Kota Kinabalu, Malaysia.Pp. 131.

APPENDICES

Appendix 1: Analysis of variance of proximate composition of depulped and fermented cocoa beans

Analysis of Variance for PROTEIN - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	0.566477	4	0.141619	2.65	0.0637
B:Fermentation Time	19.5223	3	6.50743	121.63	0.0000
INTERACTIONS					
AB	3.2078	12	0.267317	5.00	0.0008
RESIDUAL	1.07	20	0.0534998		
TOTAL (CORRECTED)	24.3666	39			

Analysis of Variance for FAT - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	460.083	4	115.021	22.30	0.0000
B:Fermentation Time	585.762	3	195.254	37.86	0.0000
INTERACTIONS					
AB	968.673	12	80.7228	15.65	0.0000
RESIDUAL	103.158	20	5.15792		
TOTAL (CORRECTED)	2117.68	39			

Analysis of Variance for ASH - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	2.7064	4	0.6766	8.90	0.0003
B:Fermentation Time	4.65457	3	1.55152	20.40	0.0000
INTERACTIONS					
AB	3.98228	12	0.331857	4.36	0.0019
RESIDUAL	1.52076	20	0.0760378		
TOTAL (CORRECTED)	12.864	39			

Analysis of Variance for CARBOHYDRATE - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	303.298	4	75.8245	15.43	0.0000
B:Fermentation Time	446.471	3	148.824	30.28	0.0000
INTERACTIONS					
AB	933.08	12	77.7566	15.82	0.0000
RESIDUAL	98.2909	20	4.91455		
TOTAL (CORRECTED)	1781.14	39			

Analysis of Variance for MOISTURE - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	9.49133	4	2.37283	52.56	0.0000
B:Fermentation Time	10.6734	3	3.5578	78.80	0.0000
INTERACTIONS					
AB	26.4269	12	2.20224	48.78	0.0000
RESIDUAL	0.902942	20	0.0451471		
TOTAL (CORRECTED)	47.4945	39			

Appendix 2: Analysis of variance of mineral composition of depulped and fermented cocoa beans.

Analysis of Variance for K - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	36372.8	4	9093.2	6.85	0.0012
B:Fermentation Time	445274.	3	148425.	111.89	0.0000
INTERACTIONS					
AB	18022.4	12	1501.87	1.13	0.3893
RESIDUAL	26530.8	20	1326.54		
TOTAL (CORRECTED)	526200.	39			

Analysis of Variance for Mg - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	4915.24	4	1228.81	6.98	0.0011
B:Fermentation Time	53919.3	3	17973.1	102.14	0.0000
INTERACTIONS					
AB	3201.97	12	266.831	1.52	0.1982
RESIDUAL	3519.26	20	175.963		
TOTAL (CORRECTED)	65555.8	39			

Analysis of Variance for Ca - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	1.8526E7	4	4.63149E6	465.99	0.0000
B:Fermentation Time	834448.	3	278149.	27.99	0.0000
INTERACTIONS					
AB	1.10797E6	12	92331.2	9.29	0.0000
RESIDUAL	198780.	20	9938.99		
TOTAL (CORRECTED)	2.06672E7	39			

Analysis of Variance for Cu - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	514.467	4	128.617	11.36	0.0001
B:Fermentation Time	281.31	3	93.77	8.28	0.0009
INTERACTIONS					
AB	122.462	12	10.2052	0.90	0.5610
RESIDUAL	226.424	20	11.3212		
TOTAL (CORRECTED)	1144.66	39			

Analysis of Variance for Zn - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	18.5682	4	4.64204	2.90	0.0482
B:Fermentation Time	8.90764	3	2.96921	1.85	0.1699
INTERACTIONS					
AB	27.4927	12	2.29106	1.43	0.2313
RESIDUAL	32.026	20	1.6013		
TOTAL (CORRECTED)	86.9945	39			

Analysis of Variance for Fe - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	79.8863	4	19.9716	5.65	0.0033
B:Fermentation Time	9.00833	3	3.00278	0.85	0.4833
INTERACTIONS					
AB	45.3197	12	3.77664	1.07	0.4328
RESIDUAL	70.7207	20	3.53604		
TOTAL (CORRECTED)	204.935	39			

Analysis of Variance for P - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	4.41355	4	1.10339	24.63	0.0000
B:Fermentation Time	0.910194	3	0.303398	6.77	0.0025
INTERACTIONS					
AB	3.08148	12	0.25679	5.73	0.0003
RESIDUAL	0.896036	20	0.0448018		
TOTAL (CORRECTED)	9.30126	39			

Appendix 3: The Physicochemical and Biochemical Composition of Depulped and Fermented Ghanaian Cocoa Beans

Analysis of Variance for Pulp volume - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	835.25	4	208.813	32.22	0.0000
B:Fementation Time	4653.97	3	1551.32	239.40	0.0000
INTERACTIONS					
AB	323.768	12	26.9807	4.16	0.0025
RESIDUAL	129.603	20	6.48017		
TOTAL (CORRECTED)	5942.6	39			

Analysis of Variance for FFA - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Percent Depulped	76.3396	4	19.0849	2079.98	0.0000
B:Fermentation Time	76.1753	3	25.3918	2767.34	0.0000
INTERACTIONS					
AB	99.0175	12	8.25146	899.29	0.0000
RESIDUAL	0.18351	20	0.0091755		
TOTAL (CORRECTED)	251.716	39			

All F-ratios are based on the residual mean square error.

Analysis of Variance for F pH - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:pH.Percent Depulped Beans	0.390435	4	0.0976088	796.81	0.0000
B:pH.Fermentation Time	5.96715	3	1.98905	16237.14	0.0000
INTERACTIONS					
AB	0.687265	12	0.0572721	467.53	0.0000
RESIDUAL	0.00245	20	0.0001225		
TOTAL (CORRECTED)	7.0473	39			

Analysis of Variance for F TA - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	6.37225	4	1.59306	115.62	0.0000
B:Fermentation Time	27.6385	3	9.21283	668.66	0.0000
INTERACTIONS					
AB	10.2037	12	0.850304	61.71	0.0000
RESIDUAL	0.275562	20	0.0137781		
TOTAL (CORRECTED)	44.4899	39			

Analysis of Variance for Total Sugar - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Percent Depulped Beans	192168.	4	48042.0	157.43	0.0000
B:Fermentation Time	1.16304E6	3	387679.	1270.43	0.0000
INTERACTIONS					
AB	362432.	12	30202.7	98.97	0.0000
RESIDUAL	6103.11	20	305.155		
TOTAL (CORRECTED)	1.72374E6	39			

Analysis of Variance for Non Reducing - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	180233.	4	45058.3	174.46	0.0000
B Fermentation Time	1.24239E6	3	414131.	1603.44	0.0000
INTERACTIONS					
AB	381970.	12	31830.8	123.24	0.0000
RESIDUAL	5165.51	20	258.276		
TOTAL (CORRECTED)	1.80976E6	39			

Analysis of Variance for Reducing - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	3156.18	4	789.044	26.64	0.0000
B:Fermentation Time	10489.3	3	3496.44	118.04	0.0000
INTERACTIONS					
AB	21281.8	12	1773.48	59.87	0.0000
RESIDUAL	592.414	20	29.6207		
TOTAL (CORRECTED)	35519.7	39			

Analysis of Variance for Lightness - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	7.42898	4	1.85724	1601.07	0.0000
B:Fermentation Time	20.1964	3	6.73214	5803.57	0.0000
INTERACTIONS					
AB	30.8204	12	2.56837	2214.11	0.0000
RESIDUAL	0.0464	40	0.00116		
TOTAL (CORRECTED)	58.4922	59			

Analysis of Variance for greeness - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	4.95228	4	1.23807	105.05	0.0000
B:Fermentation Time	17.8389	3	5.94631	504.57	0.0000
INTERACTIONS					
AB	11.3464	12	0.945531	80.23	0.0000
RESIDUAL	0.4714	40	0.011785		
TOTAL (CORRECTED)	34.609	59			

Analysis of Variance for blueness - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	5.14771	4	1.28693	79.40	0.0000
B:Fermentation Time	9.67912	3	3.22637	199.06	0.0000
INTERACTIONS					
AB	10.36	12	0.863333	53.26	0.0000
RESIDUAL	0.648333	40	0.0162083		
TOTAL (CORRECTED)	25.8352	59			

Appendix 4: Effect of Mechanical Depulping on the Polyphenolic Compounds Concentration, Degree of Fermentation and Appearance Properties of Cocoa Beans

Analysis of Variance for Total Polyphenols - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	250.258	4	62.5644	7.57	0.0007
B:Fermentation Time	935.132	3	311.711	37.70	0.0000
INTERACTIONS					
AB	133.77	12	11.1475	1.35	0.2679
RESIDUAL	165.343	20	8.26717		
TOTAL (CORRECTED)	1484.5	39			

Analysis of Variance for O Diphenols - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	1284.23	4	321.058	12.61	0.0000
B:Fermentation Time	1933.17	3	644.389	25.31	0.0000
INTERACTIONS					
AB	273.313	12	22.7761	0.89	0.5665
RESIDUAL	509.106	20	25.4553		
TOTAL (CORRECTED)	3999.82	39			

Analysis of Variance for Fermentation Index - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	0.0407172	4	0.0101793	23.50	0.0000
B:Fermentation Time	2.72315	3	0.907717	2095.73	0.0000
INTERACTIONS					
AB	0.237594	12	0.0197995	45.71	0.0000
RESIDUAL	0.00866254	20	0.000433127		
TOTAL (CORRECTED)	3.01012	39			

Appendix 5: Influence of Mechanical Depulping on the Acidification, Browning index, Anthocyanins, Colour and FFA during Drying of Fermented Ghanaian Cocoa Beans

Analysis of Variance for Anthocyanins - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:SP3.Depulped Beans	19.1125	4	4.77814	9.87	0.0001
B:Drying Time	24.357	3	8.119	16.77	0.0000
INTERACTIONS					
AB	15.7869	12	1.31558	2.72	0.0233
RESIDUAL	9.6816	20	0.48408		
TOTAL (CORRECTED)	68.9381	39			

Analysis of Variance for Browning Index - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	0.184255	4	0.0460637	39.75	0.0000
B:Drying Time	0.492611	3	0.164204	141.68	0.0000
INTERACTIONS					
AB	0.123889	12	0.0103241	8.91	0.0000
RESIDUAL	0.023179	20	0.00115895		
TOTAL (CORRECTED)	0.823935	39			

Analysis of Variance for L - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	6.94972	4	1.73743	296.24	0.0000
B:Drying Time	17.8629	3	5.95431	1015.23	0.0000
INTERACTIONS					
AB	89.1451	12	7.42876	1266.63	0.0000
RESIDUAL	0.2346	40	0.005865		
TOTAL (CORRECTED)	114.192	59			

Analysis of Variance for a - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	10.598	4	2.64951	229.99	0.0000
B:Drying Time	7.64838	3	2.54946	221.31	0.0000
INTERACTIONS					
AB	17.4139	12	1.45116	125.97	0.0000
RESIDUAL	0.4608	40	0.01152		
TOTAL (CORRECTED)	36.1211	59			

Analysis of Variance for b - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Depulped Beans	4.26919	4	1.0673	2.67	0.0456
B:Drying Time	48.4937	3	16.1646	40.51	0.0000
INTERACTIONS					
AB	36.711	12	3.05925	7.67	0.0000
RESIDUAL	15.9607	40	0.399018		
TOTAL (CORRECTED)	105.435	59			