



## Review

## Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile – A review



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## ABSTRACT

This review examined the factors that influence flavour volatiles of cocoa beans and the volume of work that needs to be done on these factors and their impact on the flavour volatiles of commercial cocoa beans. Cocoa bean flavour is one of the most important quality attributes as flavour is central to acceptability of cocoa beans and cocoa products such as chocolate. The complex composition of cocoa bean flavour depends on bean genotype, postharvest treatments such as pulp pre-conditioning, fermentation and drying, industrial processes such as roasting as well as the type of soil and age of cocoa tree. The bean genotype determines the chemical composition of the bean, specifically the contents of bean storage proteins, polysaccharides, and polyphenols. This determines the quantities and type of precursors formed during fermentation and drying processes leading to flavour formation, hence, influencing both flavour type and intensity. Cocoa bean fermentation and drying result in the breakdown of the storage proteins by endogenous proteases into amino acids and short chain oligopeptides while the polysaccharides are also degraded by invertase to glucose and fructose. The amino acids, oligopeptides, glucose and fructose react with each other during the roasting process to produce the typical cocoa flavour volatiles. Polyphenols are also oxidized by polyphenol oxidase during fermentation and drying which reduce the astringency and bitterness of the beans, thus, enhancing the flavour of cocoa beans. However, the extent to which other factors such as age of the cocoa tree and soil chemical compositions influence the formation of flavour precursors and their relationships with final flavour quality remains unclear. With increasing demand for sustainable production of high quality cocoa beans, greater understanding of factors contributing to the variations in flavour character would have significant commercial implications.

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## 1. Introduction

Cocoa (*Theobroma cacao* L.) is a cash crop of huge economic significance in the world and the key raw material for chocolate manufacturing (Krähmer et al., 2015; Ho, Zhao, & Fleet, 2015). It forms the major agricultural export commodity for several producing countries in West and Central Africa, such as Cote d'Ivoire, Ghana, Nigeria and Cameroon (Afoakwa, Quao, Takrama, Budu, & Saalia, 2011a). Cocoa belongs to the family of Sterculiaceae and the genus *Theobroma* (Prabhakaran Nair, 2010). The genus has twenty-two species of which *T. cacao* L. is commercially the most important due to the value of its seeds (Bartley, 2005; CacaoNet, 2012). The seeds, commonly known as cocoa beans, are obtained from the pods. These pods are oval in shape, measure between 12 and 30 cm long, and contain 30 to 40 beans embedded in a mucilaginous pulp, which comprises approximately 40% of the bean fresh weight (Schwan & Wheals, 2004; Lima, Almeida, Rob Nout, & Zwietering, 2011). The pulp is reported to be rich in fermentable sugars of about 9 to 13% w/w (Lima et al., 2011) such as glucose, fructose and sucrose (Lefebvre, Janssens, Camu, & De Vuyst, 2010), high acidity (pH 3.0–3.5) conferred by the presence of diverse organic acids, but mainly citric acid (Guehi, Zahouli, Ban-Koffi, Fae, & Nemlin, 2010), and a protein content in the range of 0.4 to 0.6% w/w (Lima et al., 2011).

Cocoa is cultivated on lands covering over 70,000 km<sup>2</sup> worldwide (Kim, Lee, & Lee, 2011) between 20° north and south of the equator, in areas with suitable environment for cocoa (Fowler, 1999). About 70% of the world's cocoa production takes place in the equatorial region of West Africa, and the rest in the equatorial regions of Central and South America, the West Indies, and tropical areas of Asia (Dillinger et al., 2000). The cocoa tree is a perennial tree, 8 to 15 m in height (Fowler, 1999) and requires hot, moist conditions to grow and will not withstand prolonged drought conditions without seriously depressing the tree's vegetative and reproductive functions (CacaoNet, 2012). The fruit varies among varieties in size, shape, external colour, and appearance. These characters have often been used in classifying cocoa.

According to the World Cocoa Foundation (WCF), there are 5–6 million farmers in developing countries across tropical Africa, Asia and Latin America who produce around 90% of cocoa worldwide, and the number of people who depend upon cocoa for their livelihoods worldwide is 40–50 million (World Cocoa Foundation, 2010). In West and Central Africa, cocoa continues to be an important source of export earnings contributing significantly to the Gross Domestic Product (GDP) of these producing countries. Cocoa exports generate over \$8 billion for the region's national economies (IFDC, 2014) and support about two million smallholder farm households in West and Central Africa. In Ghana, the industry employs about 70% of the national agricultural labour force in the country (COCOBOD, 2013). For these farmers, cocoa contributes about 70–100% of their annual household incomes (Anang, Mensah, & Asamoah, 2013; Nunoo, Frimpong, & Frimpong, 2014). Apart from the economic importance of cocoa, consumption of chocolate and other cocoa products is reported to contribute positively to health (Wollgast & Anklam, 2000; Afoakwa, 2010; Andújar, Recio, Giner, & Ríos, 2012). Polyphenols in cocoa beans have been reported to exhibit anti-carcinogenic (Rodríguez-Ramiro, Ramos, López-Oliva, et al., 2011; Oleaga et al., 2012), anti-atherogenic (Wollgast & Anklam, 2000) and vasodilatory (Gómez-Juaristi et al., 2011; Khawaja, Gaziano, & Djoussé, 2011) effects and they exert them mainly as antioxidants (Schinella, Mosca, Cienfuegos-Jovellanos, et al., 2010; Martorell, Forment, de Llanos, et al., 2011).

Annual global cocoa production is reported to be more than 4 million tons per season (4.4 million tons in 2013/2014 crop season) (ICCO, 2015a). However, while global demand for sustainable cocoa is growing annually by 2 to 3%, and West Africa still contributes about 70% of the global supply, this region is confronted with a 2% annual decline in production (IDH Sustainable Trade Initiative, 2015). There is low cocoa productivity in West Africa due to poor farm management

practices, planting low-yield varieties, pests and diseases, ageing cocoa trees and loss of soil fertility due to inadequate or no use of fertilizers (Abeke, Obeng-Ofori, & Egyir, 2002; Baah & Anchirimah, 2011; Akrofi, Amoako-Atta, Assuah, & Asare, 2015). The economic and health benefits of cocoa depend on the sustainable production and intensification of high quality cocoa beans. Also, sustaining the production of high quality cocoa beans in West Africa is crucial for the millions of smallholder family farmers that depend on cocoa for their livelihoods and the millions of people who enjoy chocolate and other cocoa products around the world. In promoting sustainability, the cocoa industry wants to see growth and improvement in the quantity and quality of the cocoa beans produced as well as the standard of living enjoyed by growers.

Sustainable cocoa production also involves the production of high quality cocoa beans. Cocoa bean quality is made up of several components such as flavour volatiles, nutritional composition, polyphenolic content and fermentative quality. The most important components are the flavour volatiles of the beans as these affect cocoa bean acceptability (Owusu, 2010; Magi, Bono, & Di Carro, 2012; Krähmer et al., 2015). These compounds which constitute the flavour quality of the beans are, however, influenced by several factors. This review discussed the factors that influence cocoa bean flavour quality. The current state of knowledge in terms of research findings of these factors on the flavour volatiles of cocoa beans is presented. Research gaps in terms of the volume of work that needs to be done on these factors and their impact on the flavour volatiles of commercial cocoa beans are also exposed.

## 2. Factors affecting flavour quality of cocoa beans

Several indicators are used to measure the quality of cocoa beans. These include the bean size and count, bean colour and acidity of the beans. However, the most important quality indicator of cocoa beans is the amount and type of volatile flavour compounds (Magi et al., 2012; Krähmer et al., 2015). Flavour is central to acceptability of cocoa beans and cocoa products such as chocolate (Afoakwa, Paterson, Fowler, & Ryan, 2008) and, consequently, contributes to determining the quality (Owusu, 2010). The characteristic flavours of cocoa beans are due to a very rich volatile fraction composed of a mixture of hundreds of compounds (Magi et al., 2012). Currently, more than 600 flavour compounds have been identified from cocoa beans and cocoa products (Crafack et al., 2014). These compounds comprise of nitrogen and oxygen heterocyclic compounds, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles and sulphides, pyrazines, ethers, furans, thiazoles, pyrones, acids, phenols, imines, amines, oxazoles, and pyrroles (Hoskin & Dimick, 1984; Schnermann & Schieberle, 1997; Jinap, Wan Rosli, Russly, & Nurdin, 1998; Counet, Callemien, Ouwerx, & Collin, 2002; Taylor, 2002; Granvogl, Bugan, & Schieberle, 2006; Reineccius, 2006; Frauendorfer & Schieberle, 2008; Afoakwa et al., 2008; Ziegler, 2009).

Most of these compounds possess particular flavour characteristics. Thus, while most esters confer a fruity/flowery attribute to flavour, pyrazines usually give earthy/roasted flavour (Owusu, 2010). Flavour compounds in cocoa beans are formed during roasting from flavour precursors generated during the fermentation and drying process. Flavour compounds in cocoa beans are thus influenced by factors such as type of cocoa (genotype), bean composition, soil type, age of cocoa tree, postharvest treatments such as pulp pre-conditioning, fermentation and drying, industrial processes such as roasting as well as storage and transportation (Afoakwa et al., 2008; Afoakwa, 2010; Owusu, Petersen, & Heimdal, 2011; Crafack et al., 2014).

### 2.1. Effect of genotype and origin of cocoa tree on cocoa bean flavour quality

Flavour quality of cocoa beans depends on the genotype and origin of the cocoa tree that has produced the beans. Cocoa beans from different genotypes and origin of cocoa tree have distinct flavour characteristics (Table 1). Currently, three broad cultivars of cocoa are commonly

**Table 1**  
Flavour profile of different cocoa beans from different origins.  
Source: Adopted from Reed (2010).

Origin	Cocoa type	Flavour profile
Côte d'Ivoire	Forastero	Good cocoa impact, low bitterness, low acid, fruity, nutty
Ghana	Forastero hybrids	Strong chocolate flavour
Nigeria	Forastero hybrids	Medium cacao, occasional off-notes
São Tomé & Príncipe	Forastero	Good cocoa flavour, bitter, spicy, fruity, earthy
Madagascar	Criollo	Winey, putrid, citrus
Venezuela	Criollo "Porcelana"	Mild chocolate, slightly bitter, distinct fruity notes (plum and cherry)
Brazil	Forastero	Cocoa impact, bitter, acid, astringent (sometimes rubber, hammy, smoky), some fruitiness, no nutty notes
Colombia	Trinitario and Criollo	Fruity, bitter, cacao
Peru	Forastero	Slightly bitter and fruity
Ecuador ( <i>home of Arriba</i> )	Forastero (Nacional)	Balanced profile, low chocolate, floral, fruity, grass, earthy notes
Mexico ( <i>Tabasco</i> )	Criollo/Forastero hybrids	Low chocolate, strong acid, low fruitiness
Panama	Forastero	Moderate chocolate, acidic, fruit and nut notes
Jamaica	Forastero	Fruity
Dominican Republic ( <i>Sanchez</i> )	Criollo/Forastero hybrids	Low cacao, flavourless, bitter
Dominican Republic ( <i>Hispaniola</i> )	Criollo/Forastero hybrids	Winey, earthy, can have tobacco notes
Costa Rica	Forastero	Fruity, balanced cocoa flavour
Trinidad & Tobago	Trinitario (birthplace)	High cacao, nutty and winey notes, aromatic
Grenada	Trinitario	Chocolate, fruity, floral, grassy, woody
Indonesia	Criollo/Forastero hybrids	Low chocolate, acidic, fruity
Sulawesi	Criollo/Forastero hybrids	High bitter, low sour, low cacao, astringent
Java	Criollo/Forastero hybrids	Mild, bland profile, acid, low cacao, light colour
Papua New Guinea	Hybrids/pure Criollo and Forastero	Variable strong acid, floral, mild, nutty
Malaysian	Forastero hybrids	Low to medium cacao, medium to high acidity, astringent (due to fermentation level) phenolic

recognized: Forastero, Criollo, Trinitario and a fourth variety grown in Ecuador, called Nacional (Beckett, 2000; Motamayor et al., 2008; Awua, 2002; Counet, Ouwerx, Rosoux, & Collin, 2004; Bartley, 2005; Amoye, 2006; Afoakwa et al., 2011a). The cultivars exhibit differences in the appearance of pods, yields of beans, flavour characteristics and in resistance to pests and diseases (Afoakwa et al., 2008; Afoakwa, 2010; Adeyeye, Akinyeye, Ogunlade, Olaofe, & Boluwade, 2010). Each cocoa type has a unique potential flavour character (Afoakwa et al., 2008). These differences in flavour can be attributed to inherent genetic composition of the bean, botanical origin, location of growth but growing conditions such as climate, the amount and time of sunshine and rainfall, soil conditions, ripening, time of harvesting, and the time between harvesting and bean fermentation all contribute to variations in the final flavour formation. (See Table 2.)

Forastero, native to the Amazon basin (Lima et al., 2011), comprises of 95% of the world production of cocoa (Saltini, Akkerman, & Frosch, 2013) and is commonly referred to as "bulk cocoa" in trade. Forastero cocoa is predominantly cultivated in West Africa particularly Côte d'Ivoire, Ghana, Nigeria and Cameroon. The seeds are flat, astringent, and purple in colour (more rarely ivory or pale) due to the presence of anthocyanins. Forastero cocoa trees are very productive and are

considered to be moderately resistant to pests and diseases (Ferrão, 2002; Bartley, 2005; Lima et al., 2011). Findings by Ortiz de Bertorelli, Graziani de Fariñas, and Gervaise Rovedas (2009) showed that the Forastero beans have a higher pH after fermentation and drying when compared with Criollo beans. Thus, chocolate produced from the Forastero beans are less bitter, less astringent and less acidic than chocolate produced from either Criollo beans or Trinitario beans (Clapperton et al., 1994; de Muijnck, 2005; Sukha, Butler, Umaharan, & Boulton, 2008).

Criollo is the original cultivated cocoa, indigenous to Northern, South, and Central America. The beans are white to ivory or have a very pale purple colour, due to an anthocyanin inhibitor gene (Fowler, 1999; Ferrão, 2002). Their low yields and susceptibility to many diseases make them rare to cultivate. Nowadays, its cultivation is limited to Central America and a few regions in Asia (Fowler, 1999; Ferrão, 2002; Thompson, Miller, & Lopez, 2007). Criollo cocoa is reported to contain high amount of pyrazines (Reineccius, Keeney, & Weissberger, 1972) and exhibit low pH which easily affect the flavour profile (Ortiz de Bertorelli et al., 2009).

The Trinitario type originated in Trinidad and covers all the products of natural hybridization and recombination of the Criollo and Forastero populations (Fowler, 1999; Ferrão, 2002). The beans are variable in

**Table 2**  
Summary of factors and their impact on cocoa bean flavour.

Factors	Impact on cocoa bean flavour
Cocoa genotype	Influences the type and quantity of bean storage proteins, carbohydrates and polyphenols which are degraded during fermentation and drying to form flavour precursors
Depulping	Removes portion of the pulp prior to fermentation hence, reduces fermentable sugars during fermentation leading to the production of less acids
Pod storage	Reduces pulp volume per seed due to water evaporation and inversion of sucrose; reduces total sugar content and increases micro-aeration within the pulp and eventually reduces alcohol fermentation and acetic acid formation
Fermentation	Generates flavour precursors, namely free amino acids, peptides and reducing sugars from which flavour volatiles are formed. Polyphenols are oxidized and polymerized to insoluble high molecular-weight compounds (tannins) leading to a significant reduction of its concentration and, thus, reducing the bitterness and astringency of the beans
Drying	Physical loss of acidity through outward migration of volatile acids as well as biochemical oxidation of acetic acid from the beans leading to less acids in the beans. Oxidization and polymerization of polyphenols resulting in reduction of its concentration.
Roasting	Non-enzymatic reactions, that is the Maillard reactions to form volatile fractions like pyrazines Evaporation of volatile acids from the beans causing a reduction in acidity, hence, reducing sourness. Flavour precursors namely free amino acids, short-chain peptides, and reducing sugars undergo the Maillard reaction and Strecker degradation to produce the desirable flavour compounds

NB: impact of other factors such as soil chemical composition and age of cocoa tree on cocoa bean flavour quality is not yet known. A research gap which needs to be filled.

colour, although rarely white, and the trees show a susceptibility to pests and diseases intermediate to Forastero and Criollo populations (Fowler, 1999; Ferrão, 2002; Bartley, 2005). The Trinitario cultivar is known to have strong basic chocolate characters and some typical winery type of aroma that are not found in other varieties (Afoakwa et al., 2008). Both Trinitario and Criollo varieties produce the “fine” cocoas, whose share in the total world production is below 5% (ICCO, 2015b). These cocoas are used to make high quality dark chocolate (Fowler, 1999). Nacional cacao is viewed as a fine variety producing the well-known Arriba beans with distinctive floral and spicy flavour notes (Despreaux, 1998; Luna, Crouzillat, Cirou, & Bucheli, 2002; Counet et al., 2004).

Work by Clapperton et al. (1994) noted consistent differences in overall cocoa flavour intensity, acidity, sourness, bitterness and astringency among West African Amelonado variety (AML), four Upper Amazon clones [Iquitos Mixed Calabacillo 67 (IMC67), Nanay 33 (NA33), Parinari 7 (PA7), and Scavina 12 (SCA12)], and unidentified Trinitario (UIT1) grown in Sabah, Malaysia. Work by Ziegler (1990) also suggested that monoterpenes such as linalool are part of the components or molecules responsible for fine flavour in cocoa and concluded that fine flavour cocoas contain higher amounts of linalool than bulk cocoa.

Cocoa bean genotype influences the type and quantity of bean storage proteins, carbohydrates and polyphenols (Afoakwa et al., 2008). This determines the quantities and type of precursors formed during fermentation and drying processes leading to flavour formation thus influences both type of flavour of the beans and intensity (Taylor, 2002; Luna et al., 2002; Counet et al., 2004; Taylor & Roberts, 2004). Cocoa bean is made up of two main parts namely, the testa (seed coat or the shell) and the embryo (Thompson, Miller, & Lopez, 2001). Attached to the testa is the sugary, white mucilaginous pulp that surrounds it, while the embryo is contained within the seed coat or testa. The main parts of the embryo are two folded cotyledons connected by a small embryonic axis (Biehl & Ziegler, 2003). The dry weight of cocoa bean varies considerably, but is approximately 1.0–1.2 g (Biehl & Ziegler, 2003). The chemical composition of cocoa beans has been studied extensively (Thompson et al., 2001; Biehl & Ziegler, 2003; Afoakwa, 2010; Bertazzo, Comai, Brunato, Zancato, & Costa, 2011). The fresh cocoa bean has an approximate composition of 32–39% water, 30–32% fat, 10–15% proteins, 5–6% polyphenols, 4–6% starch, 4–6% pentosans, 2–3% cellulose, 2–3% sucrose, 1–2% theobromine, 1% acids and 1% caffeine (Lopez & Dimick, 1995; Bertazzo et al., 2011). The predominant sugars in cocoa beans are sucrose, fructose and glucose (Afoakwa, 2010) with sucrose being the major component (about 90% of total sugars), followed by fructose and glucose (about 6%) (Biehl & Ziegler, 2003).

Cocoa fat contains about 95% triacylglycerols, 2% diacylglycerols, <1% monoacylglycerols, 1% polar lipids, and 1% free fatty acids (as percentages of lipids) (Biehl & Ziegler, 2003). The predominant fatty acids in cocoa butter are saturated (stearic; 18:0, 35% and palmitic; 16:0, 25%) and monounsaturated (oleic; 18:1, 35%), with the remaining fat being primarily polyunsaturated linoleic (3%) (Bracco, 1994). The cotyledon is composed of two types of parenchyma storage cells (Afoakwa, 2010). The first storage cell is the polyphenolic cells (14–20% dry bean weight) which contain a single large vacuole filled with polyphenols and alkaloids including caffeine, theobromine and theophylline (Osman, Nazaruddin, & Lee, 2004). Afoakwa (2010) argued that the pigmented polyphenols, when undisturbed, confer deep purple colour to fresh Forastero cotyledons. The other storage cells, the lipid-protein cells, on the other hand, have cytoplasm tightly packed with multiple small protein and lipid vacuoles and other components such as starch granules – all of which play roles in defining cocoa flavour and aroma characters (Kim & Keeney, 1984; Nazaruddin, Ayub, Mamot, & Heng, 2001).

Polyphenols in cocoa beans are stored in the pigment cells of the cotyledons and depending on the amount of anthocyanins, those pigment cells, also called polyphenol-storage cells, are white to deep purple

(Wollgast & Anklam, 2000). Three groups of polyphenols can be distinguished in cocoa beans and these are: catechins or flavan-3-ols (ca. 37%), anthocyanins (ca. 4%) and proanthocyanidins (ca. 58%) (Wollgast & Anklam, 2000; Bordiga et al., 2015). The main catechin in the cocoa bean is (–)-epicatechin with up to 35% of polyphenol content. Other catechins found in smaller amounts are (+)-catechin as well as traces of (+)-gallocatechin and (–)-epigallocatechin (Wollgast & Anklam, 2000; Afoakwa, 2010). The anthocyanin fraction consists mainly of cyanidin-3- $\alpha$ -L-arabinoside and cyanidin-3- $\beta$ -D-galactoside (Wollgast & Anklam, 2000), while proanthocyanidins are mostly flavan-3,4-diols, that are 4  $\rightarrow$  8 or 4  $\rightarrow$  6 bound to condensed dimers, trimers or oligomers with epicatechin as the main extension subunit (Romanczyk et al., 1997).

Other polyphenols found in cocoa beans are the flavonol glycosides such as quercetin-3-O- $\alpha$ -D-arabinoside and quercetin-3-O- $\beta$ -D-glucopyranoside (Wollgast & Anklam, 2000). Again, up to 17 phenolic acids and esters have also been reported and the total amount of seven of them comprises not more than 23 ppm of the seed dry weight (phloroglucinol, protocatechuic acid, vanillic acid, o-hydroxyphenylacetic acid, p-coumaric acid, caffeic acid, ferulic acid) (Biehl & Ziegler, 2003). Ziegler (2009) reported that epicatechin and the smaller procyanidins up to three subunits are soluble and, therefore, cause the astringent taste sensation of cocoa but molecules built up of more than three subunits are insoluble and cause no astringency.

Zak and Keeney (1976a, 1976b) found four predominant fractions of protein in cocoa beans representing 95% (w/w) of total seed proteins, and these are albumins (water-soluble), globulins (salt-soluble), prolamin (alcohol-soluble) and glutelins (soluble in dilute acids and alkali). Albumin is a major polypeptide accounting for about 52% of total bean protein and has a molecular weight of 21 kDa (Voigt, Biehl, & Wazir, 1993; Dodo & Furtek, 1994; Lerceteau, Rogers, Petiard, & Crouzillat, 1999; Kochhar, Gartenmann, Guilloteau, & McCarthy, 2001). The albumin is not degraded during fermentation (Dodo, Fritz, & Furtek, 1992). Work done by Voigt and Biehl (1995) showed that incubating the purified cocoa albumin with cocoa proteases did not produce any specific cocoa aroma upon roasting. The globulin fraction accounts for 43% of total protein in cocoa beans and it consists of three polypeptide subunits with apparent molecular weights of 47, 31 and 16 kDa (Voigt et al., 1993; Lerceteau et al., 1999; Kochhar et al., 2001) and it is almost insoluble at pH < 5.0 (Biehl & Ziegler, 2003). These are subunits of the vicilin-type (7S) globulin (VCG), a glycoprotein, each of them consisting of multiple pI-forms (Biehl & Ziegler, 2003; Lerceteau et al., 1999; Kochhar et al., 2001). The vicilin-class globulins are quantitatively degraded during fermentation (88–90% of the initial content) into flavour precursors such as peptides and amino acids, which are important precursors for the formation of cocoa flavour through Maillard reactions during drying and roasting (Voigt et al., 1993; Amin, Jinap, & Jamilah, 1998; Hue et al., 2016).

## 2.2. Effect of postharvest treatment of cocoa on bean flavour quality

Postharvest treatment of cocoa involves all the primary process harvested cocoa pods goes through before the final dried beans is obtained. These processes include pulp pre-conditioning, fermentation, and drying. These processes are usually carried out in the country of origin and they play a critical role in the flavour profile of the dried cocoa beans (Krähmer et al., 2015). While the complex composition of cocoa bean flavour depends on the bean genotype, specifically on contents of bean storage proteins, polysaccharides and polyphenols, it is possible and easier to produce cocoa beans with poor flavour profile with bad postharvest treatments.

### 2.2.1. Pulp pre-conditioning

Pulp pre-conditioning involves changing the properties of the pulp prior to the development of microorganisms in fermentation

(Afoakwa, Quao, Takrama, Budu, & Saalia, 2011b). The pulp is the substrate metabolized by a sequence of microorganisms during fermentation (Ostovar & Keeney, 1973), and since the properties of the substrate determine microbial development and metabolism, changes in the pulp may affect the production of alcohols by yeasts and subsequent production of acids by lactic acid bacteria and acetic acid bacteria. These changes may be in the form of altering the moisture content of the pulp, sugar content, and volume of pulp per seed as well as pH and acidity of the pulp. Removing portions of cocoa bean pulp or reducing the fermentable sugar content has been shown to contribute to less acid production during fermentation, leading to less acid beans (Afoakwa, Quao, Takrama, Budu, & Saalia, 2012). Studies have shown that pre-fermentation treatments have significant effects in changing the acidity and polyphenol content of the cocoa beans and thus, flavour of the beans (Meyer, Biehl, Said, & Samarakoddy, 1989; Nazaruddin, Seng, Hassan, & Said, 2006; Afoakwa et al., 2012). Pulp pre-conditioning can be done in three basic ways prior to fermentation and these are pod storage, depulping (mechanical or enzymatic depulping) and bean spreading (Biehl, Meyer, Crone, Pollmann, & Said, 1989; Schwan & Wheals, 2004; Afoakwa et al., 2011a). Cocoa pulp can be pre-conditioned either inside the pods (pod storage) before the bean–pulp mass is brought out for fermentation or outside the pods (mechanical or enzymatic depulping and bean spreading).

**2.2.1.1. Depulping of cocoa beans.** Excessive pulp on the cocoa beans leads to high acid production during fermentation which is detrimental to bean flavour quality as it makes it excessively sour (Afoakwa & Paterson, 2010). By removing a portion of the pulp, or reducing the fermentable sugar content of the beans, it has been shown that less acid is produced during fermentation, leading to less acid beans (Duncan, Godfrey, Yap, Pettiphar, & Tharumarajah, 1989; Sanagi, Hung, & Yasir, 1997; Afoakwa et al., 2012). Removal of up to 20% of the cocoa pulp from fresh Brazilian cocoa beans significantly improved the flavour quality of the beans produced (Schwan & Wheals, 2004). Depulping can be done in two ways, namely mechanically and enzymatically (Biehl et al., 1989; Afoakwa et al., 2012).

Methods for mechanical depulping of fresh cocoa beans include presses (Afoakwa et al., 2012), centrifuges (Schwan, Rose, & Board, 1995), or simply spreading beans onto a flat surface for several hours prior to fermentation, causing a significant increase in the sweating produced in the first 24 h of fermentation. Mechanical depulping is done to remove some of the pulp of the fresh cocoa beans so as to reduce substrates and therefore acids produced during fermentation with a subsequent decrease in sourness. The process causes bruising of the beans and its cell structures leading to the activation of enzyme, which might influence various biochemical processes during fermentation. In addition to reducing acidity, benefits of de-pulping include shorter fermentations and increased efficiency and the ability to use the excess pulp in the manufacture of jams, marmalades, pulp juices, wines or cocoa soft drinks (Schwan & Wheals, 2004; Dias, Schwan, Freire, & dos Santos Serodio, 2007; Afoakwa, 2010). By using a mechanical depulper, the pulp is removed substantially uniformly from the beans and the amount of pulp removed from the fresh cocoa beans in the depulper may be from 10 to 30%, preferably from 20 to 25% by weight based on the original total combined weight of the beans and pulp.

Enzymatic depulping involves the addition of pectin degrading enzymes to the cocoa bean–pulp mass prior to the fermentation process to breakdown the pectin in the pulp. The breakdown of the pectin in the pulp leads to a reduction in pulp volume which in turn increases the aeration of the fermenting mass during fermentation.

**2.2.1.2. Pod storage.** Pod storage is basically storing harvested cocoa pods for a period of time before opening the pods and fermenting the beans. Pod storage as observed by Afoakwa et al. (2011b) appears to have highly beneficial effect on the chemical composition of cocoa beans and subsequent development of chocolate flavour. Meyer et al. (1989)

reported that pulp preconditioning by post-harvest storage of Malaysian cocoa pods led to the reduction of nib acidification during subsequent fermentation, reduction of acid note and an increase in cocoa flavour in the resulting cocoa beans. Afoakwa et al. (2011a) also noted that increasing pod storage (PS) consistently decreased the non-volatile acidity with concomitant increase in pH during fermentation of Ghanaian cocoa beans. Nazaruddin et al. (2006) reported that pulp pre-conditioning of cocoa prior to fermentation was significant in affecting the changes in acidity, causing a significant reduction in the content of polyphenol compounds especially (–)-epicatechin and (+)-catechin during fermentation, hereby reducing the astringency and bitterness in cocoa and cocoa products (Nazaruddin et al., 2006).

### 2.2.2. Fermentation

Fermentation of cocoa beans is very crucial as it promotes dramatic biochemical changes in the type and concentration of flavour precursors in cocoa beans (Kadow, Bohlmann, Phillips, & Lieberei, 2013; Afoakwa, Kongor, Takrama, & Budu, 2013; Krähmer et al., 2015). Raw cocoa beans have an astringent and unpleasant taste and have to be fermented and dried to obtain the characteristic cocoa taste and flavour. The correct fermentation and drying of cocoa beans, which are carried out in the countries of origin are essential to the development of suitable flavour and/or flavour precursors (Ho, Zhao, & Fleet, 2014; Krähmer et al., 2015). During fermentation, microbial successions occur as the micro-environment (temperature, pH, oxygen availability) changes (De Vuyst, Lefeber, Papalexandratou, & Camu, 2010; Illegghems, Weckx, & De Vuyst, 2015). The flavour quality of cocoa beans depends on complex chemical and biochemical changes which occur in the beans during fermentation and drying. Fermentation generates flavour precursors, namely free amino acids and peptides from enzymatic degradation of cocoa proteins and reducing sugars from enzymatic degradation of sucrose (Lagunes-Gálvez, Loiseau, Paredes, Barel, & Guiraud, 2007; Misnawi, 2008; Afoakwa et al., 2013; Krähmer et al., 2015) from which the typical cocoa aroma is generated during the subsequent roasting process (Frauendorfer & Schieberle, 2008). Besides the formation of the flavour precursors, there is also a significant increase in volatile compounds, such as alcohols, organic acids, esters and aldehydes after fermentation (Frauendorfer & Schieberle, 2008; Magi et al., 2012). Again, phenolic compounds are oxidized and polymerized to insoluble high molecular-weight compounds (tannins) leading to a significant reduction of its concentration and, thus, reducing the bitterness and astringency of the final product to acceptable levels (Misnawi, 2008).

Cotyledon protein degradation into peptides and free amino acids appears central to flavour formation and Afoakwa (2010) reported that the combined action of aspartic endopeptidase and serine carboxy-(exo) peptidase on vicilin (7S)-class globulin (VCG) storage polypeptide yields cocoa-specific precursors. Proteolysis in the seeds mainly takes place within 24 h after destruction of the cells and acidification by acetic acid (Ziegler, 2009). The aspartic endopeptidase hydrolyses peptide bonds in VCG at hydrophobic amino acid residues, forming hydrophobic oligopeptides which then become substrates for the serine carboxy-(exo) peptidase which removes carboxyl terminal hydrophobic amino acid residues (Biehl, Heinrichs, Voigt, Bytof, & Serrano, 1996; Biehl & Voigt, 1999). Carboxypeptidase plays an important role in converting hydrophobic oligopeptides to cocoa specific aroma precursors, namely hydrophilic oligopeptides and hydrophobic free amino acids (especially leucine, valine, alanine, isoleucine, phenylalanine), which are required for the formation of the typical cocoa aroma components in the presence of reducing sugar upon roasting (Voigt, Heinrichs, Voigt, & Biehl, 1994).

The duration and method of fermentation are crucial to the formation of flavour compounds and flavour precursors. Aculey et al. (2010) noted an increased level of organic acids such as propanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid and acetic acid after 72 h of cocoa fermentation. The increased levels of organic acids are a

result of the breakdown of sugars from the pulp surrounding the cocoa beans (Bonvehí, 2005). Propanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid and acetic acid are all reported to be important odour-active compounds in cocoa (Bonvehí, 2005; Frauendorfer & Schieberle, 2006). Unfermented cocoa beans are reported to develop little cocoa and chocolate flavour when roasted while over-fermented beans produce unwanted hammy and putrid flavours (Afoakwa et al., 2008; Afoakwa, 2015). Important flavour-active components produced during fermentation include ethyl-2-methylbutanoate, tetramethylpyrazine and certain other pyrazines (Afoakwa et al., 2008; Afoakwa, 2015). Bitter notes are evoked by theobromine and caffeine, together with diketopiperazines formed from roasting through thermal decompositions of proteins (Afoakwa, 2015). Other flavour precursor compounds derived from amino acids released during fermentations include 3-methylbutanol, phenylacetaldehyde, 2-methyl-3-(methylthio)furan, 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine (Taylor, 2002; Afoakwa, 2015).

### 2.2.3. Drying

After fermentation, the beans are dried to reduce the moisture content from about 60% to between 6 and 8% (Prabhakaran Nair, 2010) to prevent mould infestation during storage and also allow some of the chemical changes which occurred during fermentation to continue and improve flavour development (Kyj et al., 2005). The drying process of fermented cocoa beans initiates major polyphenol oxidizing reactions catalysed by polyphenol oxidase, giving rise to new flavour components and loss of membrane integrity, inducing brown colour formation. This helps to reduce bitterness and astringency and also the development of the chocolate brown colour of well fermented cocoa beans. The biochemical oxidation of acetic acid from the beans continues during drying (Afoakwa, 2010; Prabhakaran Nair, 2010). Hashim and Chaveron (1994) and Cros and Jeanjean (1995) have both suggested that during the drying of fermented cocoa beans, reducing sugars participate in the thermal treatment of non-enzymatic browning reactions, that is the Maillard reactions to form volatile fractions of pyrazines. Oberparleiter and Ziegleder (1997) have confirmed earlier findings by Hashim and Chaveron (1994) and Cros and Jeanjean (1995) by identifying Amadori compounds, the first intermediates of Maillard reaction in dried, unroasted cocoa beans. These Amadori compounds are the first intermediates of the reaction of free amino acid and glucose.

Drying rate during the drying process is of crucial importance for the final quality of the cocoa beans. The drying process must not be too rapid otherwise the beans tend to retain an excessive amount of acetic acid, and this is deleterious to flavour (Hii, Rahman, Jinap, & Che Man, 2006; Bharath & Bowen-O'Connor, 2008; Zahouli, Guehi, Fae, & Nemlin, 2010; Saltini et al., 2013). Rapid drying of the beans results in case hardening which prevents outward migration of acetic acid from the beans, thus, leading to a build-up of acidity in the beans (Puziah, Jinap, Sharifah, & Asbi, 1999). On the other hand, too slow drying rate would result in low acidity, poorer colour and high presence of moulds (Hashim, Selamat, Muhammad, & Ali, 1999; Hii et al., 2006; Bharath & Bowen-O'Connor, 2008; Zahouli et al., 2010).

## 2.3. Effect of industrial processing of cocoa on bean flavour quality

### 2.3.1. Roasting

Roasting is one of the important steps which affects the quality characteristic of cocoa beans during industrial processing (Zzaman & Yang, 2013; Oracz & Nebesny, 2014). Roasting determines the character of the chemical and physical processes that occur inside the beans, as well as the quality of the final products (Krysiak & Motyl-Patelska, 2006). During roasting, there is evaporation of volatile acids from the beans causing a reduction in acidity, hence, reducing sourness and bitterness of the cocoa beans (Afoakwa et al., 2008). The high roasting temperature reduces the acidity, specifically the volatile acids with low boiling point such as acetic acid, making the cocoa beans less acidic

(Afoakwa et al., 2008; Beckett, 2008; Frauendorfer & Schieberle, 2008; De Zaen Cocoa Manual, 2009). The less volatile acids, such as oxalic, citric, tartaric, succinic and lactic acids remain largely unchanged by the roasting process (Afoakwa et al., 2008).

Again, flavour precursors namely free amino acids, short-chain peptides, and reducing sugars produced during fermentation and drying process undergo the Maillard reaction and Strecker degradation during roasting to produce the desirable flavour compounds such as pyrazines, alcohols, esters, aldehydes, ketones, furans, thiazoles, pyrones, acids, imines, amines, oxazoles, pyrroles and ethers (Counet et al., 2002; Nazaruddin et al., 2006; Frauendorfer & Schieberle, 2008; Arlorio et al., 2008; Misnawi & Teguh, 2010). The ideal conditions for the Maillard reaction to occur are high temperatures and low moisture content and these conditions can be found in roasting (Fennema, 1996). The carbonyl derivative from the Maillard reaction reacts with free amino acids during Strecker degradation. This causes degradation of amino acids to aldehydes which contribute to the aroma. Strecker degradation of each specific amino acid produces a unique aldehyde with a unique aroma (Fennema, 1996).

The choice of roasting parameters determines the character of the chemical and physical processes that occur inside the beans, and thus the quality of the end products (Świechowski, 1996; Jinap et al., 1998; Krysiak & Motyl-Patelska, 2006). Several studies revealed that the temperature and duration of roasting substantially affect the chemical and physical changes occurring in cocoa beans (Farah, Zaibunnisa, & Misnawi, 2012). Cocoa bean roasting conditions generally range from 15 to 45 min with temperatures from 130 to 150 °C (Krysiak & Motyl-Patelska, 2006; Krysiak, 2006; Krysiak, Adamski, & Żyżelewicz, 2013). Time and temperature of the roasting process depend on several factors, such as cocoa material (beans, nibs or liquor roasting), final cocoa product (dark or milk chocolates) and type of cocoa (Criollo or Forastero) (Kothe, Zimmermann, & Galensa, 2013). Ramli, Hassan, Said, Samsudin, and Idris (2006) reported that “fine” cocoa varieties such as Criollo require lower temperatures than the “bulk” ones, while Afoakwa et al. (2008) indicated that low temperature roasts are employed for milk and some dark chocolates.

## 2.4. Soil chemical composition and cocoa bean flavour quality

Cocoa trees are extremely selective about where they grow, including the type of soil they prefer. Cocoa tree needs a soil containing coarse particles and with a reasonable quantity of nutrients, to a depth of 1.5 m to allow the development of a good root system (ICCO, 2015c). Cocoa trees are more sensitive to moisture stress than other tropical crops. The cocoa tree is sensitive to a lack of water, so the soil must have both water retention properties and good drainage. The chemical properties of the topsoil are most important, as the plant has a large number of roots for absorbing nutrients. Cocoa can grow in soils with a pH in the range of 5.0–7.5. It can therefore cope with both acid and alkaline soil, but excessive acidity (pH 4.0 and below) or alkalinity (pH 8.0 and above) must be avoided (ICCO, 2015c). The influence of soil pH is critical on the solubility of minerals and nutrients and it is thus regarded as a useful indicator of other soil parameters (Ololade, Ajayi, Gbadamosi, Mohammed, & Sunday, 2010).

Soil pH provides useful information about the availabilities of exchangeable cations (e.g. Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup>) in soils (Ololade et al., 2010). The soil should also have a high content of organic matter of about 3.5% in the top 15 cm of soil. The organic matter of soils includes the remains of plants, animals and microorganisms in all stages of decomposition. The level of organic matters in soils influences a number of soil chemical and physical properties (Ololade et al., 2010). Soils for cocoa must have certain anionic and cationic balances. Exchangeable bases in the soil should amount to at least 35% of the total cation exchange capacity (CEC) otherwise nutritional problems are likely (ICCO, 2015c). The CEC is a measure of the soil's ability to adsorb (and release) cations (Ololade et al., 2010). It is highly needed for the

estimation of contaminant transport potential and sorption capacity for any soil location, i.e., the total number of cations it can retain on its adsorbent complex at a given pH. The optimum total nitrogen/total phosphorus ratio should be around 1.5 (ICCO, 2015c).

Several works have been done on soil type, soil chemical composition and nutrient requirements for cocoa production (Amusan, Amusan, Braimoh, & Oguntunde, 2005; Ololade et al., 2010; Baah, Anchirinah, & Amon-Armah, 2011; Adewole et al., 2011). However, all research works on soil effects on cocoa are focused on yield with little or no work on the effect of soil on the flavour quality of cocoa beans. There is, therefore, a big gap in terms of research work on the effect of soil chemical composition on the flavour quality of cocoa beans.

### 2.5. Age of cocoa tree and flavour quality

Like any living organism, the cocoa tree goes through different stages in its entire life cycle. According to Mahrizal, Nalley, Dixon, and Popp (2013), production life cycle of cocoa occurs in four stages which includes: (1) an early period of no yield which normally occurs in the first 3 years, (2) a period of increasing yield at an increasing rate, (3) a period of increasing yield at a decreasing rate, and (4) a period of decreasing yields. The last stage is associated with trees that are past their yield prime. Binam, Gockowski, and Nkamleu (2008) also reported that after four years from the time of planting, the cocoa tree becomes productive and the yield rate increases annually until approximately 18 years of age. Then, the yields begin to decline due to exhaustion of soil nutrients, erosion, and increasing occurrence of pests and plant diseases (Vekua, 2013).

Age is therefore an important factor in the life of cocoa trees due to biological lags inherent to the crop (Vekua, 2013). Several studies have reported on the impact of ageing cocoa farms or trees in Ghana and other West African cocoa producing countries on yield (Binam et al., 2008; Hainmueller, Hiscox, & Tampec, 2011; Vekua, 2013) with little or no work on flavour quality. There is therefore a big research gap that needs to be filled. The influence of cocoa tree age on the formation of flavour precursors during cocoa bean fermentation as well as the formation of flavour volatiles needs to be studied.

### 2.6. Research gaps

Cocoa is a crop of huge economic significance especially in developing countries. The crop is also enjoyed by millions of people around the world in the form of chocolate and other cocoa related products. As a result, several researchers have taken keen interest in developing and improving on the quality characteristics of cocoa beans as well as the wellbeing of cocoa farmers so as to ensure a sustainable production. Extensive works have been done on cocoa fermentation, drying and roasting and their impact on the polyphenolic and flavour characteristics of cocoa beans. Other factors such as pod storage and mechanical depulping which influence the flavour profile of cocoa beans during fermentation, drying and subsequent roasting have also received fair attention by some researchers.

However, works done on age of cocoa tree and soil characteristics have concentrated on the impact on yield with little or no work on the flavour quality characteristics of the beans. Therefore, works need to be done on the influence of age of cocoa tree on the formation of flavour precursors during cocoa bean fermentation, drying as well as the flavour volatiles produced during subsequent roasting. The nutrients and chemical composition of soils need to be studied and ascertain their influence on the flavour quality of cocoa beans.

## 3. Conclusions

Sustainable production of cocoa is crucial to both the small holder farmers and their families that depend on cocoa for income and also the millions of people who enjoy chocolate and other cocoa related products.

Sustainable cocoa production, however, depends largely on the quantity cocoa beans produced annually as well as the quality of the beans. Cocoa bean flavour is an important quality attribute which determines acceptability of cocoa beans and cocoa products such as chocolate. The complex composition of cocoa bean flavour depends on the bean genotype specifically on contents of bean storage proteins, polysaccharides and polyphenols. Pulp pre-conditioning, fermentation and drying enhance the formation of the flavour precursors which undergo the Maillard reaction during roasting to produce the typical cocoa flavours. Research works on age of cocoa tree and the soil chemical compositions are focused on yield with little or no work on flavour quality. More works need to be done on the impact of age of cocoa tree and soil chemical compositions on the development of flavour precursors during fermentation and drying and their subsequent formation of flavour volatile compounds during cocoa bean roasting.

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