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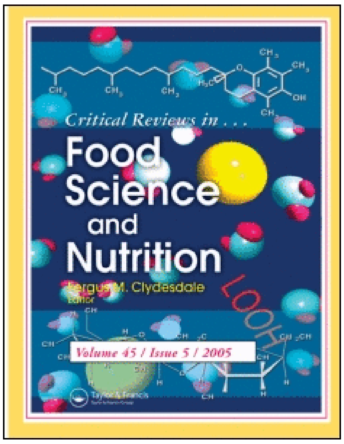

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Flavor Formation and Character in Cocoa and Chocolate: A Critical Review

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Flavor Formation and Character in Cocoa and Chocolate: A Critical Review

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Chocolate characters not only originate in flavor precursors present in cocoa beans, but are generated during post-harvest treatments and transformed into desirable odor notes in the manufacturing processes. Complex biochemical modifications of bean constituents are further altered by thermal reactions in roasting and conching and in alkalization. However, the extent to which the inherent bean constituents from the cocoa genotype, environmental factors, post-harvest treatment, and processing technologies influence chocolate flavor formation and relationships with final flavor quality, has not been clear. With increasing speciality niche products in chocolate confectionery, greater understanding of factors contributing to the variations in flavor character would have significant commercial implications.

Keywords Theobroma cacao, genetic origin, cocoa fermentation, roasting, conching, chocolate flavor

INTRODUCTION

Principal varieties of the cocoa tree Theobroma cacao (family Sterculiaceae) are: Criollo, rarely grown because of disease susceptibility; Nacional with fine flavor, grown in Ecuador; Forastero from the Amazonas region; and Trinitario, a hybrid of Forastero and Criollo. Forastero varieties form most of the "bulk" or "basic" cocoa market. The world annual cocoa bean production is approximately 3.5 million metric tons and the major producers are the Ivory Coast, Ghana, Indonesia, Brazil, Nigeria, Cameroon, and Ecuador. There are also a number of smaller producers, particularly of "fine" cocoa, which forms less than 5% of the world trade (Coe and Coe, 1996; Awua, 2002; Schwan and Wheals, 2004; Amoye, 2006).

Chocolate consumption has possible health benefits with specific claims recently identified and studied (Erdman et al., 2000; Wollgast and Anklam, 2000; Weisburger, 2001; Tapiero et al., 2002; Steinburg et al., 2003; Miller et al., 2006; Gu et al., 2006). Cocoa beans and derived products are rich in antioxidants—including catechins, epicatechin, and procyanidins—polyphenols similar to those found in wine, vegetables, and tea (Kim and Keeney, 1984; Yamagishi et al., 2001; Carnesecchia et al., 2002; Tapiero et al., 2002; Hatano et al., 2002; Kris-Etherton and Keen, 2002; Engler et al., 2004; Grassi et al., 2005; Lamuela-Raventos et al., 2005; Hermann et al., 2006; Gu et al., 2006; Buijsse et al., 2006; Afoakwa et al., 2007). These contribute as precursors to the flavor formation in cocoa and chocolate (Misnawi et al., 2003; Counet, et al., 2004; Kyi, et al., 2005).

Chocolate has a distinctive flavor character, with specific notes related to bean genotype, growing conditions, and processing factors (Clapperton, 1994; Beckett, 2003; Whitefield, 2005). Fermentation is a key processing stage that causes the death of the bean and facilitates removal of the pulp and subsequent drying. During this stage, there is initiation of flavor precursor formation and color development, and a significant reduction in bitterness. In thermal reactions of roasting important modifications occur, including Maillard reactions with contributions from reducing sugars and amino acids, each showing variations. Conching is also important for flavor development and final texture in chocolate, effecting elimination of volatile acids, removal of moisture, viscosity modifications, and color changes due to emulsification and tannin oxidation (Mermet et al., 1992; Fowler, 1999; Beckett, 2000; Awua, 2002; Beckett, 2003; Kealey et al., 2001; Reineccius, 2006).

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The chemistry of cocoa beans in fermentation is still under study (Buyukpamukcu et al., 2001; Luna et al., 2002; Misnawi, et al., 2003; Schwan and Wheals, 2004; Kyi et al., 2005) as are contributions from roasting and alkalization (Gill et al., 1984; Oberparleiter and Ziegleder, 1997; Jinap and Dimick, 1991; Dimick and Hoskin, 1999; Stark et al., 2005; Ramli et al., 2006; Granvogl et al., 2006; Stark et al., 2006a; Reineccius, 2006) and conching (Pontillon, 1995; Plumas et al., 1996 Awua, 2002; Beckett, 2000; Reineccius, 2006). Key flavour compounds in chocolate have been identified (Cerny and Fay, 1995; Cerny and Grosch, 1994; Schnermann and Schieberle, 1997; Schieberle and Pfnuer, 1999; Counet et al., 2002; Taylor, 2002; Taylor and Roberts, 2004; Reineccius, 2006). However, the biochemical and chemical processes leading to chocolate flavor formation and development, and their relationships to the final character and perceptions of quality are not fully understood.

This review discusses the sources of variation in the formation and development of chocolate flavors and suggests how the overall character is achieved.

BEAN COMPOSITION AND FLAVOR PRECURSOR FORMATION

The shell (testa) represents 10–14% dry weight of the cocoa bean. The kernel or cotyledon, most of the remaining 86–90% (Table 1), confers characteristic flavors and aromas of chocolate (Rohan and Stewart, 1967; Osman et al., 2004) and is composed of two types of parenchyma storage cells. Polyphenolic cells (14–20% dry bean weight) contain a single large vacuole filled with polyphenols and alkaloids including caffeine, theobromine, and theophylline (Osman et al., 2004). The pigmented polyphenols, when undisturbed, confer a deep purple color to fresh Forastero cotyledons. Lipid-protein cells, on the other hand, have cytoplasms tightly packed with multiple small protein and lipid vacuoles and other components such as starch granules—all of which play roles in defining cocoa flavor and aroma characters (Kim and Keeney, 1984; Nazaruddin et al., 2001).

Table 1 Bean composition of unfermented West African (Forastero) cocoa.

Constituents	Dried beans (%)	Fat-free materials (%)
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
Carbohydrates		
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

Sources: Rohan (1963); Reineccius et al. (1972).

Reineccius et al. (1972) reported that fresh unfermented cocoa beans contained 15.8 mg/g sucrose and trace amounts of fructose, sorbose, mannitol and inositol. Berbert (1979) suggested the sucrose content at 24.8 mg/g unfermented beans formed about 90% of total sugars (27.1 mg/g), with the reducing sugars fructose and glucose forming about 6% (0.9 and 0.7 mg/g, respectively) and others (including mannitol and inositol) at <0.50 mg/g. Differences have been attributed to method and time of harvesting, type, and origin of cocoa beans (Reineccius et al., 1972). Tissue components remain compartmentalized, separating flavor constituents that may interact with cell membrane and wall breakdown during the subsequent fermentation.

Cocoa is rich in polyphenols, specifically catechins (flavan-3-ols) and procyanidins, stored in cotyledon pigment cells and cocoa leaves (Osman et al., 2004). Depending on the anthocyanin content, pigmentation in polyphenol-storage cells ranges from white to deep purple. Polyphenol and alkaloids, ca. 14–20% bean weight, are central to bean flavor character (Kim and Keeney, 1983). Three groups of polyphenols can be differentiated: catechins or flavan-3-ols (ca. 37%), anthocyanins (ca. 4%) and proanthocyanidins (ca. 58%). The primary catechin is (−)-epicatechin, up to 35% of total polyphenols and from 34.65 to 43.27 mg/g of defatted freshly harvested Criollo and Forastero beans (Kim and Keeney, 1984). Less abundant is (+)-catechin with only traces of (+)-gallocatechin and (−)-epigallocatechin. Nazaruddin et al. (2001) reported that the total polyphenols ranged from 45–52 mg/gin cocoa liquor, 34–60 in beans, and 20–62 in powder: (−)-epicatechin contents were 2.53, 4.61, and 3.81 mg/g, respectively.

The anthocyanin fraction is dominated by cyanidin-3-α-L-arabinoside and cyanidin-3-ß- D-galactoside. Procyanidins are mostly flavan-3,4-diols are 4 to 8 or 4 to 6 bound to form dimers, trimers, or oligomers with epicatechin as the main extension subunit (Romanczyk et al., 1997). Fat-soluble polyphenols in dried fat-free fresh Forastero cocoa form 15 to 20%, which falls to approx. 5% after fermentation. The contents of 10% or greater are considered a sign of poor fermentation. Higher concentrations of polyphenols lead to a very astringent tasting chocolate. Criollo cocoa beans have approx. two-thirds of this content of polyphenols, and anthocyanins have not been found (Lange and Fincke, 1970; Hansen et al., 2000). Polyphenol reactions with sugar and amino acids contribute flavor and color to cocoa beans, and alkaloids to the bitterness (Lehrian and Patterson, 1983).

Cotyledons contain as storage proteins single albumin and globulin species (Biehl et al., 1982a). The globulin, with two polypeptides of 47 and 31 kDa (Pettipher 1990; Spencer and Hodge 1992; Voigt et al., 1993) is degraded in fermentation, the albumin (21 kDaltons) is not. Cocoa-specific aroma precursors can be generated in vitro from globulin in partially purified bean fractions by aspartic endoprotease and carboxypeptidase activities (Voigt et al., 1994a). Cotyledon protein degradation into peptides and free amino acids appears central to the flavor formation. The consensus is that the combined action of two proteases, namely aspartic endopeptidase and serine carboxy-(exo)peptidase, on vicilin (7S)-class globulin (VCG) storage polypeptide yield cocoa-specific precursors. The aspartic endopeptidase (EC 3.4.23) hydrolyses peptide bonds in VCG at hydrophobic amino acid residues and forming hydrophobic oligopeptides, substrates for the serine exopeptidase (EC 3.4.16.1) that removes carboxyl terminal hydrophobic amino acid residues (Biehl et al., 1993; Biehl et al., 1996; Biehl and Voigt, 1999; Voigt et al., 1994b). Kirchhoff et al. (1989) observed a correlation between free amino acids accumulation and the generation of specific aroma precursors, with pH-dependent proteolytic processes. Activities in both key enzymes are pH-dependent, near to pH 3.8—optimum for aspartic endopeptidase—more hydrophobic oligopeptides and less free amino acids are produced. Whereas close to 5.8, the optimum for serine exopeptidase, there are increases in hydrophilic oligopeptides and hydrophobic amino acids. Related storage proteins or alternative peptidases both failed to produce appropriate flavor precursors. With a rapid fall to low pH (<4.5) reduction in flavor precursors is observed and a slow diffusion of organic acids through cotyledons, timing of initial entry, duration of the period of optimum pH, and the final pH are crucial for final flavor (Biehl and Voigt, 1999). Thus bean composition interacts with fermentation in the formation of cocoa flavor quality. An analysis of VCG proteins and proteolytic degradation products in five popular genotypes (Forastero, Criollo, Trinitario, SCA 12, and UIT1) concluded that character in chocolate may vary, but all genotypes had the potential for abundant aroma content in raw cocoa (Amin et al., 2002).

Electrophoretic (SDS-PAGE) analyses showed polypeptide species at 47, 31, and approximately 14.5 kDa, all derived from post-translational modification of a vicilin (7S) storage protein precursor observed in vivo as a 139 kDa trimer (Biehl et al., 1982b; MacDonald et al., 1994). Polypeptide and cDNA sequence data showed considerable homology to other 7S class storage proteins, and specifically α-globulin in cotton seeds (Spencer and Hodge, 1992; McHenry and Fritz, 1992). Specific cocoa aroma was obtained in vitro when this vicilin globulin, was successively degraded by an aspartic endoprotease and a carboxypeptidase and the products were roasted in the presence of reducing sugars (Voigt et al., 1994a,b). Acidification during fermentation is critical for final cocoa quality since the different pH optima of endoprotease and carboxypeptidase activities determine the efficiency and products of proteolysis. The outcome is mixtures of hydrophobic and hydrophilic peptides, the latter more important for the formation of typical aroma notes. In summary it can be concluded that proteolysis of globulin is central to cocoa flavor formation.

Low molecular weight protein breakdown products and reducing sugars all contribute to Maillard reactions that produce cocoa flavor in roasting (Rohan and Stewart, 1967). Peptides and hydrophobic free amino acids, specifically leucine, alanine, phenylalanine, and tyrosine released during fermentation by aspartic proteinase and carboxypeptidase activities (Voigt et al., 1993, 1994a) contribute to flavor (Mohr et al., 1976) by reacting with fructose and glucose (Lopez et al., 1978). Cocoa fermentation protein breakdown has been characterized by Biehl and Passern (1982) and Biehl et al. (1985); Lopez et al. (1978), and Rohan and Stewart (1967) studied the changes in sugars.

During fermentation, the microbial activity on the cocoa pulp generates heat, and produces ethanol, acetic and lactic acids that kills the bean. Pulp fermentation products penetrate slowly into beans causing swelling and stimulating enzymic reactions that yield flavor precursors, and on roasting characteristic flavor and aroma notes. Fresh beans with low contents of flavor precursors will have limited commercial usage and activities in fermentation will be unable to rectify this shortfall (Rohan and Stewart, 1967; Mohr et al., 1976; Voigt et al., 1994a). Appropriate amounts and ratio of precursors are essential for optimal flavor volatiles production in roasting.

Subcellular changes in the cotyledons release key enzymes effecting reactions between substrates pre-existing in unfermented beans (Hansen et al., 1998). Enzymes exhibit different stabilities during fermentation and may be inactivated by heat, acids, polyphenols, and proteases. Aminopeptidase, cotyledon invertase, pulp invertase, and polyphenol oxidase are significantly inactivated, carboxypeptidase partly inactivated, whereas endoprotease and glycosidases remain active during fermentation (Hansen et al., 1998). Hansen et al. (2000) noted that differences in enzyme activities can be partly explained by pod variation and genotype but in general, activities present in unfermented beans seem not a limiting factor for optimal flavor precursor formation in fermentation. Significant fermentation effects may relate to factors such as storage protein sequence and accessibility, destruction of cell compartmentalization, enzyme mobilization, and pulp and testa changes.

Proteases effect the multiple cellular processes in plants, such as protein maturation and degradations associated with tissue restructuring and cell maintenance (Callis, 1995). Key aspartic proteinases (EC 3.4.23) have been characterized in a number of Theobroma cacao gymnosperms (Mutlu and Gal, 1999) and activity in the seeds of Theobroma cacao extensively studied by Biehl et al. (1993). Partially purified aspartic proteinase had activity optima at 55°C and pH 3.5. Subsequently, Voigt et al. (1995) purified Theobroma cacao seed aspartic proteinase into a heterodimer of 29 and 13 kDa polypeptides that efficiently hydrolyzed Theobroma cacao seed vicilin and (less effectively) the trypsin inhibitor into peptides (Voigt et al., 1994a).

Two cDNA species, TcAP1 and TcAP2 respectively, encoding different polypeptides of the plant aspartic proteinase gene family, have been cloned and characterized (Laloi et al., 2002). Both genes are induced early in seed development, and show significantly decreased expression as the seeds reach maturity. However, TcAP2 expression is induced to higher levels suggesting that the gene encodes the primary aspartic proteinase in the mature seed. It should also be noted that T. cacao seeds have unusually high levels of such aspartic proteinase activity (Voigt et al., 1994a). Guilloteau et al. (2005) noted that physical and biochemical properties of the active T. cacao seed TcAP2 aspartic proteinase complex are novel suggesting the highly expressed gene product may represent a previously uncharacterized activity. A purified TcAP2 gene product efficiently degrades cocoa seed vicilin into low molecular products including di- and tripeptides, implying that this gene product may play an important role during fermentation.

A processing sequence is required to produce cocoa beans with good flavor. Pulp sugar fermentation should yield high levels of acids, particularly acetic acid (Voigt et al., 1994a). As seed pH decreases, the cell structure is disrupted which triggers mobilization and/or activation of the primary aspartic proteinase activity with massive degradation of cellular protein (Biehl et al., 1982b; Biehl et al., 1985). Fermentation proteinase and peptidase activities, seem critical for good flavor quality (Voigt and Biehl, 1995; Laloi et al., 2002).

Significant differences in enzyme activities exist between cocoa genotypes but simple and general relationships have not been established between the genotype flavor potential and key enzyme activities in unfermented beans. Therefore, how enzymatic processes are regulated, and substrates and products that relate to desirable flavors, and limiting factors for the enzymatic contribution to fermentation processes remain unclear.

EFFECT OF GENOTYPE ON COCOA BEAN FLAVORS

Genotype influences both flavor quality and intensity in chocolate (Taylor, 2002, Luna et al., 2002; Counet et al., 2004; Taylor and Roberts, 2004), likely determining the quantities of precursors and the activity of enzymes, and thus contributions to flavor formation. Reineccius (2006) concluded that varietal differences were primarily due to quantitative (as opposed to qualitative) differences in the flavor precursor and polyphenol contents. The contents of sugars and enzymic breakdown of polysaccharides form an important source of precursors. However, post-harvest processes (fermentation and drying), and roasting have a strong influence on final flavors (Clapperton et al., 1994; Kattenberg and Kemming, 1993; Luna et al., 2002; Counet and Collin, 2003). Three primary cocoa types: forastero (bulk grade), criollo (fine grade), and hybrid, trinitario (fine grade) show wide variations in final flavor (Beckett, 2000; Awua, 2002; Amoye, 2006). Nacional cacao is viewed as a third fine variety: producing the well-known Arriba beans with distinctive floral and spicy flavor notes (Despreaux, 1998; Luna et al., 2002; Counet et al., 2004). These differences in flavor can be ascribed to bean composition variation from botanical origin, location of growth, and farming conditions. Bulk varieties dominate blends while fine grades, used in lesser quantities, are selected to make specific contributions to the overall flavor profile.

Table 2 Origin, cocoa variety, and fermentation duration effects on flavor character

Origin	Cocoa type	Duration (days)	Special flavor character
Ecuador	Nacional (Arriba)	2 Short	Aromatic, floral, spicy, green
Ecuador	Criollo (CCN51)	2	Acidic, harsh, low cocoa
Ceylon	Trinitario	1.5	Floral, fruity, acidic
Venezuela	Trinitario	2	Low cocoa, acidic
Venezuela	Criollo	2	fruity, nutty
Zanzibar	Criollo	6 Medium	Floral, fruity
Venezuela	Forastero	5	Fruity, raisin, caramel
Ghana	Forastero	5	Strong basic cocoa, fruity notes
Malaysia	Forastero/Trinitario	6	Acidic, phenolic
Trinidad	Trinitario	7–8 Long	Winy, raisin, molasses
Grenada	Trinitario	8–10	Acidic, fruity, molasses
Congo	Criollo/Forastero	7–10	Acidic, strong cocoa
Papua New Guinea	Trinitario	7–8	Fruity, acidic

Each bean variety has a unique potential flavor character. 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 flavor formation. Table 2 summarizes how the differences in genetic origin, cocoa variety, and the duration of the fermentation influence flavor profile but different conditions may lead to significant differences in flavor from a single cocoa variety. A good example is the difference in flavor profile between a single Forastero variety produced originally in Ghana and now grown in Malaysia (Clapperton, 1994), arising possibly through the geographic, the climatic conditions, and the duration and/or method of fermentation (Table 2).

Bulk cocoas typically show strong flavor characters, fine cocoas are perceived as aromatic or smoother (Kattenberg and Kemming, 1993; Jinap et al., 1995; Luna et al., 2002). Clapperton et al. (1994) noted consistent differences in flavor attributes, specifically overall cocoa flavor intensity, acidity, sourness, bitterness, and astringency. Bean origins include the 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 Trinatario (UIT1) grown in Sabah, Malaysia. Flavor characters in UIT1 differed from West African Amelonado, characterized by intense bitterness and astringency associated with caffeine and the polyphenol contents. Fermented beans from Southeast Asia and the South Pacific are characterized by a higher acidity (more lactic and acetic acids) than West African beans (Clapperton et al., 1994) due to varietal differences, box fermentation, and rapid artificial drying.

Cocoa liquors differ in sensory character. The West African group (Ghana, Ivory Coast, and Nigeria) are generally considered sources of standard (benchmark) cocoa flavor with a balanced but pronounced cocoa character with subtle to moderate nutty undertones. Cameroon liquors are renowned for bitterness, those from Ecuador for floral-spicy notes. American and West Indian varieties range from aromatic and winy notes from Trinidad cocoa to the floral or raisin-fruity notes of Ecuadorian stocks making unique contributions to blends. Asian and Oceanian beans exhibit a range of flavor profiles ranging from subtle cocoa and nutty/sweet notes in Java beans to the intense acid and phenolic notes of Malaysian (De La Cruz et al., 1995). Counet et al. (2004) reported fine varieties with short fermentation processes had high contents of procyanidins, while Trinatario from New Guinea and Forastero beans were specifically higher in total aroma. Aroma compounds formed during roasting were found to vary quantitatively directly with fermentation time and inversely with the procyanidin content of cocoa liquors.

High concentrations of phenol, guaiacol, 2-phenylbutenal, and γ-butyrolactone characterize Bahia beans known for typical smoked notes. Also reported are higher contents of 2-methylpropanal and 3-methylbutanal in Caracas (Venezuela) and Trinidad dried fermented beans (Dimick and Hoskin, 1999). Of Maillard products, Reineccius (2006) reported roasting yields with higher levels of pyrazines in well-fermented beans (Ghana, Bahia) than in less-fermented (Arriba) or unfermented from Sanchez (Dominican Republic) or Tabasco (Mexico). Lower in astringency and bitterness imparted by polyphenols, Criollo beans, in which anthocyanins are absent, are often less fermented than Forastero (Carr et al., 1979; Clapperton, 1994; Clapperton et al., 1994; Luna et al., 2002).

CHOCOLATE FLAVOR DEVELOPMENT: POST-HARVEST TREATMENTS

Fermentation Processes

Fermentation is essential for the development of appropriate flavors from precursors. After pod harvest, beans and the adhering pulp are transferred to heaps, boxes, or baskets for fermentations lasting from 5 to 6 days for Forastero beans but for Criollo only 1 to 3 days. On the first day, the adhering pulp liquefies and drains off, with steady rises in temperature. Under anaerobic conditions micro-organisms produce acetic acid and ethanol that inhibit germination and contribute to structural changes such as the removal of the compartmentalization of enzymes and substrates with movements of cytoplasmic components through the cocoa cotyledon generally between 24–48 h of bean fermentation. By the third day, the beans mass will have heated typically around 45°C, remaining at 45–50°C until the fermentation is complete (Lehrian and Patterson, 1983; Schwan et al., 1995; Kealey et al., 2001; Fowler, 1999).

Mucilaginous pulp of beans undergoes ethanolic, acetic, and lactic fermentations with consequent acid and heat stopping germination, with notable swelling and key changes in cell membranes facilitating enzyme and substrate movements. The differences in pH, titratable acidity, acetic and lactic acid concentrations, fermentation index, and cut test scores for cocoa beans from different origins are reported (Jinap and Dimick, 1990; Luna et al., 2002; Misnawi et al., 2003). The chemistry of cocoa beans fermentation has been reviewed (Ziegleder, 1990; Lopez and Dimick, 1991a; Buyukpamukcu et al., 2001; Luna et al., 2002; Misnawi et al., 2003; Schwan and Wheals, 2004; Kyi et al., 2005).

During fermentation, the rate of diffusion of organic acids into the cotyledons, timing of initial entry, duration of the period of optimum pH, and the final pH are crucial for optimum flavor formation (Biehl and Voigt, 1999). Beans of higher pH (5.5–5.8) are considered unfermented, with a low fermentation index and cut test score, and those of lower pH (4.75–5.19), well-fermented. Fermentation techniques can reduce the acid notes and maximize the chocolate flavors (Lopez, 1979; Holm et al., 1993; Beckett, 1999, Whitehouse, 2005). Ziegleder (1991) compared natural acid (pH 5.5–6.5) and alkaline (pH 8) cocoa extracts obtained by direct extraction—the former possessed a more intense and chocolate aroma than the latter, attributed to high contents of aromatic acids and sugar degradation products with persistent sweet aromatic and caramel notes. Cocoa beans of lower (4.75–5.19) and higher pH (5.50–5.80) were scored lower for chocolate flavor and higher for off-flavor notes respectively, and chocolate from intermediate pH (5.20–5.49) beans was scored more highly for chocolate (Jinap et al., 1995).

Sucrose and proteinaceous constituents are partially hydrolyzed, phenolic compounds oxidized, and glucose is converted into alcohols and oxidized to acetic and lactic acids during fermentation. Beans subsequently undergo an anaerobic hydrolytic phase, followed by aerobic condensation. Timing, the sequence of events, and the degree of hydrolysis and oxidation varies between fermentations. The concentration of flavor precursors is dependent on enzymatic mechanisms. Color changes also occur with hydrolysis of phenolic components by glycosidases accompanied by bleaching, influencing final flavor character (Lopez and Quesnel, 1973; Biehl et al., 1990; Lopez and Dimick, 1991b; Lopez and Dimick, 1995).

Nitrogenous flavor precursors formed during anaerobic phases are dominated by the amino acids and peptides available for non-oxidative carbonyl–amino condensation reactions promoted in elevated temperature phases such as fermentation, drying, roasting, and grinding. Although degraded to flavor precursors, residual protein is also diminished by phenol-protein interactions. During aerobic phases, oxygen-mediated reactions occur, such as oxidation of protein-polyphenol complexes formed anaerobically. Such processes reduce astringency and bitterness: oxidized polyphenols influence subsequent degradation reactions (Rohan, 1964; Dimick and Hoskin, 1999; Counet et al., 2004; Kyi et al., 2005).

The fermentation method determines the final quality of products produced, especially their flavor. Previous studies on post-harvest pod storage and bean spreading had shown marked improvement in chocolate flavor and reductions in sourness, bitterness, and astringency (Meyer et al., 1989; Biehl et al., 1990). In commercial production, similar effects were obtained through combinations of pod storage, pressing, and airblasting (Said et al., 1990). Variations in such factors as pod storage and duration affect the pH, titratable acidity, and temperature achieved during fermentation, influencing the enzyme activities and flavor development (Biehl et al., 1990).

Important flavor-active components produced during fermentation include: ethyl-2-methylbutanoate, tetramethylpyrazine, and certain pyrazines. Bitter notes are evoked by theobromine and caffeine, together with diketopiperizines formed from roasting through thermal decompositions of proteins. Other flavor precursor compounds derived from amino acids released during fermentations include 3-methylbutanol, phenylactaldehyde, 2-methyl-3-(methyldithio)furan, 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine (Taylor, 2002). Immature and unfermented beans develop little chocolate flavor when roasted and excessive fermentation yields unwanted hammy and putrid flavors (Fowler, 1999; Beckett, 2000; Zaibunnisa et al., 2000; Reineccius, 2006).

Drying

Flavor development from cocoa beans precursors continues during drying with the development of a characteristic brown color. Major polyphenol oxidizing reactions are catalyzed by polyphenol oxidases, giving rise to new flavor components, and loss of membrane integrity, inducing brown color formation. Use of artificial drying can increase cotyledon temperatures. Dimick and Hoskin (1999) reported that case hardening restricts loss of volatile acids, with detrimental effects on final chocolate flavor.

After fermentation and drying, the target for cocoa beans is ca. 6–8% moisture contents. For storage and transport moisture contents should be <8% or mould growth is possible (Carr et al., 1979; Fowler et al., 1998; Kealey et al., 2001; Awua, 2002). Indicators of well-dried, quality beans are a good brown color and low astringency and bitterness and an absence of off-flavors such as smoky notes and excessive acidity. Sensory assessment of cocoa beans dried using different strategies, i.e. sun drying, air-blowing, shade drying and oven drying suggested sun-dried beans were rated higher in chocolate development with fewer off-notes (Dias and Avila, 1993; Buyukpamukcu et al., 2001; Awua, 2002; Kyi et al., 2005; Granvogl et al., 2006; Amoye 2006). Table 3 summarizes key odorants in cocoa mass following fermentation and drying stages.

Frauendorfer and Schieberle (2006) identified similar flavor compounds in cocoa powder using molecular sensory correlations. Off-notes from incomplete drying or rain soaking may result in high levels of water activity and mould contamination, producing high concentrations of strongly flavoured carbonyls, leading to alterations in bean flavor, producing hammy off-flavors, which is also correlated with over-fermentation (Dimick and Hoskin, 1999; Misnawi et al., 2003).

Table 3 Dominant odor-active volatiles in cocoa mass

Compound	Odour quality	Flavour dilution factor
2- and 3-methylbutanoic acid^{b}	sweaty	2048
3-Methylbutanal^{a,b}	Malty	1024
Ethyl 2-methylbutanoate^{a,b}	Fruity	1024
Hexanal^{a,b}	Green	512
Unknown^{a}	Fruity, waxy	512
2-Methoxy-3-isopropyrazine^{a,b}	Peasy, earthy	512
(E)-2-Octanal^{a,b}	Fatty, waxy	512
Unknown^{a}	Tallowy	512
2-Methyl-3-(methyldithio)furan^{a,b}	Cooked meat-like	512
2-Ethyl-3,5-dimethylpyrazine^{a,b}	Earthy roasty	256
2,3-Diethyl-5-methylpyrazine^{a}	Earthy roasty	256
(E)-2-Nonenal^{a,b}	Tallowy green	256
Unknown^{a,b}	Pungent, grassy	128
Unknown^{a,b}	Sweet, waxy	128
Phenylacetaldehyde^{a,b}	Honey-like	64
(Z)-4-Heptanal^{a,b}	Biscuit-like	64
δ-Octenolactone^{a,b}	Sweet, coconut-like	64
γ-Decalactone^{b}	Sweet, peach-like	64

Sources: ^{a}Belitz and Grosch (1999); ^{b}Schnermann and Schieberle (1997).

FLAVOR DEVELOPMENT DURING COCOA PROCESSING

Effect of Roasting

Roasting of cocoa is an essential step to further develop the chocolate flavor from the precursors formed during fermentation and drying. Whole bean roasting loosens the shell which is then readily removed in winnowing. Prior to roasting, cocoa beans have bitter, acidic, astringent, and nutty flavors. Roasting further diminishes acidity reducing concentrations of volatile acids such as acetic acid (Beckett, 2000; Ramli et al., 2006; Granvogl et al., 2006) but not non-volatiles such as oxalic, citric, tartaric, succinic and lactic acids (Jinap et al., 1998; Awua, 2002). The degree of cocoa roast shows a time/temperature dependent relationship, over periods of 5 to 120 min and in the range 120 to 150°C. Low temperature roasts are employed for milk and certain dark chocolates. An alternative practice is nib roasting where whole beans are pre-heated, at just below 100°C, to loosen the shells which are then removed. The thermal operations to loosen the shell include hot air shock, steam, or infra-red heating (Kim and Keeney, 1984; Kealey et al., 2001; Awua, 2002). The nibs are then treated (e.g., alkalized) and roasted.

Maillard reactions, central to cocoa flavor development, are important in roasting, and free amino acids, peptides, and reducing sugars all participate (Rohan and Stewart, 1967). Voigt et al. (1993, 1994a) noted the hydrophobic amino acids leucine, alanine, phenylalanine, and tyrosine, released by proteinase activities in fermentation, are important contributors (Mohr et al., 1976,Voigt et al., 1993, 1994a), as are reducing sugars fructose and glucose derived from sucrose hydrolysis (Lopez et al., 1978).

Maillard reactions (Fig. 1) require heating at pH values above 3, in the presence of water, a reducing sugar such as glucose, and

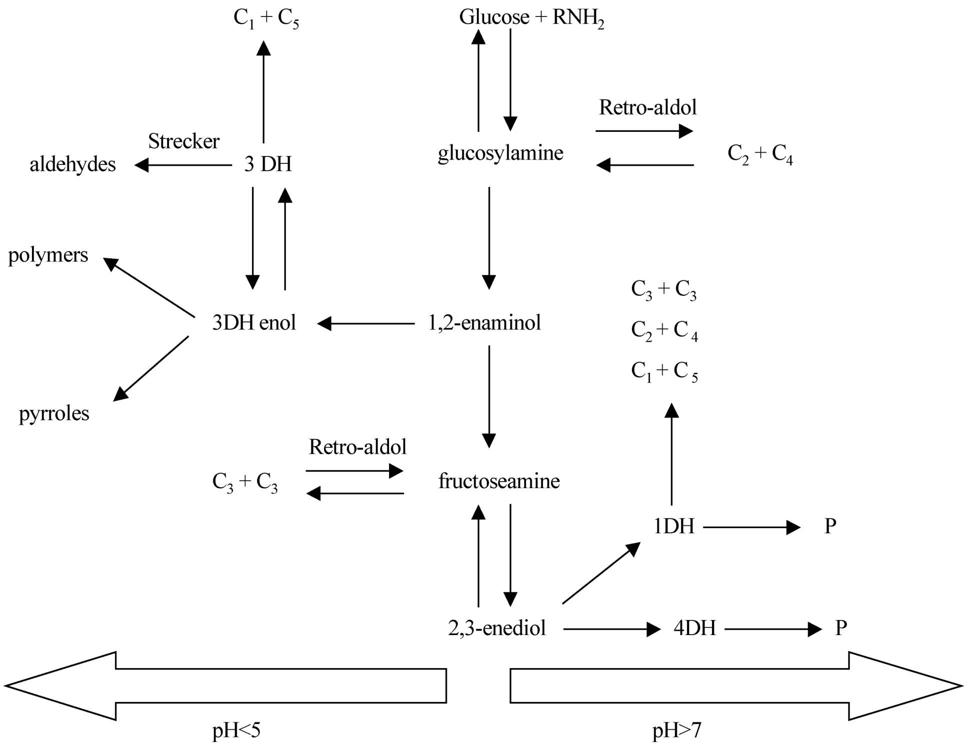

Figure 1 Model of the Maillard reaction.

an amino group, generally from protein. Reactions to the left of Fig. 1 yield flavors, to the right color formation. The 1-DH, 3-DH and 4-DH intermediates are 1-, 3- and 4-deoxyhexosuloses respectively, all dicarbonyl compounds. Initial amine-assisted degradation of a reducing sugar proceeds by a sugar-amine condensation forming a Schiff base (Fig. 2), tautomerizing to a 1,2-enaminol (Fig. 3). The link between glucose C 1 and fructose C 2 in sucrose prevents ring opening and Schiff base formation blocking participation in Maillard reactions. Reaction intermediates can act as catalysts or inhibitors for other reactions contributing to flavor (Beckett, 2000; Ramli et al., 2006; Granvogl et al., 2006).

Reducing sugars and amino acids form additional compounds, such as glucosylamines or fructosylamines with rearrangement of glucosylamines into isomerization products. At this point, the reaction pH influences the intermediates formed: acidic conditions favor 3-deoxyhexuloses (3-DH); basic or neutral pH favor formation of dehydroreductone intermediates (1-DH). Central to flavor formation are intermediates that have lost amino groups (1-DH compounds), the nature of the amine does

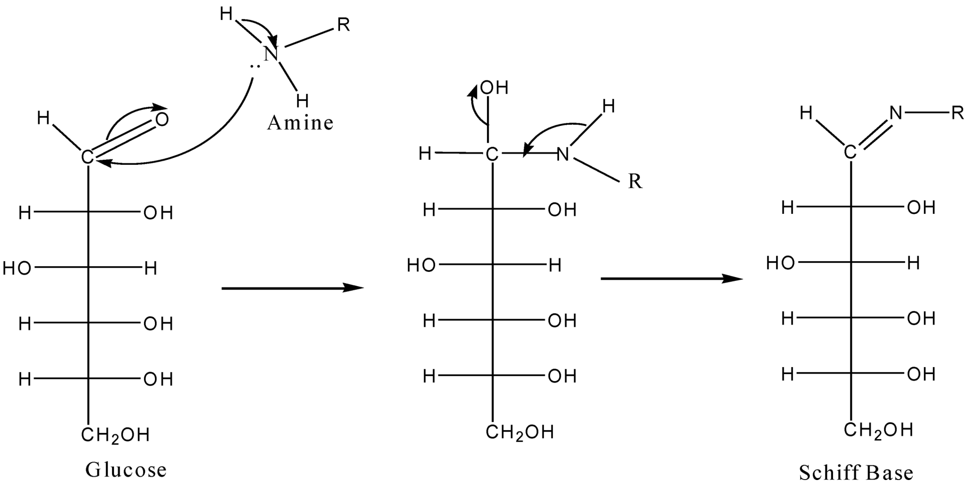

Figure 2 Mechanism of sugar-amine condensation to form a Schiff Base.

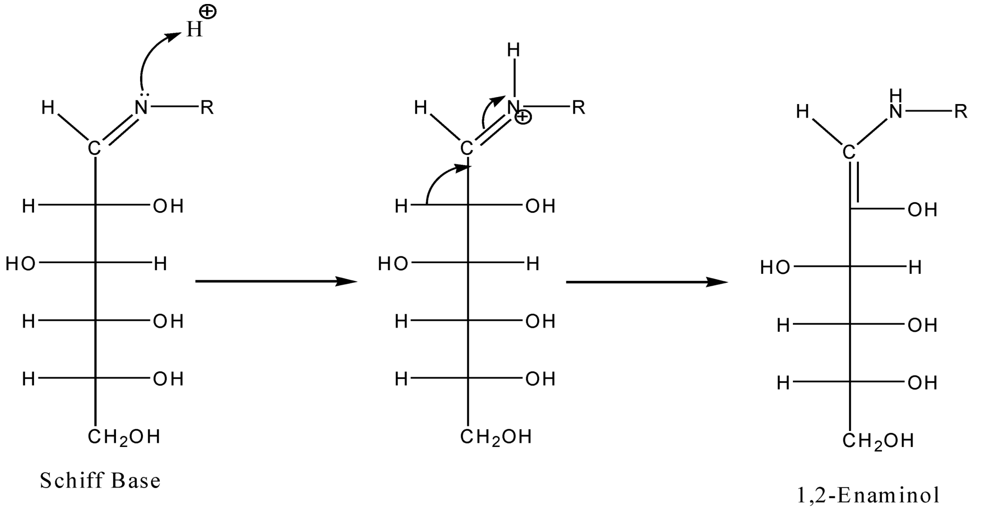

Figure 3 The mechanism for the formation of the 1,2-enaminol from the Schiff Base.

not influence the ultimate aroma character but may be important for the overall reaction rate (Williams, 2000; Stark et al., 2006a; Granvogl et al., 2006). Transformed compounds are not detectable by color or flavor changes that may be reversible at this stage, but isomerized products are key substrates for subsequent reactions.

The 1-DH compounds are dehydrated, fragmented, and transaminated yielding smaller dicarbonyl molecules, or contributing to Strecker degradation reactions, depending on the temperature and the pH (Dimick and Hoskin, 1999; Williams, 2000; Ramli et al., 2006; Granvogl et al., 2006). Strecker degradation reactions, central to the appropriate flavors for chocolate, involve interactions of numerous compounds, leading to the structure derived from amino acids being split into three parts (Fig. 4).

The nature of the amine component is crucial to chocolate flavor formation as not only are these aldehydes themselves flavor-active but further reactions yield heterocyclic compounds important to final character. Leucine and glucose yield aroma notes described as "sweet chocolate," threonine and glutamine and glucose give "chocolate" notes when heated to 100°C and valine and glucose, heated to 180°C a note described as "penetrating chocolate" (Dimick and Hoskin, 1999). Such aroma notes indicate reactions have proceeded past the initial stage. Strecker degradation reactions and subsequent formation of a model pyrazine are summarized in Figs. 5 and 6.

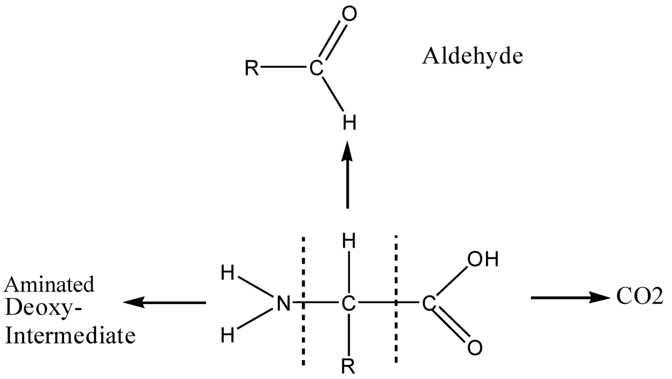

Figure 4 The formation of amino acid specific aldehydes through the Strecker degradation reaction.

In an acidic environment, generally hydroxymethylfurfurals and other furfural products are formed and at neutral pH, the results of the reaction are reductones. The intermediates are complex and little is known about their structure and the exact nature of their formation in food systems. However, the population of intermediate compounds, quantitatively individually dependent on reaction substrate and pH, polymerizes and determines the final chocolate flavor. Important compounds include pyrazines, pyrroles, pyridines, imidazoles, thiazoles, and oxazoles (Dimick and Hoskin, 1999; Counet et al., 2002; Ramli et al., 2006; Granvogl et al., 2006).

Mailard Reactions—Aldol Condensation, Polymerization, and Cyclization

These final stages of Maillard reactions are probably least understood but it is generally accepted that aldol condensation and cyclization lead to the formation of heterocyclic aroma volatiles such as pyrazines (Fig. 6) whilst polymerization produces melanoidin pigments. Dimick and Hoskin (1999) concluded that the specific pyrazine structure is dictated by side groups on dioxo compounds. Pyruvaldehyde and valine, for example, yield end products of 2-methyl-propanal and 2,5-dimethyl pyrazine, contributing nutty flavors. Precursors make chocolates rich in pyrazines, with at least 80 contributing significantly to overall flavor (Counet et al., 2002; Stark and Hofmann, 2005), but the total concentrations in roasted beans vary: Ghanaian cocoas typically have 698 μg per 100 g; Mexican beans as low as 142 μg (Reineccius, 2006). Nitrogenous component contents are a source of flavor differentiations.

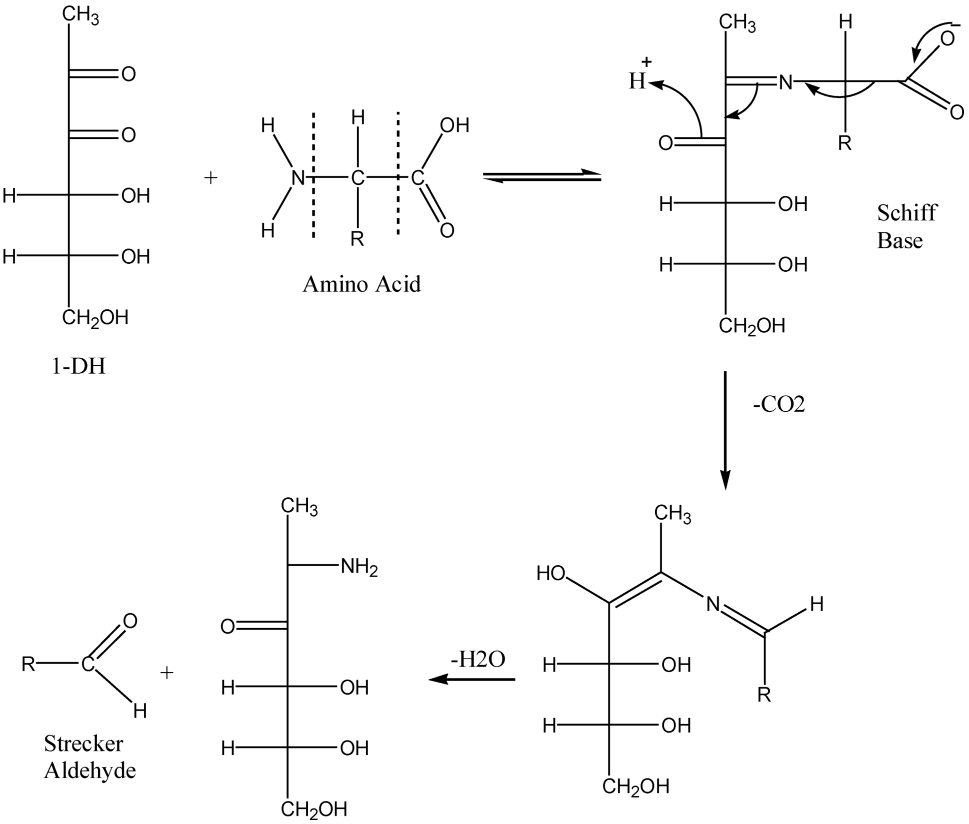

Figure 5 The mechanism of a Strecker degradation reaction.

Simple degradation products of amino acids in cocoa products are summarized in Table 4. However, there are >500 compounds identified from volatile and non-volatile chocolate fractions—hydrocarbons, alcohols, aldehydes, ketones, esters, amines, oxazoles, sulfur compounds (Heinzler and Eichner, 1991; Dimick and Hoskin, 1999; Taylor, 2002; Taylor and Roberts, 2004; Reineccius, 2006; Stark et al., 2006b). Aldehydes from amino acids play important roles in chocolate flavor balance. Aldehydes from Strecker degradation of amino acids, also produces pyrazines. The amino acid structure dictates the resulting aldehyde and also the amine and acid that can be produced from the amino acid degradation (Table 4).

Table 4 Degradation products of amino acids found in cocoa products.

	Degradation products		
Amino acids	Amine	Aldehyde	Acid
Alanine	Ethylamine	Acetaldehyde	Acetic acid
Glycine	Methylamine		Formic acid
Valine	Isobutylamine	2-Methylpropanal	2-Methylpropanoic acid
Leucine	Isoamylamine	3-Methylbutanal	3-Methylbutanoic acid
Isoleucine		2-Methylbutanal	2-Methylbutanoic acid
Threonine			2-Hydroxypropanoic acid
Phenylalanine	2-Phenethylamine	2-Phenylacetaldehyde	2-Phenylacetic acid
Tyrosine			2-(4-Hydroxyphenol)
Methionine		Methional	Acetic acid

Source: Dimick and Hoskin (1999).

Effects of Alkalization

Alkalization (treatment of cocoa nibs or liquor with solutions of alkali) is carried out primarily to change color but also influences the flavor of cocoa powder. Alkalizing is common for cocoa products such as drinks to enhance solubility or in baking or coatings (Minifie, 1989; Awua, 2002; Whitefield, 2005). Dimick and Hoskin (1999) suggested that cocoa nibs from Malaysia and Brazil are characterized by high acidity and low chocolate flavor, limiting possible character developments in processing and Sharif (1997) showed improvements in the quality of cocoa nibs and liquors from these origins could be achieved by alkali treatments reducing acidity before nib roasting or thin film processing. Sharif (1997) noted alkalizing Malaysian cocoa nibs to pH 6.0 did not significantly ($p \leq 0.05$) change flavor relative to a control but chocolates from nibs alkalized to pH of 7.2 and 8.1 were significantly different and dark chocolate prepared from Ivory Coast, Malaysian, and Brazilian cocoa had sour, bitter, and mouldy notes significantly changed by alkali treatment. The conclusion was that chocolates from alkalized and thin-film processed cocoa liquor had better flavors than non-alkalized nib-roasted chocolate (Sharif, 1997). Alkalization reduces acidity as well as astringency with aspects like typical cocoa and bouquet

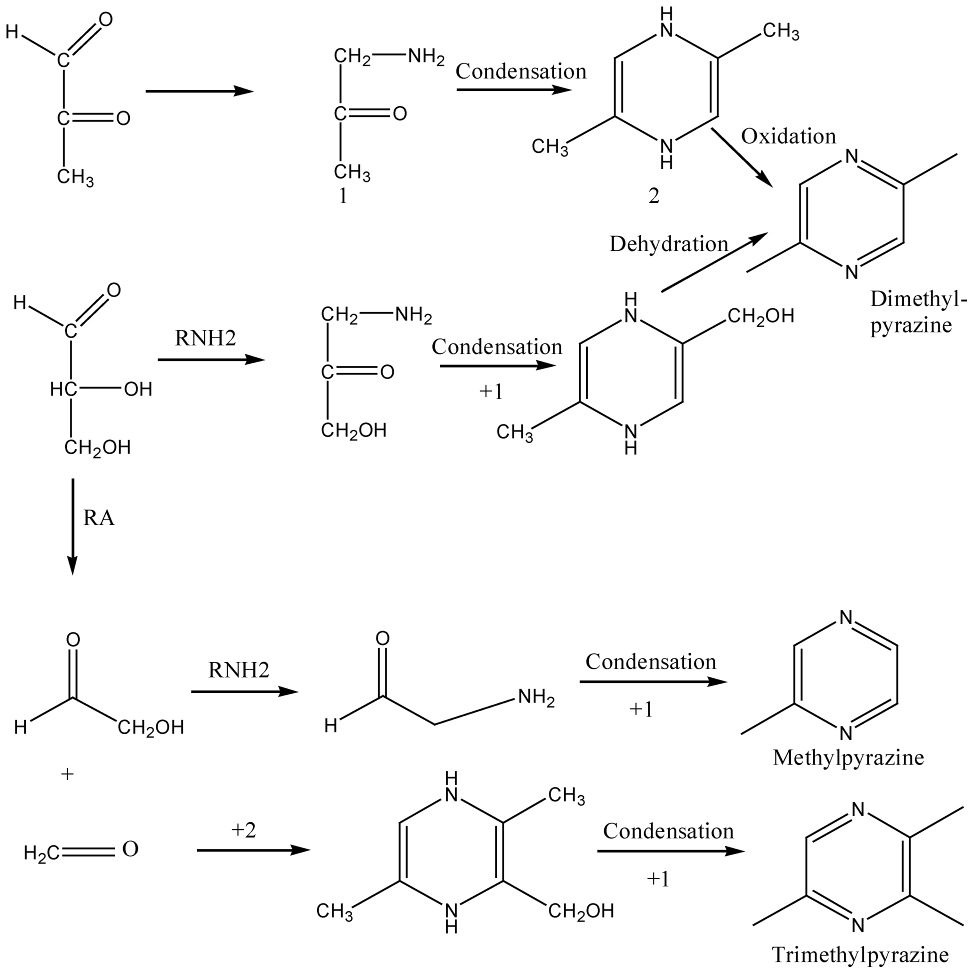

Figure 6 The formation of pyrazines through the reaction of deoxy intermediates with amino acids.

enhanced and intensified. Reductions in astringency are effected by further polymerizations of flavonoids during alkali treatments (ADM Cocoa Manual, 2006).

FLAVOR DEVELOPMENT DURING CHOCOLATE MANUFACTURE

Conching

Conching is regarded as essential for final flavor development and appropriate texture. This is the final stage in chocolate manufacture, whether dark or milk. Residual volatile acids and moisture are removed, angular sugar crystals and viscosity are modified, and the color changed due to emulsification and oxidation of tannins (Awua, 2002; Beckett, 2003; Reineccius, 2006; Afoakwa et al., 2007). Generally a two-stage process, the first stage converts flake or powder into a paste by mechanical or heat energy, driving off moisture and undesirable volatiles, effect oxidations, and distribute lipids through a continuous fat phase. Beckett (2000) suggested oxidations modify precursors developed in fermentation and roasting processes to achieve the final cooked flavor and eliminates undesirable astringent and acidic notes. The second stage converts the thick paste into a free flowing liquid through the addition of cocoa butter and lecithin.

Conching conditions show interactions between time and temperature so that higher temperatures reduce the processing time. Conching conditions for crumb milk chocolate are 10–16 h at 49–52°C but 16–24 h at 60°C for milk powder chocolates; temperatures above 70°C lead to changes in cooked flavors (Beckett, 2000; Awua, 2002; Beckett, 2003; Whitefield, 2005). Dark chocolates are typically conched at higher temperatures, 70°C or up to 82°C (Minifie, 1989; Awua, 2002). Conditions may be modified (generally shortened) by pre-treatment of chocolate liquors as thin films at temperatures >100°C (Minifie, 1989; Afoakwa et al., 2007).

The air spaces surrounding a conche in operation have an odor of acetic acid, suggesting an initial loss of short-chained volatile fatty acids, such as acetic acid, the end products of fermentation. This was confirmed by quantitative studies (Dimick and Hoskin, 1999; Beckett, 2000). Volatile phenols show 80% reductions in headspace concentrations within a few hours of conching (Beckett, 2000). Hoskin and Dimick (1984) reported phenols decreased from 21.3 μg/100 g to 10.9 μg/100 g after 44 h in

Table 5 Flavor compounds identified in milk chocolates

No.	Compound	RI		Odor description
		FFAP	SE-54	
A.	Neutral/Basic Fractions			
1	3-methylbutanal[a,p]	920	651	malty
2	2,3-butandione (diacetyl)[b,p]	984	592	buttery
3	hexanal[c,p]	1083	801	green
4	1-hexen-3-one[p]	1101	775	linseed oil-like
5	unknown[p]	1195	—	gerarium-like
6	(Z)-4-heptenal[p]	1246	899	sweet, biscuit-like
7	5-methyl-(E)-2-hepten-4-one[p]	1287	972	hazelnut-like
8	1-octen-3-one[p]	1304	980	mushroom-like
9	dimethyl trisulfide[d,p]	1384	968	sulfurous
10	nonanal[c,p]	1400	1093	soapy
11	trimethylpyrazine[ep]	1406	1000	earthy
12	unknown[p]	1422	—	fruity, waxy
13	2-methoxy-3-isopropylpyrazine[p]	1428	1097	earthy, beany
14	(E)-2-octenal[p]	1433	1060	fatty, waxy
16	2-ethyl-3,6-dimethylpyrazine[f,p]	1445	1079	nutty, earthy
17	unknown[p]	1454	—	tallowy
18	2-ethyl-3,5-dimethylpyrazine[e,p]	1461	1083	potato chip-like
20	2,3-diethyl-5-methylpyrazine[g,p]	1490	1158	potato chip-like
21	(Z)-2-nonenal[p]	1513	1148	green, fatty
23	(E)-2-nonenal[p]	1528	1161	green, fatty
24	(E,Z)-2,6-nonadienal[p]	1579	1154	cucumber-like
25	(Z)-2-decenal[f,p]	1601	1250	tallowy
27	(E)-2-decenal[p]	1647	1262	fatty, green
28	phenylacetaldehyde[e,p]	1652	1047	sweet, honey-like
30	2-methyl-3-(methyldithio)furan[p]	1667	1170	cooked meat-like
31	(E,E)-2,4-nonadienal[p]	1703	1215	fatty, waxy
33	ethyl phenylacetate[p]	1724	—	sweet, waxy
34	(E,E)-2,4-decadienal[p]	1812	1318	fatty, waxy
35	phenylethyl acetate[h,p]	1821	1244	fruity, sweet
37	2-phenylethanol[e,p]	1915	1118	sweet, yeast-like
40	R-δ-octenolactone (99%)[p]	2009	1261	sweet, coconut-like
42	R-γ-nonalactone (80%)[i,p]	2038	1663	sweet, coconut-like
43	ethyl cinnamate[i,p]	2125	1469	sweet, cinnamon-like
44	$\gamma-$decalactone[p]	2155	1470	sweet, peach-like
46	R-δ-decalactone (84%)[j,p]	2208	1469	sweet, peach-like
47	R-δ-decenolactone (99%)[p]	2241	1477	sweet, peach-like
49	3-methylindol (skatole)[p]	2494	1388	mothball-like
B.	Acidic Fractions			
15	acetic acid[k,p]	1439	600	sour
19	unknown[p]	1478	—	waxy
22	unknown[p]	1522	—	green
26	butanoic acid[k,p]	1610	821	buttery, rancid
29	2- and 3-methylbutanoic acid[l,p]	1652	873	sweaty
32	pentanoic acid[l,p]	1721	911	sweaty, pungent
36	hexanoic acid[l,p]	1829	1019	sweaty, pungent
38	unknown[p]	1936	—	sour
39	3-hydroxy-2-methylpyran-4-one (maltol)[l,p]	1961	1111	caramel-like
41	4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)[m,p]	2022	1070	caramel-like
45	3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)[p]	2182	1110	seasoning-like, spicy
48	3-hydroxy-5-ethyl-4-methyl-2-(5H)-furanone (abhexon)[p]	2250	1198	seasoning-like, spicy
50	phenylacetic acid[n,p]	2254	1262	sweet, flowery
51	3-methoxy-4-hydroxybenzaldehyde (vanillin)[o,p]	2577	1406	vanilla-like

Compound identified in milk chocolates: [a]Bailey et al. (1962); [b]Mohr (1958); [c]Rohan (1969); [d]van Praag et al. (1968); [e]Marion et al. (1967); [f]Rizzi (1967); [g]Vitzthum et al., (1975); [h]Dietrich et al. (1964); [i]Flament et al. (1967); [j]Ziegleder and Stojacic (1988); [k]Bainbridge and Davies (1912); [l]Dietrich et al. (1964); [m]Ziegleder (1991); [n]Quesnel et al. (1963); [o]Ziegleder and Stojacic (1988). [p]Schnermann and Schieberle (1997).

Table 6 Flavor compounds identified in dark chocolates

No.	Compound	RI[a]	Odor description
A.	Neutral/Basic Fractions		
	Alcohol		
1	1-pentanol[g]	757	
2	2-heptanol[g]	879	
3	benzyl alcohol[f,g]	1010	
4	3,7-dimethyl-1,6-octadien-3-ol (linalool)[b,e,g]	1086	flowery
5	2-phenylethanol[c,d,e,f,g]	1090	
	Aldehydes		
6	2-methylpropanal (isobutanal)[e,g]	566	chocolate
7	3-methylbutanal[b,c,d,e,g]	633	chocolate
8	2-methylbutanal[e,g]	643	chocolate
9	2-methyl-2-butenal[b,g]	764	
10	3-(methylthio)propionaldehyde (methional)	866	potato
11	Heptanal[e,g]	877	
12	Benzaldehyde[b,e,f,g]	935	
13	Phenylacetaldehyde[c,d,e,f,g]	1015	flowery, honey
14	Nonanal[b,d,e,g]	1082	
15	2-phenyl-2-butenal[f,g]	1242	cocoa, sweet, roasted, rum
16	2-phenyl-5-methyl-2-hexenal[b,f,g]	1485	
	Esters		
17	ethylbenzoylformate[g]	1039	
18	ethylbenzoate[b,f,g]	1156	
19	ethyloctanoate[e,g]		
20	2-phenylethylacetate[b,c,d,g]	1233	
	Furans		
21	dihydro-2-methyl-3(2*H*)-furanone[f,g]	781	
22	furancarboxaldehyde (furfural)[b,e,f,g]	805	
23	furfuryl alcohol (furfurol)[b,e,f,g]	827	
24	1-(2-furanyl)ethanone (acetylfuran)[b,e,f,g]	884	
25	5-methyl-2-furancarboxaldehyde[b,e,f,g]	931	
26	5-ethenyltetrahydro-R,R,5-trimethyl-*cis*-2-furanmethanol (linalool oxide)[g]	1076	
27	3-phenylfuran[g]	1208	cocoa, green, mint
	Hydrocarbons		
28	methylbenzene (toluene)[g]	767	
	Ketones		
29	2,3-butanedione (diacetyl)[c,d,e,f,g]	578	buttery
30	2-heptanone[e,f,g]	868	
	Nitrogen Compounds		
31	Benzonitrile[b,f,g]	951	
	Pyrans		
32	3,4-dihydro-8-hydroxy-3-methyl-1*H*-2-benzopyran-1-one[g]	1517	
	Pyrazines		
33	pyrazine[g]	731	hazelnut, green
34	methylpyrazine[b,g]	803	
35	2,5-dimethylpyrazine[b,f,g]	889	green, ether, rum
36	Ethylpyrazine[b,g]	895	hazelnut, roasted
37	2,3-dimethylpyrazine[b,g]	899	
38	ethenylpyrazine	907	
39	2-ethyl-5(or 6)-methylpyrazine[b,f,g]	973	cocoa, roasted, green
40	Trimethylpyrazine[b,c,d,f,g]	980	
41	2-ethyl-3-methylpyrazine[b,g]	983	hazelnut, roasted
42	2-ethenyl-6-methylpyrazine[g]	992	roasted, smoky, praline, rum
43	3(or 2),5-dimethyl-2(or 3)-ethylpyrazine[c,d,f,g]	1057	
44	tetramethylpyrazine[b,f,g]	1065	milk coffee, mocha, roasted, green
45	2-isopropyl-3-methoxypyrazine[c,d,g]	1081	garden peas, green, hazelnut
46	2,3-diethyl-5-methylpyrazine[c,d,g]	1135	
47	3,5(or 6)-diethyl-2-methylpyrazine[f,g]	1137	cocoa, chocolate, rum, sweet, roasted
48	3,5(or 6)-diethyl-2-methylpyrazine[f,g]	1139	
49	2,5(or 6)-dimethyl-3-(2-methylpropyl)pyrazine[g]	1184	hazelnut
50	2,5-dimethyl-3-(3-methylbutyl)pyrazine[g]	1296	roasted, sweet, green

(Continued on next page)

Table 6 Flavor compounds identified in dark chocolates *(Continued)*

No.	Compound	RI[a]	Odor description
	Pyridines		
51	pyridine[g]	724	
52	2-methylpyridine[g]	800	
53	1-(2-pyridinyl)-1-propanone[g]	1114	
	Pyrroles		
54	2-carboxaldehyde-1*H*-pyrrole[g]	986	
55	1-(1*H*-pyrrol-2-yl)ethanone (acetylpyrrole)[b,f,g]	1030	cocoa, chocolate, hazelnut, roasted
56	3-ethyl-2,5-dimethyl-1*H*-pyrrole[g]	1119	cocoa, hazelnut, coffee, roasted
57	1-(2-furanylmethyl)-1*H*-pyrrole (furfurylpyrrole)[g]	1166	roasted, chocolate, green
58	1*H*-indole[g]	1276	
	Sulphur Compounds		
59	dimethyl disulphide[g]	743	
60	dimethyl trisulphide[c,d,g]	969	onion, cabbage, sweaty
B.	ACIDIC FRACTIONS		
	Alcohols		
61	2,4-hexadien-1-ol[g]	831	
	Aldehydes		
7	3-methylbutanal[b,c,d,g]	633	chocolate (low intensity)
8	2-methylbutanal[g]	643	chocolate (low intensity)
	Furans		
23	furfuryl alcohol (furfurol)[b,e,f,g]	827	
62	2,5-dimethyl-4-hydroxy-3(2*H*)furanone (Furaneol)[c,d,g]	1023	caramel-like, sweet
	Hydrocarbons		
28	methylbenzene (toluene)[g]	772	
	Ketones		
29	2,3-butanedione (diacetyl)[c,d,e,f,g]	578	buttery (low intensity)
63	4-methylcyclohexanone[g]	998	
64	3,4,4-trimethyl-2-cyclopenten-1-one[g]	1064	
	Phenols		
65	phenol[f,g]	961	
66	4-methylphenol[g]	1031	
67	2-methoxyphenol (guaiacol)[g]	1063	smoked, sweet (low intensity)
68	4-hydroxy-3-methoxybenzaldehyde (vanillin)[c,d,e,f,g]	1366	vanilla-like
	Pyrazines		
51	2,5-dimethyl-3-(3-methylbutyl)pyrazine[g]	1289	
	Pyridines		
69	2-pyridinamine[g]	803	
	Pyrones		
70	3-hydroxy-2-methyl-4-pyrone (maltol)[d,g]	1086	
	Pyrroles		
71	2,3-dimethyl-1*H*-pyrrole[g]	804	
	Thiazoles		
72	4,5-dihydro-2-methylthiazole[g]	1151	

[a] Compound identified by GC-MS (MS) and/or by retention index on CP-Sil5-CB (RI) and/or by GC-olfactometry (GCO). Sources: [b] Maniere and Dimick (1979); [c] Schieberle and Pfnuer (1999); [d] Schnermann and Schieberle (1997); [e] Ghizzoni et al.(1995); [f] Ziegleder and Stojavic (1988); [g] Counet et al. (2002).

low roast chocolate, and 10.3 μg/100 g to 6.0 μg/100 g after 24 h in high roast in conching. In a later paper, Dimick and Hoskin (1999) concluded that polyphenols, through oxidation and enzymatic mechanisms, form complexes with amino acids, peptides, and proteins. The outcome is the withdrawal of flavor-active volatiles from headspaces and reductions in perceptions of astringency through irreversible phenol interactions, and more "mellow" final flavors.

Hoskin and Dimick (1983) suggested that in conching of dark chocolate, amino acid concentrations do not fall as temperature and/or the concentrations of amino acids and sugars are below thermal thresholds for Maillard reactions. Heinzler and Eichner (1991), however, reported that Amadori compounds formed in drying and roasting decrease during conching and Pontillon (1995) proposed caramelizations of lactose and Maillard reactions with milk proteins (in milk chocolate). A consensus is that chocolates show marked decreases in overall off-flavors after conching (Pontillon, 1995; Hoskin and Dimick, 1983; Plumas et al., 1996; Beckett, 2003; Counet et al., 2002).

Counet and his coworkers (Counet et al., 2002) concluded key dark chocolate odorants were present prior to conching, during which Strecker aldehydes were partially lost through evaporation and/or chemical reactions. On the other hand, 2-phenyl-5-methyl-2-hexenal content was increased through aldol condensation of phenylacetaldehyde and 3-methylbutanal followed by dehydration (Counet et al., 2002). Schnermann and Schieberle

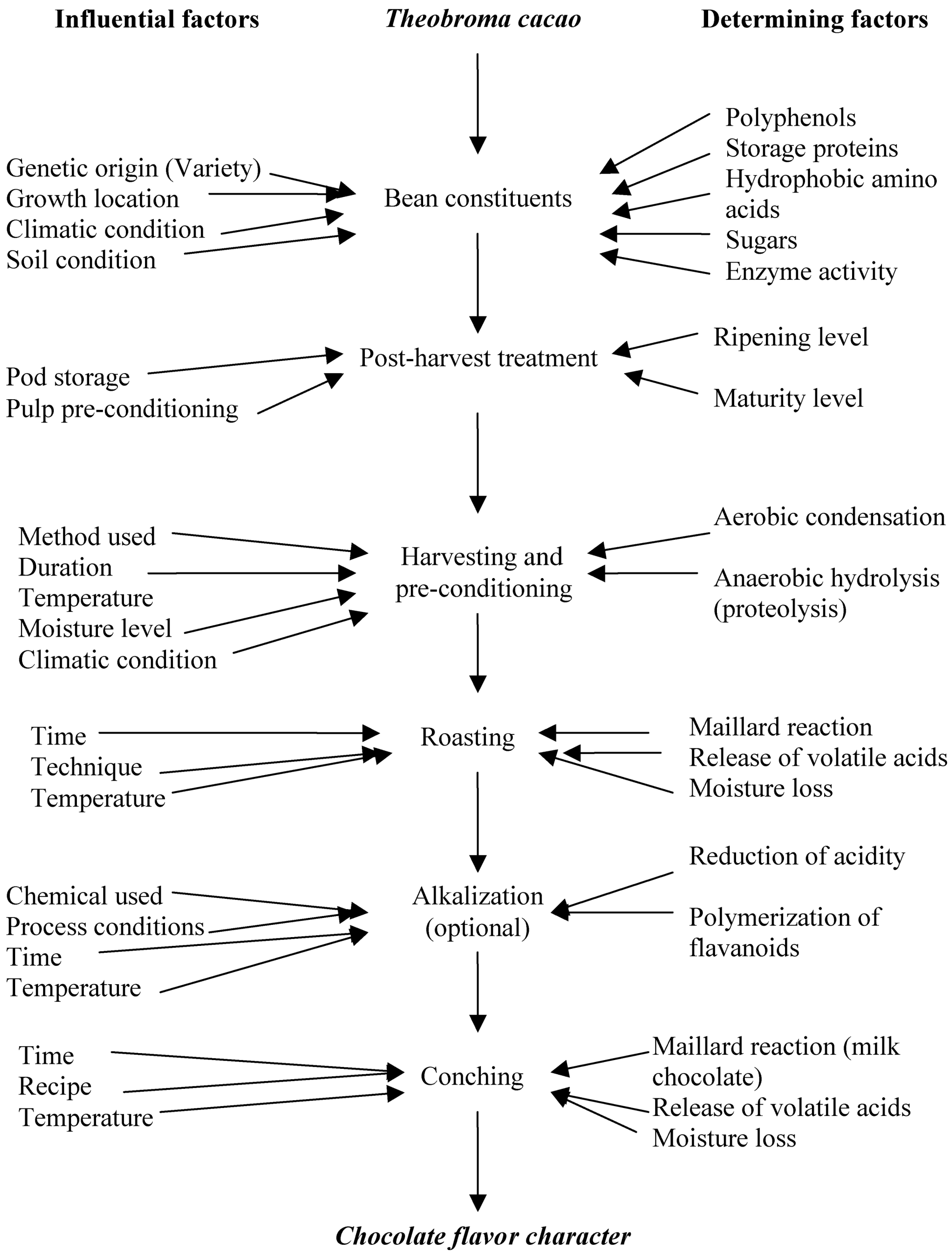

Figure 7 Mechanism of chocolate flavour formation and development process

(1997) noted furaneol and maltol (Table 5) were also generated during conching. Of heterocycles, only concentrations of the least volatile compounds were increased, notably polysubstituted ethyl-, isobutyl-, and isopentylpyrazines, tri- or tetramethylpyrazine, furans, and acetylpyrrole (Table 5).

KEY FLAVOR COMPOUNDS IN MILK CHOCOLATE

Analytical studies have identified >600 volatile compounds in cocoa and chocolate products (Schieberle and Pfnuer, 1999; Taylor, 2002; Taylor and Roberts, 2004; Reineccius, 2006), primarily pyrazines, esters, amines and amides, acids, and hydrocarbons. Schnermann and Schieberle (1997) identified as key neutral/basic flavor-active components of milk chocolate: 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine, 1-octen-3-one, 2-ethyl-3,6- dimethyl pyrazine, 2,3-diethyl-5-methylpyrazine, (*Z*)-2-nonenal, 2-methyl-3-(methyldithio)furan, (*E*,*E*)-2,4-nononadienal, (*E*,*E*)-2,4-decadienal, and *R*-δ-decalactone (Table 5). In acidic volatiles, 14 components were identified as contributing to flavor (Table 5) with vanillin (vanilla), added also in manufacture, followed by 2- and 3-methylbutanoic acid (buttery, rancid) and sotolon (fenugreek/maple syrup/caramel) showing the highest odor intensity values.

Although 1-octen-3-one and (E,E)-2,4-decadienal have been reported as primary odorants of milk products (Widder et al., 1991; Schieberle et al., 1993), these and, in addition, dimethyl trisulphide and 4-hydroxy-2,5-dimethyl-3(2H)-furanone may also be generated in conching although this is experimentally unproven. In essence the key flavor components of milk chocolate appear to primarily originate in the roasted cocoa mass.

KEY FLAVOR COMPOUNDS IN DARK CHOCOLATE

In a more recent analytical study of dark chocolate (Counet et al., 2002), a similar aroma extract dilution analysis (AEDA) approach to that of Schieberle and his colleagues was used to identify key flavor-active components and effects of conching on flavor. Of 60 compounds, nitrogen and oxygen heterocycles, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles, and sulfides (Table 6), 10 had not previously been identified as chocolate constituents: 1-pentanol (1), 3-(methylthiol)-propionaldehyde, methylbenzene, pyrazine, ethenylpyrazine, pyridine, 2-methylpyridine, 1-(2-furanylmethyl)-1H-pyrrole, 1H-indole, and dimethyl disulfide (Table 6). Two others, benzyl alcohol and dihydro-2-methyl-3(2H)-furanone, had only been reported in milk chocolates. Specific nitrogen heterocycles, from Maillard reactions, were concluded as important: 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine, 3,5-(or 6)-diethyl-2-methylpyrazine, acetylpyrrole, and furfurylpyrrole (Table 2) all with praline and chocolate notes. The ethyl group in two pyrazine compounds suggests key roles for alanine and/or its Strecker aldehyde, acetaldehyde, in chocolate flavor synthesis (Cerny and Fay, 1995; Cerny and Grosch, 1994). Four other heterocycles; 2,3-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2-isopropyl-3-methoxypyrazine were identified (Table 6). Tetramethylpyrazine, the most abundant pyrazine in dark chocolate at >6 ppm, exhibited milk coffee-mocha-roasted notes.

Of 33 particularly flavor-active components in the neutral/basic fraction (Counet, et al., 2002) three had specifically strong chocolate characters: 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal. Others were characterized by Maillard cocoa/praline/nutty/coffee notes: 2,3-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine, 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine, 3,5(or 6)-diethyl-2-methylpyrazine, and furfurylpyrrole. Character in the acidic fraction—phenolic, sweet—was very different from that of the neutral/basic with its essentially chocolate flavor. Only 6 of the 18 components (resolved by HRGC with FID/MS) were flavor-active, and one, vanillin, was added prior to conching. Furaneol was perceived as sweet and caramel in extracts from both dark chocolates (Counet et al., 2002).

CONCLUSION AND FURTHER STUDIES

Chocolate flavor resides in not only a volatile aromatic fraction of flavor-active components but also in non-volatile compounds influencing taste perception. Its complex composition depends on the cocoa bean genotype specifically on contents of bean storage proteins, polysaccharides, and polyphenols. The inheritance and regulation of such flavor origins remains an area for advanced research. Following, critical review of the entire process, a summary of the parameters important for chocolate flavor generation has been developed (Fig. 7). An appropriate starting composition can be converted through controlled post-harvest treatments and subsequent processing technologies to a high quality flavor character. Cocoa bean fermentation is crucial not only to the formation of key volatile fractions (alcohols, esters, and fatty acids) but also the provision of flavor precursors (amino acids and reducing sugars) for important notes contributing to chocolate characters. Drying reduces the levels of acidity and astringency in cocoa nibs by decreasing the volatile acids and total polyphenols. Maillard reactions in roasting convert flavor precursors formed during fermentation into two main classes of flavor-active component: pyrazines and aldehydes. Although no new key odorants are synthesized during conching, the levels of 2-phenyl-5-methyl-2-hexenal, furaneol, and branched pyrazines significantly increase and form key odorants in both milk and dark chocolates, while Strecker aldehydes are lost by evaporation. These processes suggest an important role of conching in chocolate manufacture in determining the final flavor characters. Direct relationships are thus observed between the initial composition and post-harvest treatments (fermentation and drying) of cocoa beans and subsequent processing (roasting and conching) and technological effects on the flavor formation, development, and character in chocolate.

However, a comparison of flavor characters in chocolate is complicated by variations caused by different genotypes, geographical origin, pod differences, fermentation and drying methods, and subsequent processing (roasting, alkalization, and conching). Although this review suggests major causes of variations, it is still premature to conclude that it is fully understood. There have been few systematic studies and difficulties in comparing cocoa varieties grown under different conditions with various fermentation methods. Therefore, conclusions are difficult on how enzymatic processes are regulated, which enzyme substrates/products are related to good flavor character, and limiting factors for the enzymatic processes (enzyme activity, substrate or enzyme availability, cocoa genetic background, growth conditions, or the post-harvest treatments), and finally the process controls in chocolate manufacture. To fully understand the variations in chocolate character, further research is required to optimize the post-harvest treatments (pod storage, pulp pre-conditioning, depulping, fermentation, and drying) of cocoa beans differing in genotype, subsequent manufacturing processes (roasting, alkalization, and conching) during chocolate manufacture as well as the sensory strategy in the evaluation of final flavor character in chocolate.

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