Influence of raw material and processing on aroma in chocolate

Ph.D. thesis

Margaret Owusu

Faculty of Life Science, University of Copenhagen

October 2010

To the memories of my sister Bithia & to Mehdi, my favorite technician

Preface

This PhD thesis is intended to fulfill the requirement of a PhD degree at the Faculty of Life Sciences, University of Copenhagen, Denmark. The presented work has been carried out at the Quality & Technology, Department of Food Science and at the chocolate factory of Toms Confectionery Group A/S, Ballerup, Denmark. My main supervisor has been Associate Professor Mikael Agerlin Petersen and Dr. Hanne Heimdal as co-supervisor to whom I am most indebted.

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Summary

There is no chocolate without cocoa, the main raw material for its production. The quality of the raw material is very important to obtain good quality chocolate as some 'imperfections' in it cannot be removed from the chocolate even with processing. Ghana is an important cocoa-growing country, supplying more than 20% of the world's cocoa. In spite of Ghana's cocoa beans being a standard for the Forastero variety, variation exist in the exported beans due to differences in the geographic areas of the cocoa growing regions within Ghana, as well as fermentation method/degree. There is therefore the possibility of obtaining cocoa beans of better quality with proper separation. Aside the primary processes of fermentation/drying of cocoa, the chocolate production processes of roasting and conching are also important determinants of the aroma/flavor of the final product. These processes together determine the aroma/flavor compounds that define the aroma/flavor characteristics of chocolate.

The general objective of this PhD thesis was to obtain more knowledge about the factors determining the aroma of chocolate and specifically, to enable production of chocolate with a better quality from Ghanaian cocoa beans.

The aroma compounds in dark chocolate produced from heap-fermented and tray-fermented Ghanaian cocoa beans were isolated by an optimized Dynamic Headspace Sampling Method and identified by Gas Chromatography-Mass Spectrometry (GC-MS). Odor-active compounds in the chocolates were detected by Gas Chromatography-Olfactometry (GC-O) and identified by GC-MS. The intensities of flavor characteristics of 'heap' and 'tray' chocolates were determined by a Quantitative Descriptive Analysis (QDA) using a trained sensory panel. Differences were found between the types but mainly the levels of aroma compounds in the two chocolates. There was also observed differences between the most important aroma compounds determined by their SNIF (Surface of Nasal Impact Frequency) value for 'heap' and 'tray' chocolates. Peak areas of isoamylacetate, linalool and methyl phenylacetate were significantly different at $p<0.05$ for the two types of chocolate. Phenylacetaldehyde with a bitter/green/grassy odor and a peak described as having a chocolate/cocoa/roasted odor and identified as a non-separable mixture of 2- and 3-methylbutanal seemed important to the aroma of both chocolates, based on their SNIF values.

However, Linalool (fuity/sweet/flowery) had a SNIF value in tray chocolate which is almost twice that of heap chocolate. 'Tray' chocolate was generally perceived to have a more fresh fruit, sweet and yoghurt flavor than 'heap' chocolate. These flavor attributes were also positively correlated but negatively correlated with bitter and astringent. Observed differences in the levels of some key odorants identified probably account for observed sensory differences in 'tray' and 'heap' chocolates.

The effect of fermentation duration on the aroma of four dark chocolate produced from 2, 3, 4 and 4 days tray-fermented Ghanaian cocoa beans was also investigated. Results showed a general increase in the number of aroma compounds with increasing fermentation duration. The 3 day-fermented sample, however, did not follow this trend as it had higher GC-MS peak areas in a number of compounds than the other three samples. More odor-active pyrazines were detected in the 5 day-fermented sample than in the other three samples. Increased days of fermentation also resulted in the reduction of undesirable attributes such as sourness, bitterness and astringency, whilst fruity, flowery and sweet flavors were related to 4 and 5 days-fermented samples. 3-day fermented samples seemed to be the worst chocolate as it was rated higher in all the undesirable attributes than even the 2-day fermented samples. Due to the unexpected results from the 3 day-fermented samples, the experiment is being repeated to confirm the results. In the meantime, however, it can be concluded that at least 4 days of tray fermentation is required to obtain chocolate which is less bitter, sour and astringent but sweet, flowery and fruity in flavor.

Additionally, the combined effect of cocoa fermentation method, as well as roasting and conching conditions on the aroma of 'heap' and 'tray' chocolates have been investigated in this thesis. A completely randomized design was used to study the two factors, roasting and conching conditions for the two fermentation methods, heap and tray. Three roasting conditions: $100^{o}C/100$ min, $120^{o}C/45$ min, $150^{o}C/30$ min and three conching durations, all at $80\ ^{o}C$: 6 h, 8 h and 10 h were used in combinations to produce 'heap' and 'tray' chocolates. Aroma volatiles in the chocolates were influenced by all three processing factors. A PCA (Principal Component Analysis) plot based on sixteen most important compounds detected by all judges in at least one sample during GC-O explained 81% of the variation in the samples with two PCs. The levels of

most important odorants, among them 2- and 3-methylbutanal (cocoa, roasted), 1,2/3-butanediol and benzyl acetate (sweet, flowery) were generally higher at high roasting temperature (150°C) but decreased significantly ($p<0.05$) with increased conching duration from 6-10 h. On the other hand, the level of 5-methyl-2-phenyl-2-hexenal generally increased drastically with short/medium conching duration but reduced drastically with further conching in both 'heap' and 'tray' chocolates indicating its formation at the short/medium durations of conching. Since the dataset was reduced to only represent the volatiles that were most important from a sensory point of view, it was expected that the heap and tray fermented samples in general will have different sensory quality and that this difference would become more pronounced with samples roasted at the higher temperatures (120 or 150 °C) and conched for rather short time (6-8 hours) or not conched at all.

To investigate this, a QDA was carried out on six selected samples. Eight of the sixteen flavor attributes used to describe and quantify the intensity of attributes in the samples were significantly different ($p<0.05$). Unroasted samples were rated higher in astringency than roasted samples whilst unconched samples were rated higher in fruity and banana attributes than conched samples.

Multivariate data analytical tools, PCA and PLS were used to investigate quantitative descriptive analysis and GC-O data and also to relate the two sets of data. PLS1 models based on single sensory attributes performed better than PLS2 models based on all sixteen sensory attributes. Ethyl-3-methylbutanoate (fruity, flowery); 2,5-dimethylpyrazine (popcorn), dihydro-2(3H)-furanone (sweet); linalool oxide (sweet, flowery); benzaldehyde (earthy, nutty) and 2/3-methylbutanal (cocoa, roasted) efficiently modeled fruitiness. It was also possible to model the taste sensory attribute astringent from the aroma compounds 5-methyl-2-phenyl-2-hexenal (sweet, roasted cocoa), ethyl-3-methylbutanoate and pentyl acetate (green, cucumber). Since fruity flavor was higher in unconched samples and astringent mouth-feel higher in unroasted samples, it may be possible to predict conching and roasting in dark chocolate from the concentrations of the above mentioned important aroma compounds.

The results of this investigation are presented in three papers submitted to international journals, three conference manuscripts (two of which have been published) and one manuscript under preparation.

List of Papers

Paper I: Owusu, M., Petersen, M. A., Heimdal, H. (2008). Assessment of chocolate produced from two Ghanaian cocoa fermentation types. In Expression of Multidisciplinary Flavour Science. I. Blank, M. Wüst, C. Yeretzian (Eds.). Proceedings of the 12th Wuerman Symposium. Interlarken, Switzerland, 363-366.

Paper II: Owusu, M., Petersen, M. A., Heimdal, H. (2010). Evaluation of aroma and sensory quality of chocolate produced from two cocoa fermentation methods. LWT-Food Science and Technology (submitted).

Paper III: Owusu, M., Petersen, M. A., Heimdal, H. (2010). Aroma of chocolate produced from tray-fermented cocoa at different stages of fermentation. In Recent Advances in Food and Flavor Chemistry – Food flavors and encapsulation, health benefits, analytical methods, and molecular biology of functional foods. C-T Ho, C. J. Mussinan, F. Shahidi & E. Tatras Contis (Eds.), RSC Publishing, pp. 221-223.

PAPER IV: Owusu, M., Petersen, M. A., Heimdal, H. (2010). Influence of roasting and conching on aroma volatiles of chocolate. Proceedings of the 9th Wartburg Symposium on Flavour Chemistry & Biology. Eisenach, Germany (Submitted).

PAPER V: Owusu, M., Petersen, M. A., Heimdal, H. (2010). Influence of cocoa fermentation duration on aroma of chocolate produced from tray-fermented cocoa beans (manuscript under preparation).

Paper VI: Owusu, M., Petersen, M. A., Heimdal, H. (2010). Effect of fermentation method, roasting and conching conditions on the aroma volatiles of dark chocolate. Journal of Food Processing and Preservation (submitted).

Paper VII: Owusu, M., Petersen, M. A., Heimdal, H. (2010). Relationship of sensory and instrumental aroma measurements of dark chocolate as influenced by fermentation method, roasting and conching conditions. Journal of Food Science and Technology (submitted).

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Paper I-VII

1.   Introduction

Chocolate is one of the most popular sweet-tasting treats and flavors in the world. Its popularity has been attributed to its distinguishing characteristics of flavor, texture and color. Inspite of the acclaimed health benefits (coming from the cocoa content) of chocolate, it is considered more of a luxury than a health food. The most outstanding characteristics of chocolate is its flavor/aroma which is dependent on the genotype/origin of the cocoa beans, the fermentation and drying method and the chocolate manufacturing processes, namely roasting and conching.

It is impossible to make chocolate without cocoa and its availability and quality is of paramount importance to the confectionery industry (Beckett, 2009). The world's second largest supply of cocoa beans come from Ghana with about 20% of the world's crop and the produce from here are considered the standard for bulk (Forastero variety) cocoa (Beckett, 2009). Forastero cocoa is a rather resistant variety with a good yield. Despite these achievements, exported Ghanaian cocoa is of 'average' quality because beans of different qualities are mixed, resulting in a constant 'standard' quality.

The variation in the Ghanaian cocoa beans, among other things, is due to geographic origin within Ghana, and to different methods and degrees of fermentation. The six cocoa growing regions within Ghana are mostly marked by climatic and soil differences. These are factors which impact on the aroma of cocoa produced (Aculey et al., 2010). Cocoa production in Ghana is by small holder farmers who use the traditional heap method in fermenting the beans. This is a method where upon breaking of the pod, the beans are piled on and covered by banana leaves. The heaps differ in size and may range from 20 to 1000 kg. Big heaps have to be turned once every 24-72 hours to achieve even fermentation but this is not adhered to by most Ghanaian farmers because it is tedious. Another fermentation method developed by the Cocoa Research Institute of Ghana (CRIG) is the Tray method which involves fermenting the beans in 10 cm deep wooden trays. Eight to ten trays are stacked on top of each other and the top-most tray is covered with banana leaves. This method allows aeration of the fermenting mass without having

to turn and ensures better and more even fermentation (Baker et al., 1994). Fermentation of cocoa is very critical to the flavor quality of chocolate produced from it since some of the off-flavors that occur at this stage cannot be corrected later even with the right manufacturing procedures It is therefore important that the raw material is treated and handled appropriately from the onset to obtain a final product of good quality.

The chocolate manufacturing processes of roasting and conching also affect its flavor because during these, aroma/flavor precursors formed during fermentation develop into aroma/flavor compounds (Ramli et al., 2006; Reineccius, 2006; Beckett, 2009) and undesirable volatiles are also removed from the chocolate. Therefore, optimizing these processes also ensures good quality of the final product.

The aroma of chocolate is very complex involving a lot of different compounds. More than 400 compounds have been isolated and identified in cocoa, chocolate and other cocoa products (Ramli et al., 2006). The use of Dynamic Headspace Sampling (DHS) coupled with Gas Chromatography Mass Spectrometry (GC-MS) presents a powerful tool for effective isolation and identification of volatile compounds in foods including chocolate. However, not all the compounds that are isolated are important contributors to the aroma of the food and Gas Chromatography-Olfactometry (GC-O) enables the identification of odor-active or sensorial important compounds that contribute to the aroma of any food.

Although the use of headspace sampling and Gas chromatography enables the isolation and identification of volatiles in a food matrix closer to what happens in inhaled or sniffed aroma than is obtained from other methods such as solvent extraction (Hachenberg and Schmidt, 1977; Charalambus, 1978), sensory evaluation is important for predicting the actual flavor/aroma of the food as perceived by a trained panel. Descriptive sensory analysis is a sensory technique that allows the sensory scientist to obtain complete sensory descriptions of products, help identify underlying ingredient and process variables, and/or to determine which sensory attributes are important to acceptance (Lawless and Heymann, 1999). The method is useful in situations where a detailed specification of the sensory attributes of a single product or a comparison among

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several products is desired (Gillette, 1984). Depending on the specific technique used, the description can be qualitative as well as quantitative. Quantitative descriptive analysis enables the treatment of data by statistical analysis.

Despite the fact that most multivariate data analytical methods are exploratory, they present a powerful tool for visualizing data. One of the most frequently used, Principal Component Analysis (PCA) has been used extensively in this investigation. PCA enables the reduction of large data such as is obtained from GC-MS analysis of chocolate. It aids in the visualization and graphical representation of relationships between samples and variables.

The overall objective of this PhD thesis was to obtain more knowledge about the factors determining the aroma of chocolate and to enable production of chocolate with a better quality from Ghanaian cocoa beans.
The investigation specifically aimed at determining the aroma compounds responsible for perceived sensory difference between chocolate produced from the predominant cocoa fermentation method, heap and the new improved tray method.

Chocolate production involves a combination of three important processing methods: cocoa fermentation method, bean roasting and chocolate conching, which determine the characteristics of the final product and also define quality. Until now, there is no report, on the combined effect of all three processes on the aroma/aroma characteristics of chocolate. Therefore, another objective of this investigation was to determine the individual and combined effects of fermentation method, as well as roasting and conching conditions on the aroma of chocolate.
Additionally, the relationship between odor-active compounds and sensory characteristics of chocolate as influenced by the above-mentioned processing factors was investigated in an attempt to predict one variable from the other using the multivariate data analytical tool, Partial Least Square (PLS) regression models to relate one variable to the other.

The results of the investigations carried out have been published in conference proceedings and/or submitted to international scientific journals.

Chapters 2 and 3 gives a general introduction to cocoa and chocolate, the methods for the primary processing of cocoa and the chocolate manufacturing processes in relation to the objectives and subject of the PhD thesis. Chapter 4 discusses the methods used in the experimental work and other existing methods used in the investigation of food and chocolate aroma/flavor. Chapter 5 presents the results of the study and discusses it in relation to existing knowledge, where appropriate (Papers I-VII). Conclusions and perspectives for future research are presented in Chapters 6-7

2. Cocoa

The main raw material for chocolate-making is the seeds of the fruit from cocoa tree, *Theobroma cacao*. The seeds are often referred to as the beans. A lot of myths surround the history of cocoa, but it is an agreeable fact that it originated from the equatorial Americas. Christopher Columbus is said to have introduced cocoa beans to Europe (Spain) on his return from his fourth voyage to the Americas. The Spaniards also took cocoa to Fernando Po, an island off the coast of West Africa.

Cocoa thrives in tropical climates 20° north and south of the equator. Being a tropical crop, it grows very well in areas with an average rainfall of 1250-3000 mm per annum and preferably between 1500-2000mm and a dry season of not more than 3 months. It requires high humidity, often 70-100% and varying soil conditions. Cocoa is more sensitive to soil moisture stress than other tropical crops but is also sensitive to water-logging. It is either cultivated by seeds or vegetatively by budding or grafting. The trees are relatively small, 12-15 m in height and are often sheltered by intercropping plants such as banana. The cocoa tree start bearing pods after two to three years, but it is not until six or seven years before they give a full yield (Cook, 1982 Beckett, 2008; Beckett, 2009).

The shape and color of the fruit (pod) varies for the different varieties but typically contains the seeds which are covered in a whitish mucilaginous pulp. The pulp is the main substrate of fermentation because it contains sugars to sustain microbial growth. (Cook, 1982; Beckett, 2008; Beckett, 2009).

2.1   Genotypes/varieties

Three major varieties of cocoa exist: Criollo, Forastero and Trinitario (Cheesman 1944; Wood and Lass 1985; Lachenaud 1997; Lachenaud et al. 1997), but man-made and natural causes have so mixed up some of the species that clarifying identities becomes of little interest to any except

2.   Cocoa

Fig. 1. Varieties of cocoa a: Criollo pods. b: Forastero pods

the academically inclined/plant breeders. Purposeful and accidental cross-breeding by man and animals has resulted in a lot of inter-mingling and genetic dilution of the major varieties. Thus it is more common to differentiate beans by their countries of origin. The varieties are recognized based on genetic origin, pod morphology and size as well as the color and flavor of the beans (Cook, 1982; Laurent, 1994; Schwan and Wheals, 2004; Sukha et al., 2008).

Forestero, also known as 'Bulk cocoa' is the main type of cocoa grown all over the world, accounting for about 80-90% of the world's production. It is high yielding, more resistant to pest and diseases and more tolerant to drought. Forastero cocoa bean has a strong inherent flavor, inclined to be somewhat bitter and usually dark brown in color. The variety originates from the Upper Amazon region and grows in several South American countries including Peru, Ecuador, Colombia, Brazil, Guyana, French Guyana and Southern Venezuela. It also found in West Africa mainly, Ivory Coast, Ghana, Nigeria and Cameroun, as well as in South-East Asia (Cook, 1982; Beckett, 2009).

Amelonado, a Forastero variety from the Lower Amazon is named after the melon shape of the pods (Wood, 1991; Beckett, 2009). Until the 1950s almost all cocoa plants in Ghana were of the West African Amelonado variety. This variety is however very susceptible to the swollen shoot

virus so several different planting materials had to be developed and are now extensively used in some of the main growing areas in Ghana and other West African countries (Thresh et al., 1988).

Criollo cocoa was originally cultivated by the Mayas of Central America and represents the first domesticated cocoa. In the sixteenth and seventeenth centuries cocoa was introduced into Asia and this was of the Criollo variety. Criollo cocoa beans have white cotyledons and a mild nutty flavor. They are susceptible to diseases and produce low yields. Criollo is now rare and only found in old plantations in Venezuela, Central America, Sri Lanka and Samoa. It also grows on the islands of the Indian Ocean such as Java, Madagascar and Comoros. The variety now accounts for only 1-5% of the world's production and is characterized by slight bitterness (but not unpleasantly so), mild astringency, flavor finesse, a pale color that gives chocolate a reddish tinge (Hurst et al., 2002; Beckett, 2009).

Trinitario accounts for 10-15% of the world's production. It originates from the island of Trinidad and is a result of hybridization between Criollo and Forastero. The crossing between the two varieties became necessary in the eighteenth century when the island's Criollo plantations were almost wiped out by an environmental disaster. Trinitario variety is now grown wherever Criollo is found and also in Cameroon (Beckett, 2009). It has characteristics which are between the parent varieties.

A variety which is only grown in Ecuador is called Nacional or Arriba and may have originated from the Amazonian area of Ecuador. It is highly aromatic but more tannic than Criollo and darker in color. Pure Nacional varieties are almost extinct. The variety has been subjected to a lot of 'genetic erosion' mainly due to successive introductions of foreign germplasm which has resulted in a decrease in quality. The varieties that have 'Arriba' flavor in Ecuador are known to be hybrids between Nacional and Trinitario (Beckett, 2009; Loor et al., 2009).

In spite of the existence of these broad classifications, the commercial cocoa bean grinder is more interested in the many sub-species or hybrids that have resulted from cross-breeding and seed selections over the years (Cook, 1982). Table.1 shows the genetic lineage of three such

hybrids developed by the Cocoa Research Institute of Ghana (CRIG). The hybrids are characterized by high yield and disease resistance (Jonfia-Essien et al., 2008).

Table 1. Genetic lineage of three cocoa hybrids developed by CRIG (Jonfia-Essien et al., 2008)

Cocoa type	Variety
Amazon/Trinitario	HV1
Inter Amazon hybrids (Amazon/Amazon)	HV2; HV3
Amazon/Amelonado hybrids	HV4

Each variety of cocoa comes with inherent characteristics which contribute to the aroma/flavor of chocolate produced from it. The individual flavor potential of a raw cocoa determines its classification as fine or flavor cocoa with a particularly characteristic, aromatic flavor or as bulk cocoa without such a specific flavor note (Elwers et al., 2009).

2.2   Origin

There are three major cocoa regions of the world: South America, West Africa and South-East Asia (Fig. 2). Cocoa from different regions/countries often has distinct flavor characteristics (Powell, 1983) as a result of different planting materials, climatic and soil conditions, agricultural and processing practices. (Jinap et al., 1995).

2.2.1   Central and South America

The history of cocoa dates back to the Mayan of South America who created a ritual beverage from the ground cocoa beans mixed with water, black pepper, vanilla and spices. Cocoa beans also served as money in South American civilizations and were only consumed as they wore out Today cocoa is grown in Mexico, Brazil, Colombia, Venezuela, Ecuador, Costa Rica, Peru, Bolivia, Jamaica, Dominican Republic, Trinidad and Tobago, among others. Although South America was the origin of the highest quality cocoa in the world, today what it furnishes is

frequently either of sub-standard quality or good quality but of undependable quantity and continuity. Attempts to revitalize the cocoa growing industry in these areas have been hampered by diseases and pests of various kinds (Cook, 1982; World Cocoa Foundation, Internet source, accessed May 10, 2010).

Mexico, from where Cortez found cocoa in 1519 used to be the hub of fine quality Criollo. However due to the fastidious nature of this variety, in Mexico as in other countries where Criollo is grown, planters prefer to replace diseased or dead Criollo with more hardy Forastero varieties and hybrids. Thus farms here are usually a heterogeneous mixture of the two varieties and those in-between. The problem resulting from this is the improper segregation of varieties before fermentation, in view of different fermentation duration requirements of the different varieties. Thus, there is the risk of over-fermentation of Criollo beans and under-fermentation of the Forastero variety. Realising this, most growers do not ferment their crop with the result that the beans do not produce a true chocolate flavor. Attempts have recently been made to segregate and properly ferment the beans and the results have been fairly good (Cook, 1982).
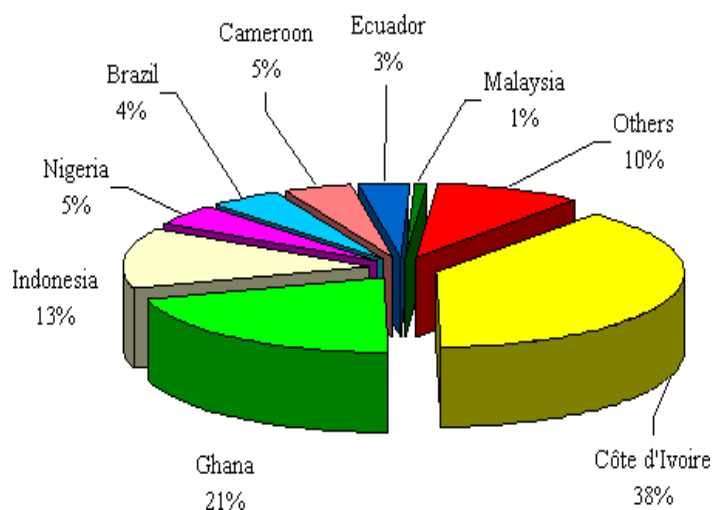
Fig. 2. Share of countries in total cocoa bean production 2005/2006 crop year forecast. (Source: UNCTAD based on data from International Cocoa Organization, quarterly bulletin of cocoa statistics.

2.   Cocoa

In the eighteenth century the variety Amelonado was introduced into Bahia in Brazil. Bahia was a major cocoa growing area in the mid-1980s, producing more than 400 000 tonnes but it now produces less than half of this mainly due to destruction by witches' broom disease.

Trinidad and Tobago is a known major producer of fine or flavor cocoa (Table 2). Cocoa from this origin, if optimally processed has a fruity, mildly floral, winey even raisny overtones with mild acidity. These attributes are well sought after and gives cocoa from here a premium world international market price. (Cook, 1982; Beckett, 2008; Sukha, 2003).

Table 2. Exporting countries of fine or flavor cocoa (ICCO Annual Report, 2007/2008).

Exporting Countries of Fine or Flavour Cocoa	Share of Fine or Flavour Cocoa of total exports Decision March 2005	Share of Fine or Flavour Cocoa of total exports Decision May 2008
Colombia	100%	100%
Costa Rica	n.a.	100%
Dominica	n.a.	n.a.
Dominican Republic	Not included	40%
Ecuador	75%	75%
Grenada	100%	100%
Indonesia	1%	1%
Jamaica	100%	100%
Madagascar	100%	100%
Papua New Guinea	25%	75%
Peru	n.a.	100%
Saint Lucia	100%	100%
São Tome and Principe	35%	35%
Trinidad and Tobago	100%	100%
Venezuela	75% or 100%	75%

Ecuador is known for its unique 'Arriba' flavor cocoa – a Forstero variety with an unusual floral aroma and flavor. Ecuador was the world's largest producer of cocoa from 1830-1840, providing 40% of the total supply. However, in 1917, a fungus disease called Monilia pod swept through part of the growing area. Further decline in production was caused by another fungal disease known as the Witches' Broom. There has been significant recovery since then but the 'Arriba'

flavor keeps declining progressively with the result that only a small percentage of Ecuadorian cocoa is Arriba at all (Cook, 1982).

2.2.2 West Africa

Cocoa was first introduced into West Africa by the Basel Missionaries who came to the then Gold Coast (now Ghana) in 1868. These trees were planted in gardens just for pleasure. It took a blacksmith named Tetteh Quarshie to bring back home cocoa seeds from Fernando Po where he had been employed as a plantation worker to disseminate the crop. This became the origin of African cocoa with the result that West Africa now supplies about 70% of the world's cocoa. Ivory Coast, presently the world's largest supplier, produces about 40% of the crop, most of which is grown on small-holdings. Recent political instabilities, however, may make future supplies less certain (Cook, 1982; Beckett, 2008).

Although Ghana was the world's largest producer in the early 1960s, by the early 1980s, the country's production had dwindled mainly due to aging trees, widespread disease, bad weather, bush fires and low producer prices. However, institution of marketing reforms by the government-established Cocobod, restructuring of cocoa production, provision of seedlings to farmers to replace lost and old trees, among others, resulted in increased production in the early 1990s. Ghana now produces about 20% of the world's supply of the crop. The country is known for the production of well-fermented and cured cocoa, and thus has a reputation for the supply of quality beans with a standard against which other 'Bulk' beans are measured (Beckett, 2008; Anonymous, 1994). Other West African countries that produce substantial amounts of the world's cocoa include Nigeria and Cameroun. The establishment of the oil and other industries in Nigeria providing alternative employment and the fact that most of the trees are old have resulted in a reduction in cocoa production.

2.2.3 South-East Asia

Countries in this part of the world that produce cocoa in substantial commercial quantities include Indonesia and Malaysia. Indonesia has expanded it cocoa industry with the result that they are now the world's third largest producer. Small-holders contribute about 87% of national

production as against 8% from state plantation and 5% from large private estates. Some of the smallholder cocoa has been originally bred in Malaysia and has been developed mainly for high yield but not for its flavor. 'Edel' (fine flavored cocoa) is planted by state or large private plantation. Cocoa is the third largest earning from the agricultural sector in terms of Indonesian exports. In the global market, Indonesian cocoa is mainly traded as unfermented, fat, bulk bean and volume base (Beckett, 2008; Yasa, 2002).

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Cocoa has been commercially grown in Malaysian since the 1950s whilst cocoa processing began in the 1970s. Malaysia built up a big cocoa production in the 1980s, but this has declined rapidly, partly due to pod borer infestation and also due to focus on other crops of greater profitability such as oil palm. The country is currently the fifth largest producer in the world (Beckett, 2008; Internet source, accessed June 15, 2010).

2.3   Primary processing of cocoa

The processing of cocoa beans proceeds harvesting of the pods from the tree. The processes involved include opening the pods to extract the beans, fermenting and finally drying the beans. The way the pods and beans are handled during these processes is important to ensure optimum formation of flavor precursors, development of color and the exclusion of off-flavors, some of which cannot be removed later even with further processing.

2.3.1   Pod opening

It is a common practice in some cocoa-producing countries including Ghana to store the pods for a few days before they are broken. This is reported to ensure a faster fermentation by causing a more rapid increase in temperature (Rohan, 1963; Dougan, 1980; Tomlins et al., 1993). During pod storage, the beans within the pod lose moisture, allowing more air to penetrate the beans once the pods are broken for initiation of fermentation. Faster fermentation and temperature rise results in good quality cocoa (AusAid, Online article, accessed July 2, 2010).

Breaking of the pods is done by machete or by wooden clubs. The former is used by experienced workers and care is taken not to damage the beans by cutting through them. The beans covered by a mucilaginous pulp are scooped out from the pods and fermented.

2.3.2 Fermentation

Fermentation of cocoa is the most critical process that results in the formation of flavor precursors and the development of the chocolate brown color. Fermentation is carried out in different ways depending on the producer and the cocoa variety as different types of cocoa require different amounts of fermentation (Beckett, 2008).

Prior to fermentation, cocoa beans are astringent and bitter with no hint of chocolate flavor. They have a slaty, grey color rather than the brown or purple-brown color of fermented dried cocoa beans. During fermentation, the mucilaginous pulp surrounding the beans which is rich in sugars undergoes ethanoic, acetic and lactic acid fermentation by yeasts, acetic and lactic acid bacteria respectively. The acid and heat generated kills the bean, making the cell membrane permeable. This allows a diffusion of acids into the bean and an increased temperature (up to $50^{o}C$), culminating in the formation of flavor precursors, namely amino acids, peptides and reducing sugars as well as some flavor compounds (Gill et al., 1984; Hansen et al., 1998; Thompson et al., 2001; Schwan and Wheals, 2004; Nielsen et al., 2007).

The method of fermentation varies from country to country and even from region to region within the same country. The most common methods, however, include box, basket, heap and recently, tray fermentations (Lehrain and Patterson, 1983). In all instances, the bottom of the fermenting container has holes to allow drainage of the drippings from the pulp. The duration of fermentation also depends on the type of cocoa being fermented. Two to three days is sufficient for Criollo cocoa whereas Forastero cocoa is fermented for 5-8 days with periodic mixing to homogenize the treatment and aerate the fermenting mass (Lopez and Dimick, 1991; Biehl and Ziegleder, 2003).

2.3.2.1 Heap

The predominant method practised by farmers in Ghana as well as in other West African countries is the heap system (Fig. 3) in which the beans are piled and covered with banana leaves or plastic sheet to protect the beans from insect-infestation and also to conserve heat (Wood and Lass, 1985; Aneani and Takrama, 2006). The heaps differ in size and may range from 20 to 1000 kg. Big heaps have to be turned every 24-72 hours to achieve even fermentation (Baker et al., 1994). Cocoa fermentation by this method is carried out for 4-6 days depending on the size of the heap.

Fig. 3 Traditional heap cocoa fermentation. a: big heap; b: small heap (Picture courtesy: Lene Hjort Lorenzen).

2.3.2.2 Box

Cocoa beans fermentation can also be carried out in wooden boxes which may be lined on the inside with banana leaves or polystyrene in some cases to hold in the heat (Fig. 4a). In both instances, the bottom of the box is provided with drainage holes to remove the liquid from the pulp.

2.3.2.3 Basket

In basket fermentations the beans are fermented in baskets lined on the sides, bottom and top with banana leaves (Fig. 4b). This prevents the cocoa from drying and also acts as insulation to hold in heat.

2.3.2.4 Tray

A different method of cocoa fermentation developed by the Cocoa Research Institute of Ghana (CRIG) is the Tray system (Fig. 5). This was developed to resolve the issue of uneven fermentation that sometimes arises with big heaps that are not turned. This is a method in which the cocoa beans are fermented in 10 cm deep wooden tray; 8-10 trays can be stacked on top of each other and the topmost tray covered with banana or plantain leaves. Air is allowed to circulate between beans in the trays without having to turn. This is reported to give higher quality fermented beans in shorter time. (Allison and Rohan, 1958; Allison and Kenten, 1963).

Fig. 4. Cocoa beans fermentation methods. a :Box fermentation ; b : Basket fermentation (picture source**:**
http://www.card.com.vn/news/Projects/013VIE05/Cocoa%20fermentation%20manual.pdf

Fig. 5. Tray cocoa fermentation showing a: four; b: eight wooden trays stack on top of each other. The topmost tray is covered with banana leaves and in the night or in the event of rain also with aluminium sheet to prevent beans getting wet. (Picture courtesy: Lene Hjort Lorenzen).

2.3.3 Drying

Following fermentation, the beans are either dried in the sun or by artificial means. The method of drying is critical to preserve the delicate flavor precursors which have been formed during fermentation. Chemical changes taking place in the beans during fermentation continues during drying until the moisture content drops from about 60% to about 7.5%. Both the high drying temperature and drop in moisture level causes enzymes to be inactivated. This is necessary to stabilize the beans for storage and shipment. Drying is also important for further development of the chocolate brown color due to the quinone protein reaction and for loosening of the shell from the bean (Cook, 1982; Hashim et al., 1998; Ramli et al., 2006).

Drying should not take place too quickly as exposure to intense heat causes the skin of the beans to wrinkle and promotes oxidative changes and destruction of flavor precursors. This may result in development of off-flavors and the retention of acetic acid, giving beans acidic and bitter flavor. On the other hand, if drying takes place too slowly, molds and off-flavors can develop. The quality of dried beans depends on temperature, rate of airflow, and the depth of the beans during the drying process (Jinap et al., 1994; Hashim et al., 1998). There are reports of development of flavor compounds during drying. Hashim et al., 1998 observed a significant decrease in free amino acids, peptide-N, total reducing sugars and sucrose and an increase in

trimethylpyrazine, tetramethylpyrazines and total pyrazines in cocoa beans as drying temperature was increased up to 80 $^{\circ}$C. They obtained the best drying treatment, however, with a combination of bean depth/temperature of 8.3cm/40 $^{\circ}$C. The development of the pyrazines was attributed to the occurrence of Maillard non-enzymatic browining reactions during the drying process. Rohan, 1963 has suggested the generation of tetramethylpyrazine in particular even at a temperature of 50 $^{\circ}$C.

2.3.3.1 Sun drying

In areas where the weather permits, the fermented beans are sun-dried. In this method, the beans are spread out an inch or two deep on raised platforms, mats, trays or a terrace on the ground and exposed to the sun until dry. The beans are occasionally turned over to ensure uniform drying and also to remove those with obvious defects. In the event of rain and during the night, they are covered with banana leaves or if they are on platforms, these are sometimes roofed. If the beans happen to get wet, they must be stirred well and re-dried quickly. Raised wooden racks which support drying mats made of bamboo are common in many African cocoa producing areas.
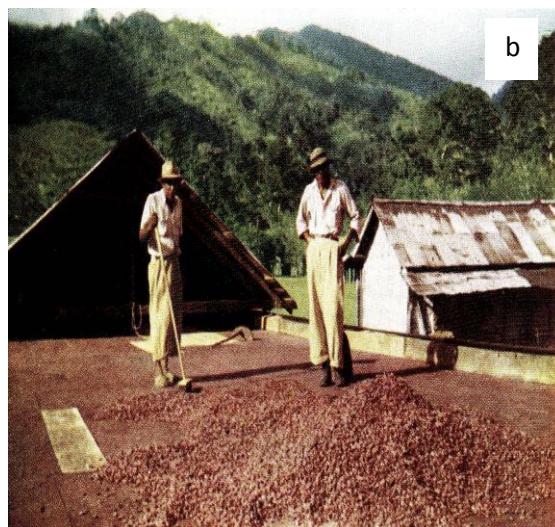
Fig. 6. Sun-drying cocoa beans. a: on mats supported by wooden racks; b: on raised platforms

The mats provide ventilation holes beneath the layer of beans and can easily be rolled up into a heap which is easier to handle at night or in adverse weather (Wood and Lass, 1985). Sun-drying is environmentally friendly, cheap and gives beans of a good quality and is therefore the preferred method of drying. However, it is a method which involves more time - a minimum of several days, labour and uncertainty with the weather and the risk of contamination from the surroundings and from farm and wild animals.

Fig. 7. Solar dryers (Internet source: http://www.worldcocoafoundation.org/what-we-do/success-stories/SolarDryer_success-story.asp; http://catalogs.indiamart.com/products/solar-dryer.html

Solar dryers may be classified according to the modes of heat collection employed and the heat transfer (Sodha et al., 1987). The mode may be direct in which the product contained in a closed chamber is covered with transparent heat of either glass or plastic and ventilated by holes. Thus heating is by direct absorption of radiation by the cocoa beans. In an indirect mode dryer, the air is heated in a collector and is directed to a dehydration chamber containing a batch or batches of the product.

Where an air heating collector is coupled with a drying chamber which exposes the product to solar radiation the dryer is termed "mixed-mode"(Bhatnagar and Ali 1989).

In the either of the above described modes, the dryer may also be constructed to move moisture from the product environment by naturally induced air currents or utilize fans to move drying air through the product (Bassey et al, 1986; Sodha et al., 1987).

2.3.3.2 Artificial drying

In instances or countries where sun-drying is not possible, artificial means are used to dry the beans after fermentation. Artificial drying is also mostly used where large lots of cocoa beans are being processed. Many types and sizes of mechanical dryers have been developed over the years. The method is common in some South American and Asian countries where cocoa is cultivated on large plantations and where the weather may be too wet for sun-drying. In some instances, cocoa beans are dried by fire. Wooden fires are lit in a chamber below the drying area, and the hot gas is led through a duct or pipe beneath the drying platform and then out through a vertical chimney. The problem with this method is the risk of smoke leakage from the fire which can contaminate the beans. The use of forced-air dryers and efficient heat exchangers can prevent smoke reaching the beans (Cook, 1982; Beckett, 2008).

Experiments have shown that during artificial drying of cocoa beans, volatile fatty acids are not reduced to the same level as that during sun drying and therefore the former tends to give beans of a higher acidity (Jinap et al., 1994; García-Alamilla et al., 2007).

Although sun-drying is mostly preferred over mechanical drying, the latter has the following advantages:

- Freedom from dependence on the weather with consequent dependability on production time schedule.

- Completion of drying in shorter time.

- Reduction in vulnerability to contamination by foreign matter (sticks, stones, visits by domestic animal, etc.).

- Less possibility of mold growth.

- Better potential control over final moisture content of beans.

Disadvantages of mechanical drying include the following:

- Often by too fast drying or too high temperatures the drying period is shortened so much that enzymatic action is not fully completed resulting in incomplete development of the chocolate flavor precursors.

- Excessive heat and rapid drying may not allow for adequate loss of volatile acids, especially acetic acid and this will affect the flavour quality.

- If smoke comes into contact with the beans during drying, a smoky off-flavor can result because cocoa easily absorbs volatile phenols from smoke.

- Costly investment especially for the small grower. (Cook, 2008).

2.3.4 Influence of fermentation and drying on flavor

Aroma formation begins with fermentation of the pulp surrounding the beans which contains mainly sugars. The first of the flavor compounds undoubtedly is ethanol, acetic and lactic acids from the activities of yeast, acetic acid bacteria and lactic acid bacteria, respectively (Hansen et al., 1998; Schwan, 1998; Schwan and Wheals, 2004; Camu et al., 2008). Acetic and lactic acids have been implicated as the cause of acidic flavor or sourness in cocoa and products produced from it (Jinap et al., 1995). Whilst some researchers ((Rohan and Stewart, 1964; Biehl, 1965; Lopez, 1983; Jinap and Dimick, 1990) suggest that acetic acid is more important for sourness in cocoa products because of its relatively higher concentration and acid taste, others ((Dittmar, 1963; Li and Lee, 1982; Li et *al.,* 1983) believe that lactic acid is more responsible for acid flavor because of its low volatility. However, Carr and Dougan (1977), as well as Lopez (1983)

suggest that both acids contribute to acidic flavor. Also associated with the fermentation process are alcohols and esters, mainly of microbial origin. Gill et al., 1984 found a significant increase in volatile compounds, mainly alcohols, organic acids and aldehydes after fermentation. In particular, increases in concentrations of 2-phenylethanol and 3-methylbutanal reported to be important aroma compounds in cocoa mass and roasted cocoa respectively, have been reported (Darsley and Quesnel, 1972; 1985; Schnermann and Schieberle, 1997).

Two methyl pyrazines, tetramethylpyrazine and trimethylpyrazine have been isolated from fermented, unroasted cocoa beans by Reineccius et al., 1972; Gill et al. 1984 and Ramli et al., 2006. They attributed the presence of these pyrazines to microbial source, especially *Bacillus subtilis* metabolism. Reineccius et al., 1972 and Gill et al., 1984 have also suggested the possibility of a thermal generation of particularly tetramethylpyrazines in the fermenting mass, the temperature of which can go up to 50 $^{\circ}$C.

Both under-fermentation and over-fermentation are detrimental to the flavor quality of the beans. The former results in what has come to be known as 'purple beans' with bitter, astringent, acidic flavour, incomplete development of the chocolate brown color and flavour. On the other hand, over-fermentation can result in a detrimental hammy off-flavor defect caused by a direct aerophilic microbial attack on beans, destroying the cocoa flavor potential, increasing pH and blackening the beans (Beckett, 2009). The hammy off-flavor can be explained by the formation of a surplus of propanoic acid, methyl propanoic acid and methyl butanoic acid (Lopez and Quesnel, 1973), although there are suggestions that these acids in usual levels of concentration are important in cocoa flavor (Ziegleder, 1991a; Schnermann and Schieberle, 1997).

Flavor development continues with the drying process. During drying, some amount of the acidic content of the beans diffuses out and is lost through evaporation. Incomplete drying may result in mold contamination which gives the final product an off-flavor. Heavy mold growth has been linked to the production of high concentrations of methyl ketones and volatile aldehydes which have a very detrimental effect on bean flavor (Hansen and Keeney, 1970). On the other hand,

formation of some flavor compounds and precursors, namely Maillard products is reported to take place with proper drying. Gill et al., 1984; Heinzler and Eichner, 1991; Oberparleiter and Ziegleder, 1997 have reported the presence of Amadori compounds, the first intermediates of Maillard reaction in dried, unroasted cocoa beans. These Amadori intermediates are important because they decompose into numerous volatile components during roasting of the beans (Beckett, 2009).

2.3.5 Storage

Cocoa beans must be stored such that they do not pick up moisture or off-flavors in the environment. Proper bean storage is a vital factor in both economy and quality because infestation and mold can develop to a level that will make the beans unusable and subjected to confiscation and destruction. Even minor development of off-flavors can cause damage to the flavor which will be noticeable in the finished chocolate.

Cocoa beans are typically packed into 60-65 kg jute sacks in some producing countries including Ghana, after drying. These are strong but also allow moisture to pass through, they can be stacked on top of each other and they are also biodegradable. Where the beans have to be shipped, they are transported in the holds of ships with good ventilation to remove moist air. (Cook, 1982; Beckett, 2008).

The beans arrive in the chocolate factory in the jute bags in which they were packed in the tropics and they are either stored in a cool, dry and well-ventilated room or immediately dumped into silos. Protection against rodents, insects, high humidity and odors must be ensured and fumigation done if infestation occurs. A novel method of cocoa beans storage has been proposed by Jonfia-Essien et al., 2010. This is a hermetic storage based on the principle of an oxygen-depleted, carbon dioxide enriched interstitial atmosphere caused by respiration of the living organisms in the ecological system of a sealed storage. A sufficiently low oxygen and elevated carbon dioxide atmosphere is created through a natural metabolic process based on insect respiration and where the commodity has high moisture, the respiration of the microorganisms

2.   Cocoa

within the sealed storage system. Trials for the method have been successfully undertaken in Indonesia and Ghana. (Jonfia-Essien et al., 2010).

3. Chocolate manufacturing

Chocolate is the most popular cocoa product. Its popularity has been attributed to its unique flavor as well as appealing color. It is a food that is solid at normal room temperature but easily melts in the mouth. The history of chocolate dates back to 600 AD when the Aztecs of Mexico and the Incas of Peru used cocoa beans as money and to produce a drink called 'Chocolatl' meaning warm liquid. This was the preserve of nobles and the rich as drinking it was literally drinking money. Processing of the beans, probably pot-fermented and sun-dried, was done using rudimentary tools. The beans were roasted in earthenware pots, de-shelled by hand and crushed or ground on slightly concave stones, sometimes with a fire beneath. Vanilla, spices and different herbs were added to suit the flavor of the producer. The resultant semi-liquid paste was molded by hand and placed on leaves in the shade of trees to cool and harden. The molded cakes were later broken up, dissolved either in hot or cold water and beaten into a foamy consistency. The resultant chocolate drink was rather astringent, fatty and unpleasant.

The chocolate drink recipe seemed bitter for the Spanish so subsequent recipes included sugar. In 1727 milk was being added to the drink in London, an invention generally attributed to Nicholas Sanders. A problem with this chocolate drink was that it was too fatty. The Dutch, however, improved this by partially de-fatting it. In 1828 Van Houten developed the cocoa press which could press the cocoa nibs to reduce the fat content to about 50%. The resulting cake was milled into powder to produce a drink with less fat. The Dutch also developed what has come to be known as 'the Dutching process' by treating the beans with alkali during roasting. This ensured better dispersion of the chocolate powder in hot water or milk. Chocolate producers found a market for the fat extracted from the production of cocoa powder when confectioners realised that 'eating' chocolate could be made by adding it to cocoa nibs and sugar. Adding of the extra fat ensured that all the solid particles are coated with fat to obtain the uniform product that easily melts in the mouth (Cook, 1982; Beckett, 2008).

With advancement in research, technology and machinery, chocolate production today has seen a lot of improvement far from what it used to be, when it was first introduced. Manufacturers are

Dried fermented cocoa beans
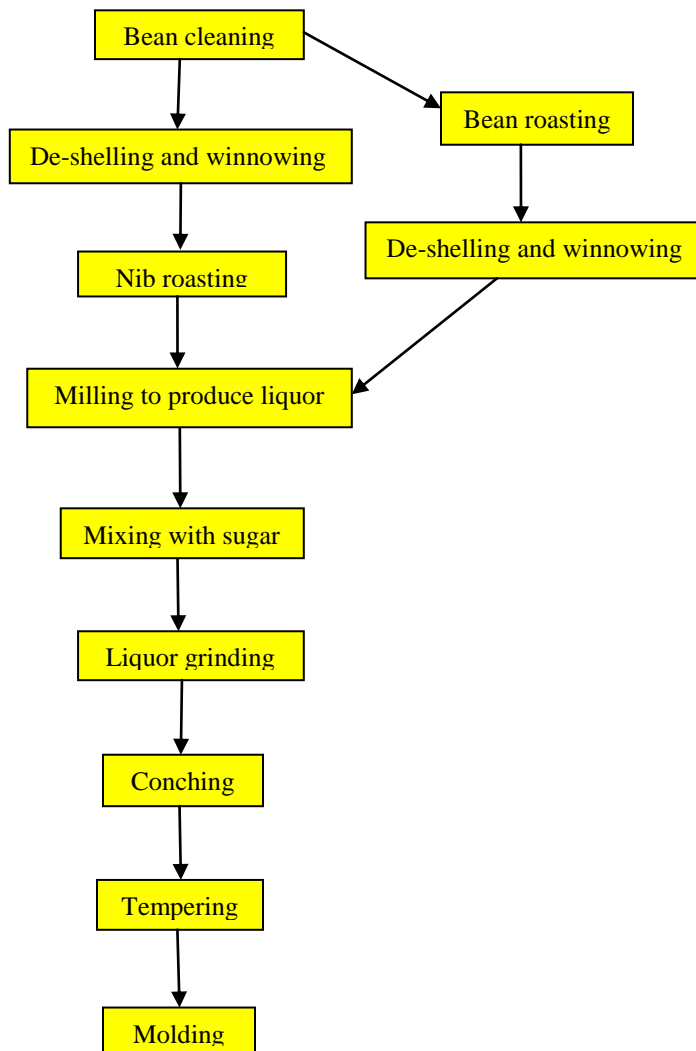
Fig. 8 Flow chart of chocolate production

constantly looking for avenues to produce chocolate of improved and varied flavor to meet the requirements of the modern consumer.

3.1   Bean cleaning

Flavor development is very important in chocolate production and most of the processes are geared towards this goal. Prior to this, the beans are cleaned to make them free of foreign objects

which may include sticks, stones, string, iron weights, nails, bullets, coins, finger rings and numerous other items, many of which may have fallen from the clothing or body of workers in the primary processing areas. Whilst some producers roast the whole bean, others de-shell and separate the shell from the cotyledon (known as nibs) and the latter is then roasted. In such situations, the beans are broken in a cracking machine and the nibs separated from the shell in a winnowing machine. Also eliminated in the cleaning process are dust, loose shell, pieces of cocoa pods and clumps of cocoa beans.

3.2   Roasting

The first process in flavor development involves roasting of the beans. Roasting is the most important technological process of chocolate production. Roasting virtually sterilizes the beans and this is important as the conditions under which they (beans) are fermented are naturally full of bacteria and fungi. The process also ensures the development of a characteristic brown color. However, the most important effect of roasting is flavor development. Both the temperature and duration of roasting are critical in determining the amount and types of thermally-generated aroma/flavor compounds (Ramli et al., 2006). Different kinds of roasting are required for different purposes or products (Cook, 1982).

While some chocolate manufacturers roast the whole bean, others practise nib roasting where the beans are de-shelled and broken before roasting. Nib roasting is reported to have certain advantages such as more uniform distribution of the heat, rapid evaporation of water from the nib and increase in output for the same amount of energy input. (Dimick and Hoskin 1981; Beckett, 2008). Roasting the whole bean on the other hand helps separate the husk from the cotyledons and makes cracking and winnowing much easier. However, the method is known to result in the loss of free cocoa butter which migrates into the shell when it is heated and is thrown away following winnowing. It is estimated that up to 0.5% of cocoa butter is lost in this way (Beckett, 2008).

3.2.1   Effect of roasting on aroma/flavor

Roasting of cocoa beans is the single most important process in chocolate manufacturing that result in the formation of the most aroma compounds. During roasting, Maillard reaction, also called non-enzymatic browning, play a major role in the formation of the cocoa flavor (Ziegleder, 1991; Misnawi et al., 2004; Ramli et al., 2006). This reaction involves free amino acids, peptides or proteins with reducing sugars, major precursors developed during fermentation (Ziegleder and Biehl 1988) and gives food products their colors and flavors when they are baked, toasted or roasted. Roasting removes undesired compounds such as acetic acid and forms the typical roasty, sweet odorants of cocoa.
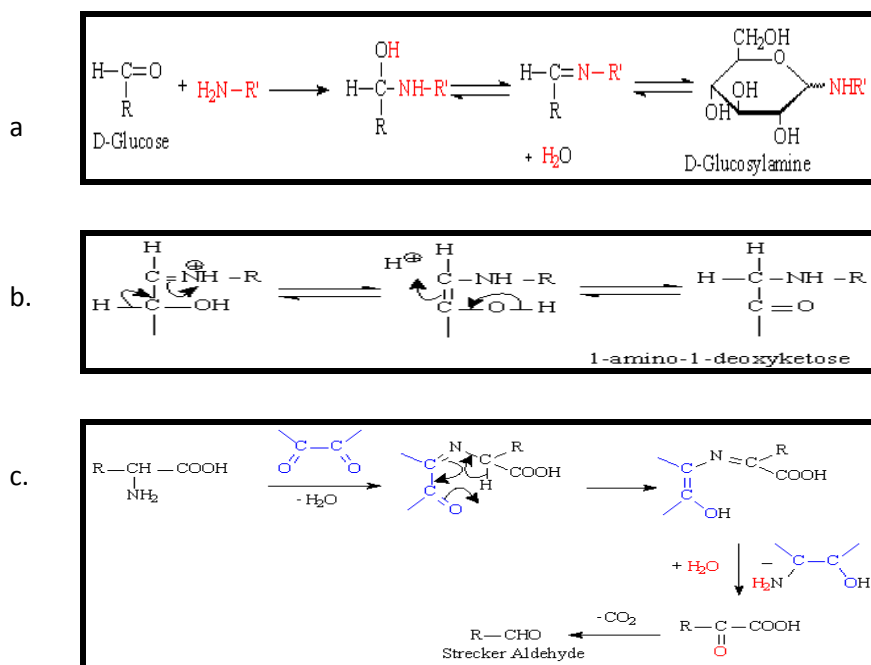
Fig. 9. Maillard reaction and Strecker degradation. a: Formation of N-glycoside; b: formation and isomerisation (Amadori re-arrangement) of immonium ion to form ketosamine; c: The ketosamine products either dehydrates into reductones and dehydro-reductones or hydrolytic fission   products   which   undergo   Strecker   degradation (http://www.chm.bris.ac.uk/webprojects2002/rakotomalala/maillard.htm).

Pyrazines and other heterocyclic compounds formed during roasting impact greatly on the aroma of cocoa and cocoa products because of their relatively low odor thresholds. Also formed are alcohols, aldehydes, ethers, furans, thiazoles, pyrones, acids, esters, imines, amines, oxazoles and pyrroles (Hoskin and Dimick, 1984; Jinap et al., 1998). These are formed via the Maillard reaction and Strecker degradation of amino acids and sugars (Keeney, 1972; Heinzler and Eichner, 1992; Bonvehí and Coll, 2002; Bonvehí, 2005).

3.3   Cocoa liquor production

After roasting, cocoa nibs are milled into a paste. The heat generated by the milling process causes the cocoa butter in the nibs to melt giving a cocoa fluid or liquor. Cocoa liquor is thus pure cocoa in its liquid form. In spite of its name, cocoa liquor contains no alcohol. It contains both cocoa solids and cocoa butter in roughly equal proportions. The aims of the grinding process are to make the particles small enough for chocolate production and to release as much fat as possible from the cells within the cotyledons (nibs). Releasing as much cocoa butter from

Fig. 10. Chocolate mixer (Melangeur) used for milling roasted cocoa beans to produce cocoa liquor.

the nibs as possible is essential due to the relatively high cost of cocoa butter. The cost of finer cocoa liquor grinding is less than that of the additional cocoa butter released (Cook, 1982; Beckett, 2008). If for instance, a relatively coarser chocolate is to be produced, it is prudent to grind the liquor quite fine in order to free all the expensive cocoa butter which will contribute maximally to viscosity reduction. (Cook, 1982; Beckett, 2008). Whilst some manufacturers process their own cocoa liquor for the production of chocolate and other products, others buy this intermediary product and use it to make the final product.

3.4   Mixing and refining

The various ingredients including cocoa liquor and sugar are mixed and kneaded until well blended. The amount of the individual ingredients used depends on the type of chocolate being produced and the manufacturer's recipe. For dark chocolate, the ingredients that are mixed are cocoa liquor and sugar whilst for milk chocolate, milk is also added either in a powdered or condensed form, depending on the manufacturer's recipe.

Although traditionally, chocolate was made by milling the sugar separately to a mean particle size of about 100 microns before adding it to the other ingredients for combined grinding, the trend now is to mix all the ingredients before grinding. The former method is however still carried out in some factories (Beckett, 2008).

After mixing, the mixture is passed through a series of heavy rollers to press or refine the ingredients into a dry flake. The sole aim of the refining process is to reduce the particle size of the ingredients to the dimensions called for by the specifications of the particular product involved and to give it a rounded mouth-feel or smoothness.

The refining process is in no doubt an important step to ensure that the chocolate mixture is refined to a particle size that is optimal for the release of important flavor compounds in the final product.
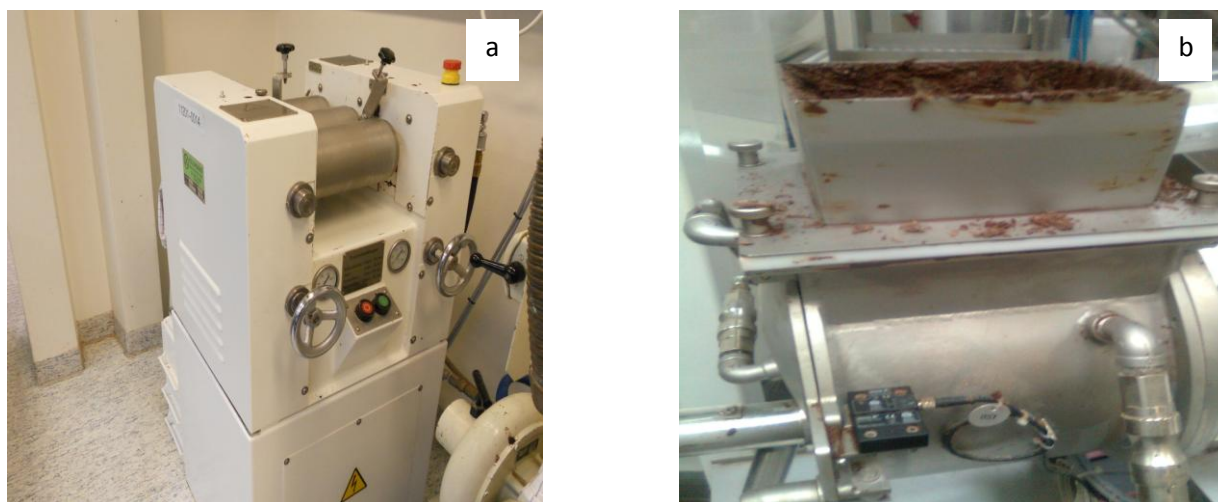
Fig. 11. a: Three-roller refiner used to reduce the particle size of chocolate ingredients b: Conche

Afoakwa et al., 2009 reported the effect of particle size on higher alcohol, aldehyde, ester, ketone and pyrazine concentrations. He observed a significant reduction in the release of all selected abundant compounds characterised by distinct aroma with the exception of 3,7-dimethyl-1,6-octadien-3-ol (linalool) and 2-carboxaldehyde-1-H-pyrrole. This, he related to increased matrix retention through structural, rheological and textural differences. A too coarse chocolate tastes too gritty whilst a too fine one may be too sticky.

3.5   Conching

The chocolate conche was invented by Rudi Lindt in Switzerland in 1878. When the conche was invented, the ability to mill chocolate was poor so the particles were probably broken in the conche, which made the chocolate smoother. Conching is a mixing step that involves volatilization of fatty acids and aldehydes and development of smooth texture. Volatilization reduces the sourness of the chocolate and develops the typical chocolate flavor. The solid particles such as sugar, non-fat cocoa and milk powder are coated with fat and become rounded. The coating of particles by fat promotes the smooth texture and snap desired in chocolate (Prawira and Barringer, 2009).

Conching involves two distinctive processes that take place within the same machine. The first, known as dry conch, is flavor development by the removal of moisture, some undesirable astringent and acidic components. The second process involves turning the chocolate from a powdered, flaky or thick dry pasty form into a free-flowing liquid, which determines the final viscosity of the liquid chocolate by breaking up large particle masses (agglomerates), coating the solid particles with fat so that they can easily flow. This stage is known as wet conch where an emulsifier, usually lecithin, is added together with extra cocoa butter. The addition of lecithin reduces the amount of cocoa butter needed to achieve the desired texture, thus reducing the cost of production. Cocoa butter is added to give smooth texture in chocolate. Increasing fat content is related to a richer mouth-feel, faster melting rate and thus, smoother chocolate (Talbot, 1999). The addition of cocoa butter coats the bitter compounds, and thus bitterness level decreases as the level of cocoa butter increases (Mazzucchelli and Guinard, 1999).

Fig. 12. Chocolate conching. a: dry flaky chocolate at the onset of conching; b: smooth, free-flowing chocolate at the end of conching (Photo source: Ziegleder, 2005).

3.5.1   Effect of conching on aroma/flavor

Conching enables maximum release of chocolate flavor during consumption. In summary, the following are the effects of conching:

- Reduction of the moisture content.
- Removal of volatile acids.
- Removal of undesirable and some desirable volatile flavors.
- Breaking up of agglomerates.
- Rounding of particle edges.
- Reduction of viscosity.
- Formation of some flavor components.

3.6   Tempering and molding

The fats in cocoa butter can crystallize in six different forms or polymorphs which have different properties. These crystals have been named I, II, III, IV, V and VI. Although type VI is stable, under normal circumstances, it is only formed by a solid to solid transformation and not directly from liquid cocoa butter (Beckett, 2008).
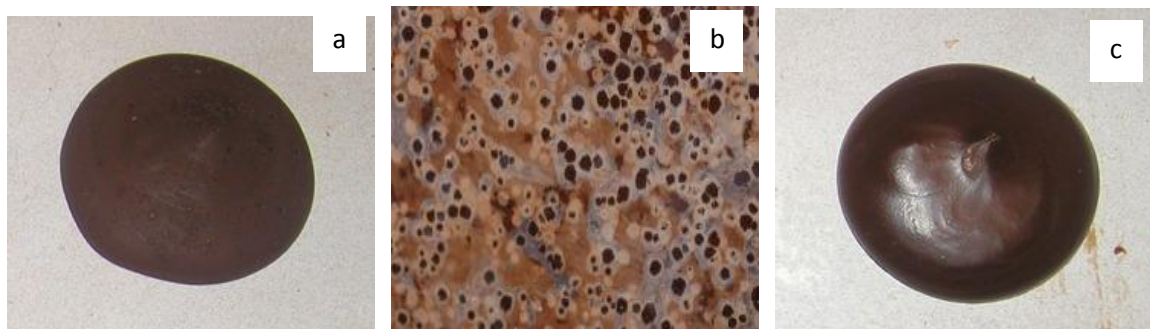
Fig. 13. Effect of tempering on the appearance of chocolate. a: untempered chocolate b: chocolate that has developed a fat bloom; c: shiny appearance of tempered chocolate (picture: internet source. http://www.chocolatealchemy.com/temperingmolding.php

The type V crystal melts at near body temperature, has a glossy look, and a good snap. The aim of tempering is to form as many type V crystals as possible so that the chocolate will have a glossy appearance and the most possible stable crystal. This ensures that the appearance and texture does not degrade over time.

Typically, the chocolate is heated to about 45 ^{o}C to ensure that all the six forms of crystals are melted. It is then cooled to about 28 ^{o}C which allows crystal types IV and V to form and then heated again to about 31 ^{o}C to eliminate the type IV crystals. Untempered chocolate develops fat blooms which are unsightly within a few days and also results in the chocolate becoming crumbly instead of snapping when broken. Once the chocolate has been tempered it can be molded into desired shapes (Beckett, 2008¸ Cook, 1982).

4. Food aroma/flavor

When food is presented to us, the first thing we usually experience is its aroma. Even before the food is close enough to be seen, sometimes we can smell it coming. The aroma of any food plays an important role in its acceptance and in most cases it is a determinant of quality. The aroma of foods may be perceived nasally, i.e. directly through the nasal cavity or retronasally, i.e. perceived by receptors when drawn in through the nose via the throat after chewing. The interaction of taste, odor and textural feeling when food is consumed is termed 'flavor'. Flavor therefore encompasses taste and odor or aroma (Belitz et al., 2004).

Every food is characterised by a distinct flavor and this may develop during processing or home preparation. Examples occur in such foods as yoghurt, coffee, chocolate, baked and fried foods. The routes for flavor formation may, for example, be non-enzymatic browning, fermentation or lipid oxidation. Important as such processes are to the flavor of foods, they may result in flavor defects in same if not carried out properly. Whilst a particular flavor attribute may be acceptable in one food, in another it may be a taint or off-flavor. Off-flavors are sensory attributes that are not associated with the typical aroma and taste of a particular food. Their occurrence in food may also be due to incidental contamination of the food from environmental sources (air, water, packaging materials) or may arise in the food itself from degradation of some food components (eg. Lipid oxidation and enzymatic action). Furthermore, they may arise as a result of ingredient mistakes during production. Another cause of unacceptable flavor which is often poorly defined is the loss of characteristic flavor which may be due to evaporation or reaction of characterizing flavor components within the food itself (Saxby, 1996; McGorrin, 2002; Reineccius, 2003).

The aroma/flavor of foods has often been used as a measure of its quality, to determine its degeneration or shelf life.

4.1 Aroma compounds

Substances that comprise aroma occur in nature as complex mixtures of volatile compounds. They stem from different compound groups and may have simple structures as in the case of

some alcohols or complex as in the case of some heterocyclic organic compounds. Groups of compounds in which aroma constituents have been identified include acids, alcohols, aldehydes, esters, furans, ketones, terpenes, pyrazines, pyrroles, pyrans, pyridines and sulfur compounds, among others (Dimick and Hoskin, 1981; Belitz et al., 2004). Most compound groups of aroma compounds possess particular flavor characteristics. Thus, whilst most esters confer a fruity/flowery attribute to foods, pyrazines usually give foods earthy/roasted/potato attribute. The aroma compounds of any food are usually those present in the food at concentrations higher than its odor threshold. Other volatile compounds present in foods at concentrations lower than their odor thresholds can also contribute to the aroma due to interaction with other aroma compounds (Belitz et al., 2004).
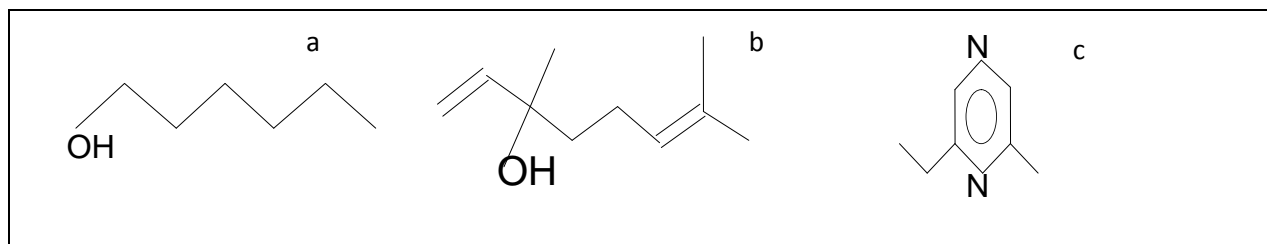
Fig. 14. Chemical structures of some aroma compounds identified in chocolate ; a:1-hexanol; b: 1,6-octadien-3-ol, 3,7-dimethyl- (Linalool); c: 2-ethyl-6-methyl pyrazine.

A total of 8000 compounds have been identified in the volatile fraction of 300 foods (Belitz et al., 2004; Nijssen et al., 1996). Inspite of this large number of identified volatiles, it is a known fact that only a limited number of these contribute to the characteristic sensory attribute of each food. Such compounds that uniquely describe the aroma quality of any food are called 'character impact compounds' or key odorants (Chang, 1989). Knowledge of the key odorants of any food is important in flavor research because it enables flavor duplication through biosynthetic pathways which can facilitate better quality control of raw materials and production processes by screening of appropriate target compounds. After the identification of the first character flavor compounds: Benzaldehyde (almond, cherry), vanillin (vanilla), methyl salicylate (wintergreen), and cinnamaldehyde (cinnamon) (Pickenhagen, 1999), many others have also been identified. Examples of such compounds are listed in Table 3.

Table 3. Character impact compounds of some foods. (Ref: Belitz et al., 2004; McGorrin, 2002).

Compound	Aroma	Occurrence
(R)-Limonene	Citrus-like	Orange juice
Benzaldehyde	Bitter, almond-like	Almonds, cherries, plums
(R)-(-)-1-Octen-3-ol	Mushroom-like	Champignons, Camembert cheese
(E,Z)-2,6-Nonadienal	Cucumber-like	Cucumbers
Geosmin	Earthy	Beetroot
trans-5-Methyl-2-hepten-4-one	Nut-like	Hazelnuts
2-Furfurylthiol	Roasted	Coffee
D-Linalool	flowery	Coriander, wine grapes

4.1.1   Character impact compounds

In spite of the identification of single impact compounds in some foods, in others, the aroma is provided by a mixture of compounds many of which may or may not have the flavor character of the particular food. The aroma of cocoa and cocoa products including chocolate, for instance, is known to be provided by a mixture of compounds from different compound groups (Dimick and Hoskin, 1981; Nijssen et al., 1996; Schnermann and Schieberle, 1997; Papers I-VI).

It is also known that a vast majority of volatile compounds that have been isolated from natural aroma extracts do not by themselves have aroma qualities reminiscent of the flavor material. An example has been reported of n-hexanal, a known component of natural apple juice. When smelled in isolation, it is described as 'green, painty, rancid oil odor'. Mahattanatawee et al., 2005, also observed that no single aroma character impact compound contributed to the aroma of carambola, guava and ripe mango and that a combination of esters, aldehydes, alcohols and ketones contribute green and sweet notes to the aroma of the fruits. Similarly, the aroma of cocoa and chocolate is composed of a number of different volatile compounds but no single one of them can represent the aroma character of real cocoa or chocolate, although mixtures of a few of the volatiles have been reported to give a chocolate aroma. Although Van Praag et al., (1968) as well as Lopez and Quesnel (1974) have shown that 3-methylbutanal together with trimethyl

disulfide give a cocoa-like odor, Van Elzakker and Van Zutphen (1961) had reported that none of the twelve odorants isolated from a high-vacuum distillation of cocoa butter had a cocoa-like odor.

In a reconstitution experiment to mimic the aroma of cocoa powder, Frauendorfer and Schieberle (2006) showed that 24 odorants which showed high Odor Activity Values (OAV) together gave the typical sweet, cocoa-like odor of the real cocoa powder sample.

In roasted cocoa and cocoa products including chocolate, the most influential compounds as far as the aroma is concerned are known to be heterocyclic compounds especially pyrazines (Counet et al., 2002; Bonvehí, 2005) which often give earthy, green, nutty, roasted aromas (Belitz et al., 2004; Owusu et al., 2008). In the study of the aroma volatiles of chocolates produced from heap- and tray-fermented cocoa beans, odorants of sensory importance detected during GC-O included compounds such as phenylacetaldehyde (bitter/green/grassy) and 3-methylbutanoic acid (unpleasant/old cheese/sweaty), 2,5-dimethylpyrazine (popcorn), tetramethylpyrazine (potato/earthy), and linalool (sweet/flowery/fruity) which have no cocoa or chocolate aroma (Paper 1). Obviously all twenty-nine odorants, and not any single one, detected by at least three judges jointly contributed to the aroma of the chocolates.

Interaction between aroma compounds have been reported and may have a role to play in most instances where a mixture of compounds contribute to the aroma of a particular food. The expression of some aroma compounds is known to be enhanced by the presence of others.

The concept of 'odor impact compounds' although applicable in a few exceptional cases, in most foods the odor is provided by an addictive effect or contribution from a number of compounds.

4.1.2   Sources of aroma compounds

The sources of aroma compounds in foods are very diverse. They may be inherent in raw plant or animal materials, or develop from microbial and/or technological processes that are part of the

food production. Thus, the constitution and nature of aroma compounds in any food material is likely to change with processing.

4.1.2.1 Aroma compounds from plant biosynthetic pathways

One often finds aroma constituents of plants that are inherent. Few if any natural aroma compounds found in plants are known to serve a biological function but are mostly a result of degradation reactions. The typical aroma of fruits is not present at the early formation periods but develops during the brief ripening period. During ripening of fruits, enzymes attack various substrates resulting in the formation of a number of aroma compounds, many of which have significant sensory properties (Reineccius, 2006). The source of the fruity-flowery aroma of most ripe fruits is usually esters and in some cases also aldehydes, alcohols and ketones and other groups of aroma compounds. The genetic make-up of a plant determines the precursors, enzyme systems and their activity in aroma formation which also determines the aroma compounds formed (Reineccius; 2006; Belitz et al., 2004). Gill et al. 1984 found benzyl thiocyanate, among others, .in raw unfermented cocoa beans. These obviously form the inherent compounds and precursors for subsequent aroma formation in cocoa beans during fermentation. These inherent aroma potentials distinguish one variety of cocoa from another in terms of their flavor.

A number of volatile aroma compounds are also formed from lipids via several different pathways. The pathways include α- and β-oxidation, and oxidation via lipoxygenase enzymes. The latter results in the production of the widest variety of aroma compounds. Oxidative degradation of linoleic and linolenic acids in fruits, for example, results in the formation of esters, alcohols, acids, and carbonyl compounds (Reineccius, 2006).

In vegetables, aroma compounds are formed during growth but also mostly via enzymatic reactions upon cellular damage or disruption. The enzymatic processes may be so rapid that the aroma compound(s) can be formed within seconds of the cellular damage such as occurs in onion. Table 4 shows some naturally occurring aroma components of some fruits and vegetables.

Table 4. Naturally occurring odorants of some fruits and vegetables (Reineccius, 2006; Belitz et al., 2004)

Compound	Occurrence
Methyl butyl,- acetate (isoamylacetate)	Banana
Ethyl 2-methylpropanoate	orange
Methyl 2-methylbutyrate	Apple (Elstar)
Ethyl-(S)-2-methylbutyrate	Pineapple
2-methyl-3-isobutyl pyrazine	Bell pepper
Dimethyl disulfide	Cabbage

4.1.2.2 Aroma compounds formed via fermentation

Fermentation is one of the processes by which volatile aroma compounds are produced in foods. Such foods include – besides cocoa - sausage, yoghurt, cheese, beer, bread, wine and soy sauce. The flavor may either be developed from the primary metabolism of microorganisms such as occurs in cocoa fermentation or from residual enzymatic activity following lyses of the microbial cell as in aged cheese. Using specific substrates, microorganisms can perform various reactions such as oxidations, reductions, hydrolytic reactions, formation of new carbon-carbon bonds and degradations to produce flavors (Scharpf et al., 1986). Aroma compounds commonly synthesized via microbial activities include acids, esters, carbonyl compounds (aldehydes and ketones), alcohols, terpenes, pyrazines, lactones, sulfur compounds, skatole and p-cresol (Landaud, 2008; Belitz et al., 2004). Examples of such compounds are shown in Table 5. A number of industries are harnessing this potential of microorganisms to produce flavor compounds in commercial quantities (Janssens et al., 1992).

Table 5. Microbial species associated with the synthesis of some aroma compounds

Aroma compound	Microorganism	Reference
Acetic acid Diacetyl	*Leuconostoc citrovorum,* L. *creamoris*	Kempler, 1983
3-methylbutanal	*Streptococcus lactis* var. *maltigenes*	Morgan, 1976
Phenylacetaldehyde	*Streptococcus lactis* var. *maltigenes*	MacLeod and Morgan, 1958
Tetramethylpyrazine	*Bacillus subtilis, Corynebacterium glutumicum,*	Kosuge and Kamiya, 1962; Demain, 1967; Ziegleder, 1982; Gill et al., 1984
Citronellol	*Ceratocystis* sp.	Collins and Halim, 1977
Butyric acid	*Clostridium butyricum*	Dziczak, 1986
Linalool	*Ceratocystis sp.*	Janssens et al., 1992

4.1.2.3 Aroma compounds formed via Maillard reaction

An invaluable source of aroma compounds in foods that have been processed by heating is the Maillard reaction (Fig. 15). This is a non-enzymatic reaction involving reducing sugars and amino acids or peptides. The reaction results in the formation of various flavor components of heated food including pyrazines, pyrroles, furans and thiazoles among others (Table 6). The reaction also results in the formation of brown pigments which are desirable particularly in foods that are baked, fried or roasted (Blitz et al., 2004; Krysiak, 2006). An 'off-shoot' reaction, the Strecker reaction, results in the formation of aldehydes and ketones. Table 6 shows aromatic products of the Maillard reaction in some foods. Factors such as pH, temperature and water activity affects the reaction and so in cases where it is undesirable, as in processed fruit juices, it is possible to inhibit its occurrence by changing the levels of these factors (Wolfrom et al., 1974; Belitz et al., 2004; van Boekel, 2006).
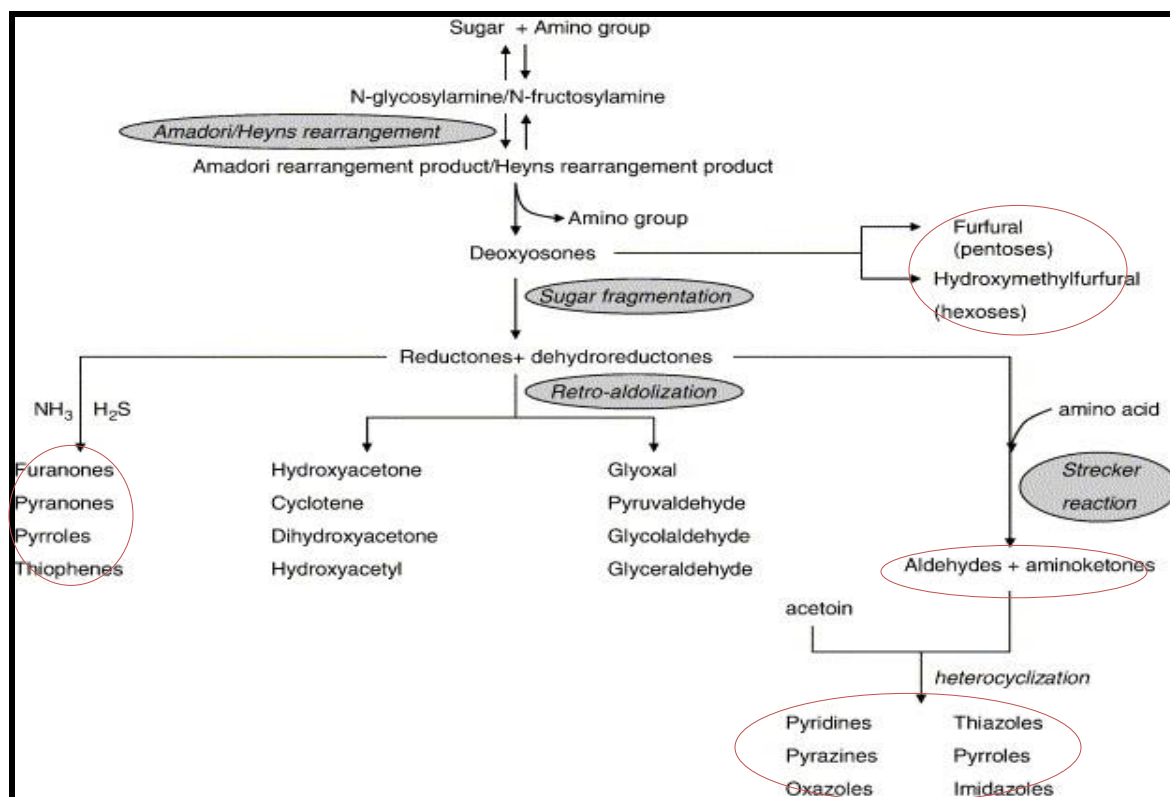
Fig. 15. Formation of Maillard products (ref. van Boekel, 2006)

Table 6. Examples of some aroma compounds formed in foods via the Maillard reaction (Reineccius, 2006; Belitz et al., 2004))

Compound class	Associated flavor/aroma	Food examples
Pyrazines	Cooked, roasted toasted, baked cereals	Heated foods in general
Alkylpyrazines	Nutty, roasted	Coffee, chocolate
Alkylpyridines	Green, bitter, astringent, burnt	Coffee, barley, malt
Acylpyridines	Cracker-like	Cereal products
Pyrroles	Cereal-like	Cereals, coffee
Furans, furanones, pyranones	Sweet, burnt, pungent, caramel-like	Heated foods in general
Oxazoles	Green, nutty, sweet	Cocoa, coffee, meat
Thiophenes	Meaty	Heated meat

4.2   Analysis of food aroma

Frequently it becomes necessary to analyse the aroma of food either to isolate and identify the compounds contributing to perceived aroma or to identify the characteristics of the aroma. The former involves instrumental analysis whilst the latter employs sensory evaluation techniques.

4.2.1   Methods for the isolation of food aroma volatiles

Aroma compounds first have to be isolated from the food matrix before they can be identified. The choice of isolation method depends on the nature of the food matrix, the volatiles of interest and the general advantages and disadvantages of the method.

Methods that have been used to extract volatiles from food include solvent extraction and distillation techniques, headspace analysis and solid phase micro-extraction (SPME). (Belitz, 2004; Wampler, 2002; Werkhoff et al., 2002).

4.2.1.1 Solid-Phase Microextraction (SPME)

The length of time it takes to isolate aroma volatiles from a food matrix is a usually a matter of consideration in the choice of method, especially when it is required to analyse a large number of samples. Solid-Phase Microextraction (SPME) is a robust technique for rapid, solventless extraction or pre-concentration of volatiles. SPME involves the partitioning of organic componenets between a sample and the thin polymeric films coated onto fused silica fibers. The odorants are adsorbed initially onto the fibre immersed in a liquid food, a food extract or in the headspace above a food sample for a certain duration. After adsorption is completed, the compounds are thermally desorbed into a GC injector block for further analysis.

SPME techniques are independent of the form of the matrix; solid, liquid and gases can all be readily sampled. Since the techniques does not require the use of a solvent and can be performed without heating the sample, the formation of chemical artifacts is greatly reduced or completely eliminated. The technique is sensitive to experimental conditions – the stationery phase (the coated fibre), volume concentration of odorants, sample matrix and uniformity as well as temperature and time of adsorption and desorption processes. These influence the yield but can

be eliminated by the use of labelled internal standards in quantitative SPME (Kataoka et al., 2000; Blitz et al., 2004, Wardencki et al., 2004). One of the major limitations of SPME is its reduced concentration capability mainly due to the small volume of polymer that coats the fiber Other disadvantages of the method include the fragility of its fused silica and its unprotected stationery phase coating. Attempts have however been made to overcome these limitations. In 1997, for instance, Ralf Eisert and Janusz Pawliszyn are reported to have successfully introduced in-tube SPME-LC in which sampling is conducted through an open tubular fused-silica capillary column (Christ et al., 2007).

4.2.1.2 Distillation and extraction techniques

Different extraction and distillation techniques are reported in the literature for isolation of aroma volatiles. These techniques basically involve removal of the volatile aroma compounds together with some water from an aqueous suspension of the sample by distillation. The organic compounds in the distillate are separated from the water by extraction. The extraction phase involves the use of a solvent and the choice of an appropriate solvent is an important consideration. The use of a solvent presents a problem especially where the analyte of interest elutes early or is also a solvent. In such a case, the presence of a solvent peak will dilute and mask the analyte peak (Wampler, 2002).

4.2.1.3 Headspace sampling

Aroma compounds are by their nature volatile and therefore advantage is frequently taken of this to isolate them by techniques of headspace analysis. The technique has the advantage of not involving the use of a solvent thereby avoiding a solvent peak in the chromatogram which might dilute and mask solvent analytes or early eluting analytes of interest. Headspace sampling may either be static or dynamic. In the former, the food is placed in a sealed vessel and heated to enhance vaporization of the volatiles. Once equilibrium has been attained by allowing it to stand for some time, an aliquot of the headspace is withdrawn and injected into a gas chromatography injection port.

In dynamic headspace sampling, a purge gas used to sweep over the headspace above the sample ensures that a large volume of the volatiles is collected onto a trap with an adsorbent material. Instead of allowing the sample volatiles to come to equilibrium with the sample matrix and the surrounding headspace, the atmosphere around the sample material is constantly swept away by a flow of purge gas, taking the volatile analytes with it. This prevents the establishment of an equilibrium state causing more of the volatiles to leave the sample and pass into the headspace. Furthermore, it increases the size of the headspace beyond the limit of the sample vessel (Wampler, 2002). The method is also known as purge and trap and has been used frequently to separate the volatiles in a wide variety of food matrix from the non-volatile portion (Marsili et al., 1994; Juric et al., 2003; Aishima, 2004; Nielsen et al., 2004; Varming et al., 2004; Aculey et al., 2010; Papers I-VI).

The trapping stage of the method enable increased sensitivity and the analysis of volatiles present at a level of parts per trillion (ppt) with careful consideration to contaminants and instrumentation. This can be checked with the use of blind control samples to each set up. Additionally, selectivity in adsorbent trapping material coupled with an optimized temperature enables trapping of particular analytes of interest whilst venting undesirable ones (Lepine and Archambault, 1992; Wampler, 2002). This method permits the isolation and identification of volatiles in a food matrix closer to what happens in inhaled or sniffed aroma than is obtained from solvent extraction (Hachenberg and Schmidt, 1977; Charalambous, 1978).

4.2.1.3.1   Breakthrough volume of trapping material

During headspace sampling of volatiles onto traps, the analytes are adsorbed on the packing material of the trap. This is a reversible process that allows desorption of the volatile compounds for analysis later. The degree of adsorption, however, differs for different compounds/compound types. While some compounds are firmly adsorbed, requiring high temperatures to desorb them, others are not so firmly 'trapped' and may even pass through the trap at room temperature along with the purge gas. This presents a challenge for analysing a food matrix such as chocolate with a complex aroma that is made up of very volatile compounds and not so volatile ones by purge and trap method. The volume of purge gas that is required to elute or allow a volatile compound

to pass through the trap has been termed 'Breakthrough volume'. This depends on the nature of the compound, its volatility, interaction between the compound and the sorbent material, amount of sorbent material in the trap and the temperature of the trap (sampling temperature). Therefore breakthrough volume is usually expressed as the volume of purge gas required to elute the volatile compound off a unit weight (eg. 1.0 g) of the adsorbent material at a particular temperature.

In general, the higher the sampling temperature and flow of purge gas, the lower the breakthrough volume. Different compound groups also have different breathrough volumes and within the same group, the smaller the number of carbons, the smaller the breakthrough volume.

Table 7. Breakthrough volumes* of some compounds in Tenax TA traps (Scientific Instrument services, 1996-2010).

Temperature/^{o}C	0	20	40	60	80	100	120	140	160	180	200
Acetaldehyde	2.20	0.650	0.200	0.070	0.031	0.014	0.007	0.004	0.002	0.001	
Acetic acid	28.0	5.60	1.40	0.427	0.137	0.045	0.017	0.008	0.004	0.002	0.001
Methyl acetate	43.0	8.0	1.60	0.443	0.154	0.054	0.020	0.009	0.004	0.002	0.001
Pentyl actetae	56000	4700	480	63.0	12.0	2.50	0.736	0.205	0.055	0.020	0.009
2-octanone	250000	20000	2000	250	40.0	7.80	2.00	0.556	0.154	0.052	0.019
1-nonanol	1.76E+06	89000	7900	1000	141	28.0	5.60	1.50	0.45	0.14	0.05
Nonanal	1.00E+06	63000	5000	530	90.0	18.0	4.00	1.00	0.320	0.090	0.033

*The blue highlighted areas indicate that the breakthrough volume is greater than 10 litres (10,000 ml) per gram of resin adsorbent material which is an acceptable value for the trapping of the analyte at that temperature. The red areas indicate that the breakthrough volume is less than 0.010 litres (10 ml) per gram of resin. This is a good temperature for complete desorption of the analyte off the adsorbent during thermal desorption.

Although there is the risk of a few compounds such as acetaldehyde, acetic acid and methyl acetate (Table 7) 'breaking through' the trap at even very low temperatures and with high flow of purge gas, most other compounds are well retained even at relatively high temperatures and high purge gas volumes.

In sampling the aroma compounds of chocolate by purge and trap, a compromise had to be sought on a good sampling temperature, purge gas flow and sampling time as well the choice of a good sorbent material that will allow as much of the analytes as possible to be retained in the trap and at the same time reduce the risk of losing the highly volatile ones via the 'breakthrough' concept.

The use of an appropriate adsorbent material in the trap is also a matter for consideration. The choice of the appropriate trapping material depends on:

- Chemical nature of the analytes

- Thermal stability of the analyte

- Adsorption and desorption characteristics of the sorbent

- Availability and cost of the sorbent material

- Presence of contaminants including water vapour

While some sorbents materials may not be suitable in retaining the trapped volatiles, others may be able to trap the volatiles of interest but also some unwanted materials. Still, others are thermally unstable and produce artifacts during the desorption process or retain analytes so strongly that very high temperature are required to desorb, resulting in thermal degradation of some analytes. The choice of a good sorbent material is therefore important to ensure the integrity of analytes during dynamic headspace sampling.

Various trapping materials are used for specific compounds and these include silica gel and activated charcoal but these have the tendency to absorb water which will be transferred to the GC. Graphitized carbon sorbents are also suggested for use in the analyses of very small molecules.

Tenax TA, a multi-purpose trapping material, is a porous polymer resin based on 2,6-diphenylene-oxide and is the most widely used sorbent material used with purge and trap for trapping volatile organic compounds in air and liquids. Due to its low breakthrough volume for

water, it is especially useful in trapping volatile organic compounds from samples containing high moisture. Tenax is known to be capable of sorbing a fairly wide range of organic volatiles, especially aroma volatiles and may be heated to relatively high temperatures for desorption.

Various combinations of some of the already mentioned sorbent material with Tenax are also sometimes used to provide good trappjng efficiency (Wampler, 2002).

To ensure optimal isolation of analytes, including the bigger molecules which are particularly known to be important for the aroma of chocolate, a rather high purge flow of 200 ml/min for 60 min coupled with a temperature of 50 ^{o}C was used. Although these relatively extreme sampling conditions have the potential of resulting in the 'breakthrough' of a few small, very volatile molecules, most of the volatile components of chocolate were not affected. Results of a method optimization (discussed later in Chaper 5), showed that increasing the purge flow volume, sampling temperature and time coupled with an increase in sample size resulted in the isolation of more analytes from chocolate, an indication that the volume of purge flow was still below the breakthrough volume of most of these volatiles and that the Tenax TA traps used were efficient sorption material for them. Acetic acid, one of the compounds known to have a low breakthrough volume, was consistently isolated from all chocolate samples analysed (Papers I-VI).

4.2.1.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of volatiles

GC-MS offers a very sensitive and efficient way of identification of isolated volatiles. It is a method of separating, quantifying and identifying the various components of a complex mixture of volatiles in the vapor phase. The trapped volatiles are desorbed using a solvent and introduced through an injection port where the analytes vaporize with heating, or desorbed using thermal desorption and often transferred directly to the GC-column using a special thermal desorption unit. The analyte molecules are swept through a column by a carrier gas which is often helium. Although hydrogen is known to be the most efficient, providing the best separation, helium is

preferred because it has a large range of flow rates comparable to hydrogen and the added advantage that it is non-flammable and works with a greater number of detectors.

GCs today almost exclusively use capillary columns which have very small internal diameter and gives separation efficiency. Most capillary columns are made of fused silica with a polyimide outer coating. These columns are flexible so a very long column can be wound into a small coil.

There is movement inhibition of the analytes by the column material and interaction between the two is dependent on the physical properties of the individual molecules. The column material serves as a stationery phase which retains the volatiles but allows the carrier gas to pass through. The stronger the interaction between a molecule and the column material, the longer the retention time, which is the time it takes a molecule to pass through the column. Both the interaction between a molecule and the column and rate of passage through the latter are temperature-dependent. Although a low temperature is known to provide the best level of separation, it can result in long elution times. This is often resolved by a temperature programming that involves a continuous or stepwise ramping to achieve the desired separation. Typical GC operating temperatures ranges between 40 and 300°C.

From the column, the analytes are identified and their levels determined by a detector. The detector may be a Mass Spectrometer (MS), Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), Electron Capture Detector (ECD), Flame Photometric Detector (FPD) or
Photoionization Detector (PD).

In the case of a mass spectrometry, the detection is based on the mass/charge ratio of an analyte. The molecules of the analytes in the vapor phase are bombarded with a high-energy electron beam. The positive ions produced from the bombardment are separated in a magnetic field on the basis of their mass-charge-ratio (m/z) and the signal from the recorded mass spectrum is presented as a computer-plotted graph of abundance (peak intensity) versus m/z.

GC-MS has been used to qualitatively and quantitatively determine the analytes in all kinds of foods that may be responsible for flavor or causing off-flavor. The use of GC-MS has enabled the effective identification of volatiles in cereals, vegetables, fruits, wine, coffee, chocolate and other cocoa products, dairy products, meat and meat products, fish and fish products, herbs and spices, among others (Yau and Liu, 1999**;** Noble and Ebeler, 2002; Juric et al., 2003; Varming et al., 2004; Berna et al., 2005). Today's GC-MS has become more sensitive and can analyse dozens of samples per day, each with hundreds of chemical components (Wampler, 2002).

4.2.1.5 Gas-Chromatography-Olfactometry (GC-O)

The aroma of any food consists of many volatiles but only a part of them are sensorially relevant. Although GC-MS will effectively identify and quantify the volatiles in a food sample, it does not give information as to which of these are key contributors to perceived aroma. GC-O offers a means of identifying the potent odorants of an aroma. It is a known fact that some aroma compounds exist at very low concentrations that are below the detection limit of a GC but are sensorially important to the aroma of food because of their low odor threshold. GC-O which involves sniffing by human subjects enables the detection of such potent components of the food which might be over-looked by the Odor Activity Value (ratio of concentration of a volatile compound in the food to odor threshold in water) concept. (Marsili, 2002).

In GC-O techniques, the human nose is used as the detector. The system is usually set up such that the flow from the column may be split or splitless. In the former, a part of the column effluent goes to a sniffing port and the other part goes to a detector which may be a Flame Ionization Dectector (FID) or to an MS whilst in the latter all the effluent goes to a sniffing port at one time and a second run is made in which all the effluent goes to the instrument detector. This enhances the performances of both detectors – the human nose and the FID or MS by providing maximum amount of sample but has the disadvantage of increasing analysis time

Fig. 16. A judge at a sniffing session

and difficulty in matching the GC peaks with the perceived odors. Inspite of its potentials, GC-O has been criticised as a subjective method especially where few judges are used. The statistical error can however be reduced by using more judges for sniffing (Pollien et al., 1997; Chaintreau, 2002; Reineccius 2006).

The detection frequency method (Pollien et al., 1997; Acree et al., 1984; Linssen et al., 1993) is a GC-O method that gives a measure of the intensity based on the number of assessors detecting a similar odor simultaneously (Petersen et al., 2002). Using this method, it is possible to rank perceived odorants in order of importance. Other ranking methods used in GC-O are dilution to detection threshold which include Aroma Extract Dilution Analysis (AEDA) (Ulrich and Grosch, 1987) and Charm analysis (Acree et al., 1984); and perceived intensity criterion made up of posterior intensity method (Casimir and Whitfield, 1978), the cross modality matching finger span technique (Etievant et al., 1999), the time intensity method (McDaniel et al., 1990) and GC Odor Profiling (Tønder et al., 1998).

The detection frequency method also called GC-Snif (Surface of Nasal Impact Frequency) or NIF (Nasal Impact Frequency) method has been used both qualitatively and quantitatively by several researchers (Pollien et al., 1997; Varming et al., 2004; Nielsen and Poll, 2004; Paper II). It uses one dilution level but the sniffing of a sample is repeated by a number of panelists and

therefore treatment of data is based on frequency of detection of sniffed peaks (Varming et al., 2004). Panelists may record information in a sniffing session manually by writing or electronically using sound recording software (Fig. 16). The method has been found to have a good correlation with sensory odor intensities (van Ruth and O'Connor, 2001).

Using the detection frequency method, it was possible to construct Nasal Impact Frequency (NIF) profiles (Fig. 17) based on the average responses (olfactogram) of all panelists for each retention time using an Excel spreadsheet. The height of a peak is a measure of the detection frequency, that is, the number of judges detecting the odor at the particular time. The area of a peak referred to as SNIF (Surface of Nasal Impact Frequency) was calculated as the total time in minutes it was perceived (summed over all judges). The area of a peak can be used as an indication of its importance to the aroma of the food being analysed (Pollien et al., 1997; van Ruth and O'Connor, 2001; Paper II).
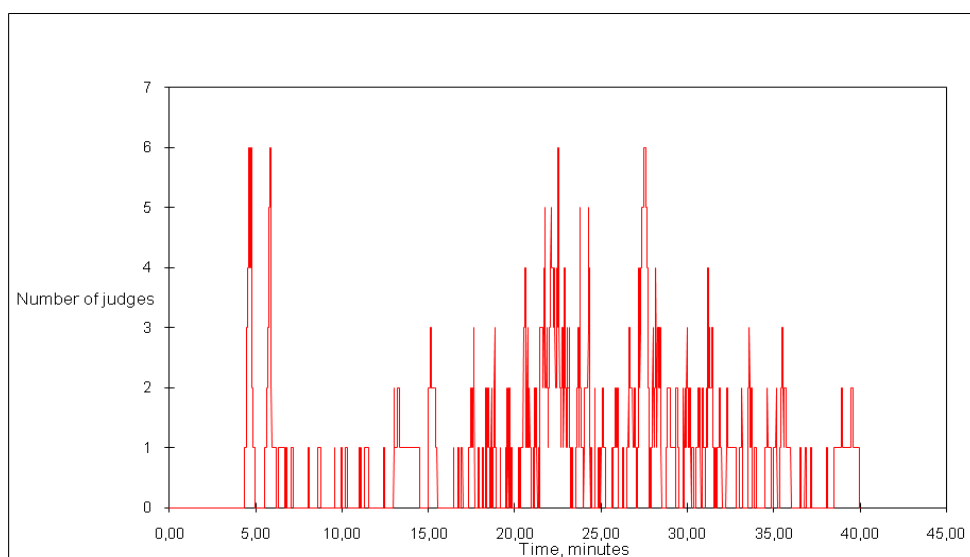

Fig. 17. Nasal Impact Frequency (NIF) profile of odorants from a chocolate sample sniffed by a panel of seven judges.

4.2.1.6 Quantitative Descriptive Analysis (QDA) of food flavor

Flavor is a sensory perception. Inspite of the increased sensitivity of various instruments to determine the composition of inhaled or sniffed aroma of food, the perception of a trained sensory judge is indispensable because of the interactions between volatile components, between volatiles and the matrix as well as taste aroma interactions (Noble and Ebeler, 2002). Sensory tests provide useful information about the human perception of product changes due to ingredients, processing, packaging or shelf-life.

Descriptive sensory analysis is a sensory technique that allows the sensory scientist to obtain complete sensory descriptions of products, help identify underlying ingredient and process variables, and/or to determine which sensory attributes are important to acceptance (Lawless and Heymann, 1999). The method is useful in situations where a detailed specification of the sensory attributes of a single product or a comparison among several products is desired (Gillette, 1984). Depending on the specific technique used, the description can be qualitative as well as quantitative. Quantitative descriptive analysis enables the treatment of data by statistical analysis.

During QDA, a selected panel of pre-screened individuals (usually 8-10) are trained to generate the lexicon to describe the product. Adequate training of quantitative descriptive panelists is very important to make them consistent and reproducible and so the method is never used with consumers. The panel also work together to identify exact descriptors and appropriate intensity scales specific to the product under study. The selected descriptors should be effective in discriminating the samples but they should not be redundant, have little or no overlap with other terms and as much as possible be orthogonal i.e uncorrelated (Lawless and Heymann, 1999).

During the evaluation the panelists identify and score product flavor attributes, in the case of flavor evaluation. QDA allowed the evaluation of the flavor of chocolates produced from cocoa that have undergone different fermentation methods- heap and tray fermentation. The method was therefore used to discriminate between these two types of chocolates (Paper II). It was also successfully used to discriminate between chocolates produced with different processing

conditions (Paper VII). Misnawi et al., 2004 used QDA to determine the effect of polyphenol concentration and roasting duration on the sensory properties of cocoa liquor.

QDA has been used as an investigative sensory technique for studying conventionally pasteurized milk (Phillip et al., 1995), ice cream (Ohmes et al., 1998; Roland et al., 1999), cheese (Ordonez, 1998), chocolate appearance and texture (Andrae-Nightengale et al., 2009), cocoa liquor (Misnawi et al., 2004) flavor, rice aroma (Yau and Liu, 1999), tomato aroma (Berna et al., 2005), among others.

4.2.1.7 Multivariate data treatment of food aroma analysis

Multivariate data analysis is a tool used to simplify and aid in the interpretation of complex data. It is useful in reduction of large sets of data and for establishing relationships between different data sets. Both instrumental and sensory food aroma analyses involve large data of volatiles and attributes that require multivariate techniques to model relationships between the two sets of variables. The ultimate goal of most multivariate analysis is to develop a model to predict a property of interest (Marsili, 2002). Multivariate analysis is useful in the interpretation of the sensory significance of complex volatile data because aroma perceptions in most foods are complex and produced by a combination of different volatiles rather than by one impact compound (Noble and Ebeler, 2002). Univariate statistical analyses are therefore not very useful in such cases.

Most multivariate analyses are exploratory and are powerful visualization tools. Principal Component Analysis (PCA), the most frequently used multivariate tool, can graphically represent relationships between samples and between variables, and simplify large data sets. To look for patterns in a large data set with a large number of variables, PCA (Noble et al., 1980), discriminant analysis (Noble et al., 1980; Rapp and Hastrich, 1978) and clustering methods have been used (Kwan and Kowalski, 1980; Schaefer et al., 1983). Methods such as Partial Least Squares Regression (PLS-R) (Martens and Martens, 1986) are used to find relationships between two data sets such as GC-MS and sensory data. PLS is a 'soft modelling' method to extract factors or latent variables which are linear combinations of one set of variables (eg GC-MS data)

that predict much of the variation in another set of variables (eg sensory attribute intensities). PLS models indicate how well the variables in one data set predict the variation in a second set of data (Noble and Ebeler, 2002).

Mulitvariate data analysis has been used to find correlations between instrumental and sensory data in a variety of food/beverages including wine (Noble and Ebeler, 2002), in tomatoes (Berna et al., 2005), bread (Sabanis et al., 2009), cheese texture (Drake et al., 1999) and textural changes in chocolate (Andrae-Nightingale et al., 2009) and chocolate aroma (Paper VII). Although the presence of a correlation may be informative, it does not always mean that a cause and effect relationship exist but rather that both variables (sensory and instrumental data) change in a similar manner. There may be several reasons for such co-variation.
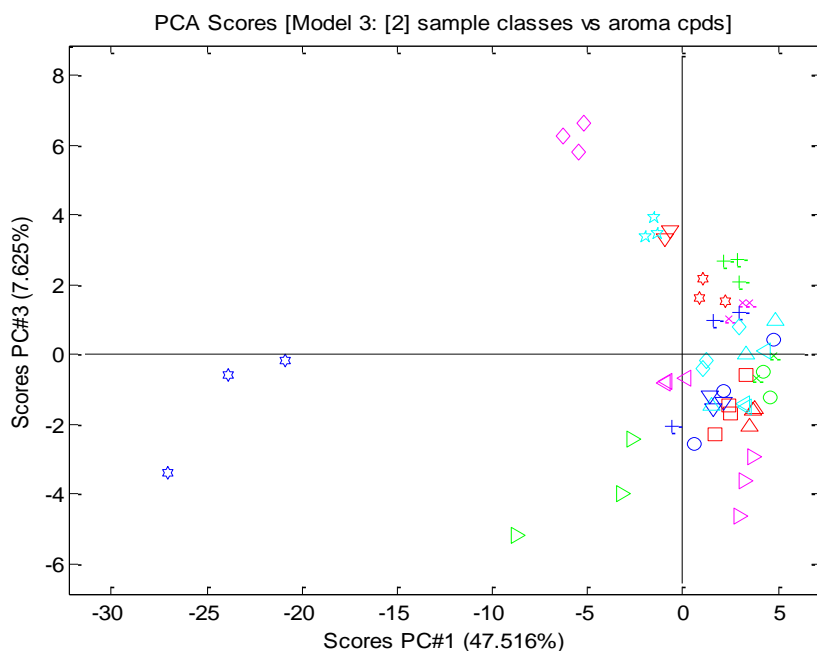
Fig. 18. PCA score plot showing clustering of replicate samples of chocolate.

PCA was used to visualize the clustering of replicate samples to establish confidence in a sampling method. A good clustering of replicates meant that mean values could be used in

subsequent PCA models. Fig. 18 shows a score plot obtained from a PCA of replicate (triplicate) samples of dark chocolates produced from heap and tray-fermented cocoa beans and with different roasting and conching conditions. PCA models were used to visualize complex datasets from GC-MS analysis of chocolate. These enabled relationships between different chocolate samples and identified aroma compounds to be established (Paper IV-VI).

In the investigation of the effect of fermentation method, roasting and conching conditions on the aroma compounds of chocolate, a reduction of GC-MS data from 86 compounds to 16 important compounds selected based on the number of judges that detected them during GC-O, and subsequent PCA on these resulted in an increase in the total variance explained by two PCs from 60 to 81%. This meant that as much as 81% of the variation in the samples could be explained by these 16 important compounds, leaving only 19% unexplained (Paper VI).

5. Chocolate aroma

One of the most important characteristics of chocolate that has made it popular among many people is its unique and complex aroma. Unfermented beans have the odor and taste of vinegar, are astringent and bitter (Belitz et al. 2004). The aroma of chocolate may vary depending on the origin/genotype of the cocoa, the fermentation method and the chocolate manufacturing processes used. Chocolate manufacturers are constantly seeking for ways to improve the flavor of their products and to introduce new and more exciting chocolate flavors. Cocoa has a delicate aroma/flavor and may easily be tainted by off-flavors from the environment during fermentation, drying, storage and/or transportation which affect the quality of the final product. These may include smoky and moldy off-flavors. It is important, therefore, to avoid such contamination to maintain the flavor quality of the finished product.

5.1   Composition of chocolate aroma

The volatile compounds that make up the aroma of chocolate are diverse and mainly derived from the primary processing of cocoa and the chocolate production processes. Following the pioneering work of Bainbridge and Davies, (1912) who isolated about 20 g of aroma oil by steam distillation of 2000 kg of cocoa beans, several researchers have analysed volatiles from cocoa and cocoa products. More than 600 of these volatiles have been found (Van Praag et al., 1969; Maniere and Dimick, 1979; Dimick, 1983; Gill et al., 1984; Ziegleder, 1991a; Nijssen et al.,1996; Schnermann and Schieberle, 1997). The processes that lead to the formation of aroma compounds and precursors in cocoa and chocolate have been well researched and reported by these workers.

The aroma component is mostly composed of pyrazines, thiazoles, oxazoles, pyrroles, pyridines, furans, amines, aldehydes, ketones, esters, alcohols and acids (Dimick and Hoskin, 1981; Schnermann and Schieberle, 1997; Bonvehi, 2005; Paper I-VI). Among the groups of compounds that make up the aroma of chocolate, alkylpyrazines are considered very important contributors due to their low odor threshold and their sensory significance (Silwar, 1983; Belitz et al., 2004)).

The components of chocolate aroma may be inherent with the genotype or type of cocoa beans used, derived from the primary processing of fermentation and drying or from the chocolate manufacturing processes namely roasting and conching. Proper handling and optimization of these processes is therefore paramount to produce a final product of good aroma quality. This is a continuous and major objective for researchers, cocoa producers as well as chocolate manufacturers.

5.2 Optimization of sampling method for isolation of chocolate volatiles

In view of the already mentioned advantages of dynamic headspace sampling (4.2.2), this method has been used to isolate the volatile fraction of cocoa and chocolate samples in a number of reported investigations (Aculey et al., 2010; Papers I-VI). To use this method however, some important factors have to be considered to get the best out of it. These include the nature of sample, sample size, sampling temperature and the volume of carrier gas (Wampler, 2002). Increasing purge time of dynamic headspace sampling is known to result in the recovery of more volatile analytes from a sample (Varming et al., 2004).

Chocolate presents a difficult matrix in view of its high fat content and the complexity of its volatile fraction ranging from small highly volatile molecules to less-volatile heterocyclic compounds. In addition, chocolate is a product that has already gone through processes that involve the use of high temperatures such as occurs during roasting and conching and much as high sampling temperatures will ensure volatilization of the aroma compounds in the sample, there is also the risk of forming new compounds or destroying temperature-unstable ones. Pini et al (2004) reported a sharp increase in the extracted amounts of some pyrazinic compounds from cocoa using Headspace Sampling by Solid Phase Micro-Extraction (HS-SPME) at temperatures higher than $60\ ^o$C and attributed this to the onset of Maillard reaction between residual substrates in the sample.

It was also essential to obtain a purge gas flow volume that will enable as much volume of the sample headspace containing the volatiles to be trapped and at the same time prevent the analytes from 'breaking through' the adsorbent material in the trap instead of being adsorbed or trapped.

Sample size was another factor that was considered in the optimization since all chocolate samples used in the investigations were hand-made and takes time to produce enough for experiments.

5.2.1   Dynamic headspace running conditions

The different conditions investigated were:

- Sample size: 5, 10, 20 and 50g
- Sampling temperature: 30, 40 and 50°C
- Purge time: 30 and 60 min
- Purge flow: 100, 150 and 200 mL/min

Each sample was analysed in triplicate and identification done by GC-MS. Other details of the sampling procedure and the GC-MS analysis of the volatiles are as described in paper II, IV and VI.

5.2.2   Formulation of chocolate samples for method optimization

All chocolate samples were produced at the Innovation Laboratory of Toms Confectionery Group A/S, Ballerup, Denmark from Ghanaian cocoa beans. The semi-sweet dark chocolates contained about 62% cocoa mass. Details of the procedure used in producing the chocolates are described in Paper II.

5.2.3   Results of optimization experiment

Fig. 18 shows a typical chromatogram obtained from GC-MS analysis of a chocolate sample.

Twenty and 50 g of chocolate sampled at 50°C for 60 min with a carrier gas flow of 200 ml/min gave more number of volatiles than sampling under the other conditions (Table 8). Based on these results and the fact that least amount of sample size was preferred, the sampling parameters chosen for subsequent experiments were 20 g at 50°C for 60 min with a flow of 200 ml/min.
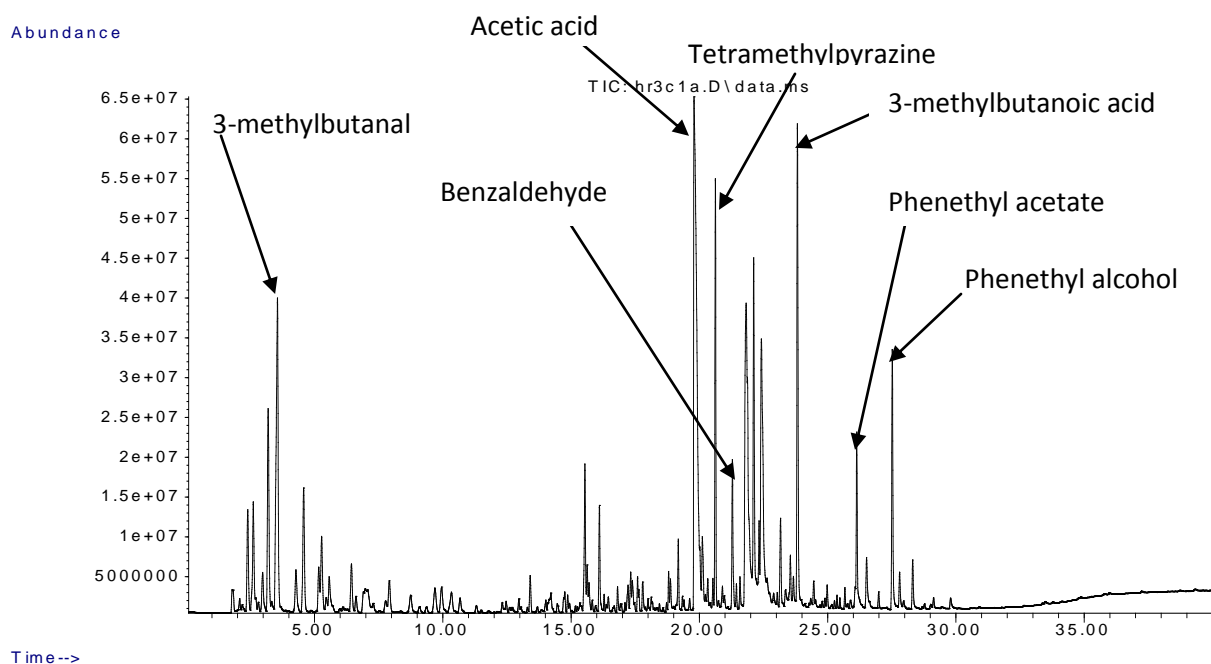
Fig. 19. Typical GC-MS chromatogram used to identify the aroma volatiles in dark chocolate isolated by the optimised dynamic headspace sampling method.

5.2.4   Reproducibility of optimized dynamic headspace sampling method

To determine the reliability of the optimized dynamic headspace sampling method for the isolation of chocolate volatiles, three samples of dark chocolate were produced from the same batch of cocoa beans using the same recipe. Aroma volatiles were isolated using the optimized method and analyzed by GC-MS. Each sample was analyzed in triplicate. ANOVA showed that only three (Table 9) out of fifty-one aroma volatiles isolated and identified had significantly different (p<0.05) peak areas for one particular sample of chocolate.

5. Chocolate aroma

Table 8. Aroma volatiles isolated by DHS of chocolate with different sampling conditions.

	Peak area x 10⁶															
	40ºC, 100ml/min				40ºC, 200ml/min				50ºC, 100ml/min,				50ºC, 200ml/min			
	30min		60min		30min		60min		30min		60min		30min		60min	
Compound	20	50	20	50	20	50	20	50	20	50	20	50	20	50	20	50
Butanal, 2/3-methyl-	26	32	24	32	38	29	26	31	61	60	31	30	53	44	103	53
Pentanal	5	5	6	4	5	4	4	4	9	9	7	7	10	9	19	12
Hexanal	3	5	7	2	5	3	5	6	6	0	5	0	7	10	17	11
2-Heptanone	.	-	-	-	-	-	-	-	-	-	-	-	6	1	2	4
Heptanal	-	-	2	-	-	-	-	-	-	-	-	-	-	-	4	5
1-pentanol	3	-	-	2	3	-	-	5	5	-	-	-	-	-	7	14
3-hydroxy-2-butanone	1	1	1	1	-	1	-	-	3	3	5	5	5	4	10	11
2-octanone	-	1	-	-	-	-	-	-	-	-	-	-	1	2	2	2
Octanal	-	-	-	-	-	-	-	-	-	-	-	7	-	1	2	2
Hexanol	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4	4
2,3,5-trimethyl pyrazine	4	14	14	2	3	3	8	12	3	3	12	12	11	20	19	18
Nonanal	41	26	18	104	37	44	19	141	168	219	98	98	143	72	144	174
Acetic acid	3	7	14	2	3	3	6	12	5	-	10	10	11	18	22	24
1-octen-3-ol	-	1	2	-	-	4	-	-	-	-	2	2	1	2	3	3
Tetramethylpyrazine	-	-	1	-	-	-	-	-	-	-	1	1	1	1	2	2
2-decanone	4	7	13	2	4	-	9	2	5	-	8	8	9	14	19	21
Benzaldehyde	-	-	-	-	-	-	-	-	1	1	3	3	2	3	4	5
Propanoic acid	-	-	-	-	-	-	-	-	-	-	4	4	2	4	3	43
1,3-butanediol	-	3	8	-	-	-	2	2	2	1	7	7	4	11	11	12
N-octanol	3	5	8	2	2	3	2	1	5	4	15	15	9	17	26	28
2-methylpropanoic acid	-	3	7	-	-	-	-	-	-	-	27	27	10	27	37	44
2,3-butanediol	-	-	-	-	-	-	-	-	-	-	-	-	-	10	23	18
1,2-propanediol	-	-	-	-	-	-	-	-	2	2	7	7	5	-	11	10
2(3H)-furanone, dihydro-	-	-	-	-	-	-	-	-	-	-	-	-	12	3	3	2
Butanoic acid	-	-	-	-	-	-	-	-	-	-	1	1	4	3	2	2
Furfural alcohol	2	6	10	2	2	-	-	11	5	0	15	0	12	21	25	29
3-methylbutanoic acid	-	-	-	-	-	-	2	5	-	-	1	1	1	1	2	2
Ethanone, 1-phenyl-	3	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-
Benzeneacetic acid, ethyl ester	1	2	5	-	-	-	2	33	-	-	3	3	2	6	6	6
2-phenylethyl acetate	-	-	-	-	-	-	-	-	-	-	2	2	-	5	4	4
Hexanoic acid	8	2	3	5	-	-	2	17	7	-	18	18	20	39	12	13
Butanoic acid, butyl ester	-	-	2	-	-	-	-	-	-	-	1	14	1	3	3	3
Phenethyl alcohol	-	-	1	-	-	-	-	1	-	-	-	-	-	2	2	2
Phenol	-	-	1	-	-	-	-	-	-	-	-	-	-	5	-	2

Nine of the aroma volatiles were significantly different for replication. The results indicate that the optimized method could be reliably used to isolate aroma compounds from chocolate.

Table 9. Analysis of variance on aroma volatiles with significantly different peak areas for chocolate samples.

Compound	Chocolate sample		
	CHOCO1	CHOCO2	CHOCO3
Benzyl alcohol	78803[b]	57480[b]	135586[a]
Nonanone	156400[b]	142389[b]	236279[a]
1-Phenyl-ethanone	184533[b]	177039[b]	259085[a]

*Values with different letters across a row are significantly different ($p < 0,05$).

5.3 Influence of cocoa fermentation method on chocolate aroma

Cocoa fermentation is the most critical process for the development of aroma precursors that develop into specific aroma compounds during the chocolate manufacturing processes of roasting and conching (Jinap et al., 1995; Counet et al., 2002; Bonvehi, 2005).

In Paper I and II, the influence of two cocoa fermentation methods - heap and tray - on the aroma of dark chocolate produced from them was studied. These are the two methods currently practised by farmers in the fermentation of cocoa in Ghana. It has been shown that aeration of the fermenting mass has an effect on the microbial population of the fermentation (Camu et al., 2008) and this will eventually affect the types and levels of aroma compounds formed. Good aeration during fermentation is ensured by the tray method (Allison and Rohan, 1958; Allison and Kenten, 1963). Jespersen et al., 2005 as well as Nielsen et al., 2007 have reported on the microbial composition/dynamics of heap and tray-fermenting Ghanaian cocoa beans using molecular methods. Recently, Aculey et al. (2010) reported on the volatile compounds in tray-fermented beans from different cocoa-growing regions of Ghana. Since dried, fermented cocoa beans are not consumed on their own but are raw materials for making other products, especially chocolate, it is relevant to know the fate of these volatiles in the final product. In addition

knowledge of the important contributors to the perceived flavor of these chocolates is also necessary.

In spite of the numerous publications on the aroma compounds identified in cocoa and chocolate, scarcely is the fermentation method used to produce the samples mentioned or identified compounds and perceived flavor of chocolate related to the type of fermentation used in producing the raw material, cocoa. In this thesis, the investigation was therefore undertaken to:

- Investigate and compare the aroma volatiles in dark chocolate produced from heap and tray-fermented Ghanaian cocoa beans.
- Identify the important odorants in the two types of chocolate
- Determine the sensory differences, if any, between the flavor of the two types of chocolate and the reasons for the observed differences as far as the identified aroma compounds are concerned.

The results of the investigation are reported in Paper I and II.

5.3.1   Aroma volatiles in 'heap' and 'tray' dark chocolates (Paper I and II)

Two chocolate samples were produced from traditional heap-fermented Ghanaian cocoa beans and experimental tray-fermented Ghanaian cocoa beans from the Cocoa Research Institute of Ghana (CRIG), using the same recipe. Volatiles in the 'heap' and 'tray' chocolates were isolated by the optimized dynamic headspace sampling method already described and identified by GC-MS. Only volatiles which are potential aroma compounds were considered in this study.

Aroma volatiles identified in the two types of chocolate included alcohols, acids, aldehydes, esters, furans, ketones, pyrazines, a pyrrole, a sulphur compound, and a phenol. Similar groups of compounds have been identified by other studies on cocoa, chocolate and other cocoa products (Dimick and Hoskin, 1981; Counet et al., 2002; Frauendorfer and Schieberle, 2006; Afoakwa et al., 2009; Aculey et al., 2010). Differences in the number and levels of these compounds identified by different authors may be a result of different varieties/origins of cocoa used, the chocolate processing methods and different sampling methodologies used. For instance,

Counet et al., 2002 identified eighteen pyrazinic compounds but in the present study, only ten pyrazines were identified. The fewer number may be as a result of the mild temperature of 106°C used to roast the beans.

GC-MS peak areas of most aroma compounds identified were generally higher in the chocolate sample produced from heap fermentation than in that produced from tray fermentation (Paper 1). On the other hand, the levels of a few aroma compounds were higher in 'tray' than in 'heap' chocolate. This included acetic acid and linalool. The peak area of the latter was significantly higher in 'tray' than in 'heap' chocolate. The higher level of acetic acid in 'tray' chocolate could be an indication of a better fermentation by the aerobic acetic acid bacteria due to the improved aeration of the fermenting cocoa mass. The acid has been recognized as a key metabolite for the cocoa bean fermentation process (Camu et al., 2008; Schwan and Wheals, 2004). Camu et al., 2008 reported increased production of acetic acid in a cocoa heap that was fermented with turning after 48 h and 72 h. Fermenting in trays may be synonymous with turning heap fermentation as they both result in better aeration and increased growth of acetic acid bacteria (Schwan and Wheals, 2004; Camu et al., 2008). However due to high levels of acetic acid being linked to sourness in chocolate (Lopez and Dimick, 1991; Schwan and Wheals, 2004; Camu et al., 2007), it is also important to combine tray fermentation with roasting and conching regimes that will ensure its reduction.

5.3.2 Important odorants and flavor of 'heap' and 'tray' dark chocolates (Paper II)

The important odorants or odor-active compounds in 'heap' and 'tray' chocolates were evaluated using GC-O. Only odorants perceived by at least three judges were considered and the GC-SNIF method (Pollien et al., 1997; Varming et al., 2004; Nielsen and Poll) used to identify the key odorants in the two chocolates (Paper 1I).

Twenty-nine odorants were detected by at least three judges in heap and tray chocolate samples during GC-O. Phenylacetaldehyde described as bitter/green/grassy odor and a non-separable mixture of 2- and 3-methylbutanal with a chocolate/cocoa/roasted odor were considered important to the aroma of both 'heap' and 'tray' chocolates based on their SNIF values. These

compounds have frequently been found by other researchers. Counet et al., 2002; Bonvehi, 2005; Frauendorfer and Schieberle, 2006; Afoakwa et al., 2009 among others identified these compounds as important components of cocoa and cocoa products.

Two acids, 3-methylbutanoic acid (unpleasant/old cheese/sweaty) and acetic acid (sharp/vinegar) were also among the important odorants identified probably due to their high levels in both chocolates. These acids have frequently been identified as important odorants in chocolates and other cocoa products by Schnermann and Schieberle, 1997; Counet et al. 2002; Bonvehi, 2005; Frauendorfer and Schieberle, 2006. Acetic acid in particular, is implicated as the cause of acidic/sour flavor in chocolates and other cocoa products (Lopez, 1983; Jinap and Dimick, 1990; Camu et al., 2008) and it is therefore important that its concentration is kept to a level that does not give the finished product an undesirable flavor. The level of acetic acid was found to be higher in 'tray' than in 'heap' chocolate but the difference was not significant ($p>0.05$). The higher level of acetic acid associated with the tray sample is probably a result of the improved aeration in the tray fermentation method. Camu et al., 2008, reported increased production of acetic acid with enhanced aeration of cocoa fermentation due to increased population of acetic acid bacteria. A trained quantitative descriptive panel did not find the intensity of sourness in 'tray' chocolates any more than that in the heap sample (Fig. 20). This indicates that the level of the acids in both chocolates were not undesirably high.

Linalool also identified as one of the most important odorants in 'tray' but not 'heap' chocolate is known to give a flowery, tea-like odor to cocoa and is related to the fermentation method (Gill et al., 1984; Hansen et al., 1998). The compound is reported to be odor-active in cocoa and cocoa products (Bonvehi, 2005; Frauendorfer and Schieberle, 2006; Afoakwa et al., 2009). 'Tray' chocolate is generally perceived to have a fruitier/flowery flavor than 'heap' chocolate (various personal communications). This was confirmed by the trained panel as they scored 'tray' chocolate significantly higher for fruity and yoghurt flavors.
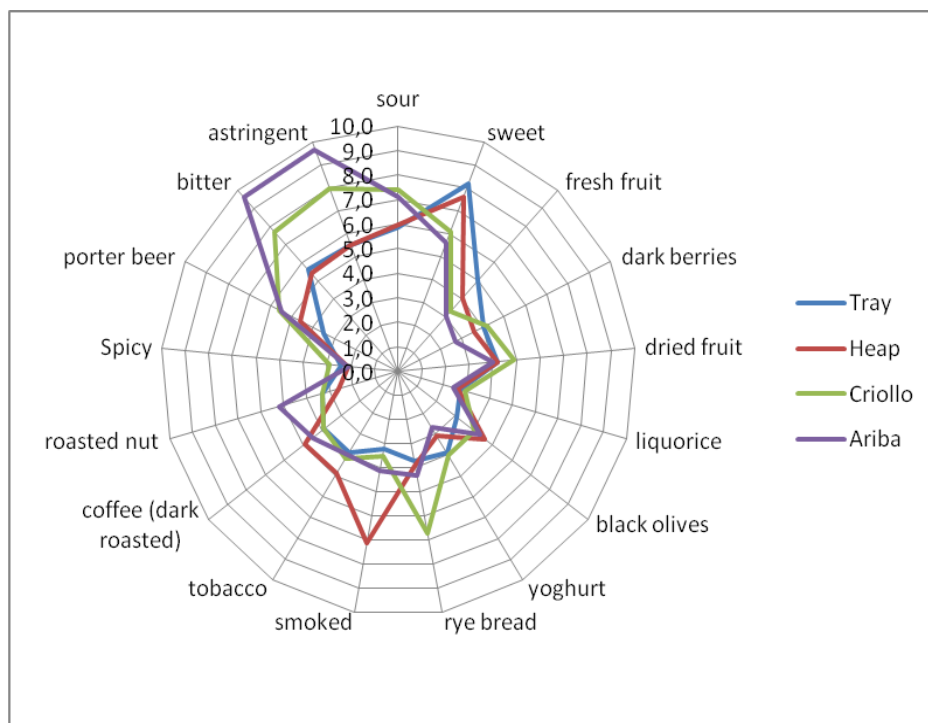
Fig. 20. Sensory profile of 'Heap' and 'Tray' chocolates ('Criollo' and 'Ariba' chocolates were used as references) (paper II).

This corresponded well with the observed higher SNIF (Surface of Nasal Impact Frequency) value and a significantly higher GC-MS peak area of linalool in 'tray' chocolate than in 'heap' chocolate. A PCA plot (Fig. 21) showed fresh fruit, yoghurt and sweet flavors to be positively correlated. These were related to 'tray' chocolate. The three afore-mentioned attributes were however negatively correlated with bitterness and astringency, meaning that chocolate being high in bitterness and astringency was typically low in fruity, yoghurt and sweet notes. Misnawi et al., (2004) found an inverse correlation between astringency and sweetness in cocoa. High levels of bitterness and astringency probably mask these desirable attributes in cocoa and chocolate (paper II).
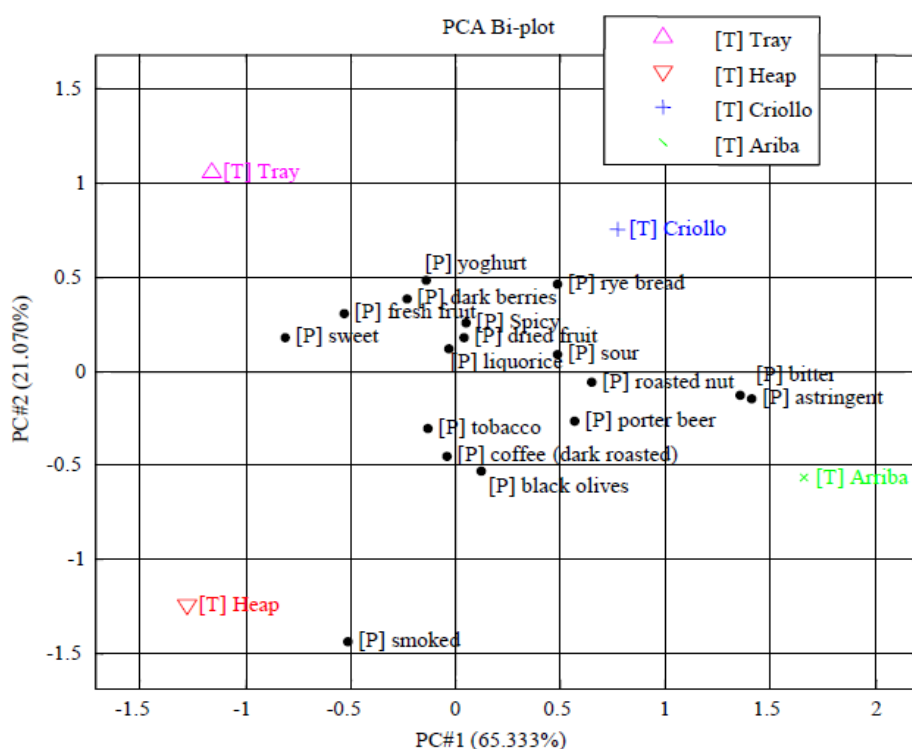
Fig. 21. PCA bi-plot of quantitative descriptive analysis of 'Heap' and 'Tray' chocolates (Criollo and Ariba were used as references) (Paper II).

It can be concluded that chocolates produced from heap and tray-fermented Ghanaian cocoa beans differ in flavor characteristics. This difference is probably as a result of differences in the levels of some of the most sensorially important odorants in the two chocolates, among others linalool. It is therefore possible to obtain desirable flavor characteristics in chocolate produced from Ghanaian cocoa beans (close to that obtained from fine-flavored beans like Criollo) by using a cocoa fermentation method that ensures better aeration of the fermentation such as occurs in tray fermentation (Paper II). This however has to be combined with proper fermentation of the beans coupled with optimization of the chocolate manufacturing processes of roasting and conching.

5.4   Influence of cocoa fermentation duration in trays on chocolate aroma (Paper III and IV)

Fermentation of cocoa beans occurs in stages, each stage being critical for the growth of particular microorganisms and/or the formation of flavor precursors. Under-fermentation results in purple beans and undeveloped chocolate flavor whilst over-fermentation results in a rise in bacillus growth and moulds which can cause off-flavors (Schwan and Wheals, 2004).

At the onset of fermentation, a succession of yeasts, acetic acid bacteria and lactic acid bacteria colonize the fermenting cocoa at particular durations (Fig. 25). Each of these groups of microorganisms is responsible for the production of metabolites that influence the aroma of fermented cocoa beans. Schwan and Wheals, 2004; Jespersen et al., 2005,Camu et al., 2007; Nielsen et al., 2007 and other researchers have reported on the succession as well as the activities of various microooorganisms during cocoa bean fermentation. Jespersen et al., 2005; Nielsen et al., 2007 and Kostinek, 2008, in particular have studied the dynamics and/or diversity of various microorganisms involved in tray fermentation.

Aculey et al., 2010 investigated the volatile compounds isolated from Ghanaian cocoa beans tray-fermented for different durations and reported the formation of some important compounds such as 2-phenethyl acetate, propanoic acid and acetoin (3-hydroxybutanone) and the breakdown of others such as diacetyl (2,3-butanedione) during fermentation. The formation and breakdown of different compounds during different stages of the cocoa fermentation process culminate in aroma/flavor precursors that define the sensory characteristics of chocolate or other products produced from it. Whilst the traditional heap cocoa fermentation method has been well-researched, the tray method is relatively new, especially to farmers; therefore there is the need for more investigations in order to optimize the method for obtaining good quality cocoa beans from it.
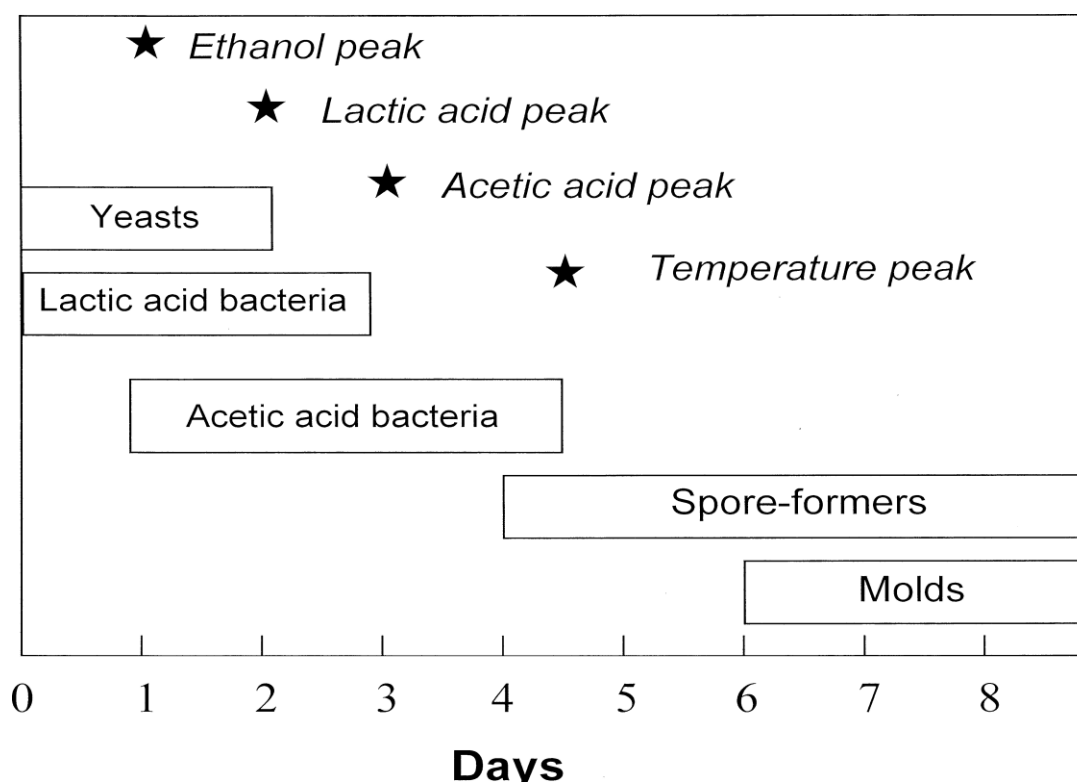
Fig. 22. Schematic diagram of a microbial succession during cocoa bean fermentations in Bahia, Brazil. Stars indicate timing of peak of metabolites and temperature (Ref. Schwan and Wheals., 2004).

Although the main source of most aroma compounds in chocolate is the roasting process, proper fermentation is necessary for the formation of the precursors of these compounds. Moreover, some aroma compounds are formed during fermentation mostly by microbial synthesis. These include mostly alcohols, esters and acids. There are also reports of microbial synthesis of some known maillard reaction compounds such as 2- and 3-methylbutanal, trimethylpyrazine and tetramethylpyrazine (Singh et al., 2003; Smit et al., 2004; Gill et al., 1984; Ramli et al., 2006).

5.4.1   Influence of fermentation duration on aroma compounds

In Paper IV it was found that generally, increasing number of days of fermentation from 2-5 days resulted in an increase in the number of aroma compounds. Chocolate produced from cocoa

fermented for five days (5D) had the highest number of aroma compounds whilst the 2-day fermented sample (2D) had the lowest. However, there was no clear trend in the levels of most aroma compounds with fermentation duration. On the other hand, chocolate sample produced from 3 day-fermented beans had higher peak areas for most aroma compounds than the other three samples and this was reflected in a PCA scores and loadings plot (Fig. 23) (Paper IV).
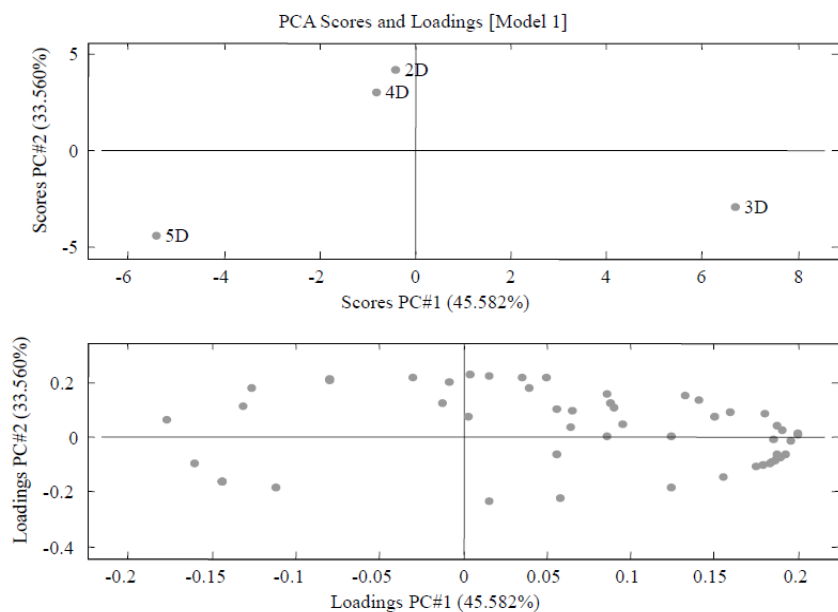

Fig. 23. PCA scores and loading plot showing distribution of aroma compounds in chocolate produced from cocoa beans tray-fermented for 2, 3, 4 and 5 days. Data is based on GC-MS peak areas (Paper IV).

The unexpected results from the 3D sample may have been caused by bad quality beans due to weather conditions during fermentation/drying or conditions during storage prior to shipment, since the samples were fermented and dried on different days.

A decrease in the level of acetic acid was observed with increasing fermentation duration. This also reflected in the sensory evaluation of the samples as 2 and 3 day-fermented samples were rated higher in sourness than 4 and 5-day fermented samples (Paper IV).

5.4.2   Influence of fermentation duration on odor-active compounds (Paper III and IV)

Fermentation duration also influenced the type of odor-active compounds detected by GC-O/GC-MS (Paper IV). Tray fermentation for 5 days produced chocolate with more odor-active pyrazines than 2, 3, or 4 days fermentation. Probably, more of the precursors of these compounds have been formed within five days of tray fermentation. Two unidentified odorants, one with a baked potato and the other with a sweet/flowery note respectively were also detected in only 4 and 5 days fermented samples (Paper III and IV).

5.4.3   Influence of fermentation duration on flavor attributes (Paper IV)

Aside the formation of aroma compounds and precursors, fermentation is also important for the modification of the bitterness and astringency associated with unfermented cocoa beans. These undesirable flavors are associated with polyhydroxyphenols such as catechins, flavan-3-ols, anthocyanins, and proanthocyanadins. The greater part of the polyphenols diffuse out of the beans during fermentation and drying whilst a part are oxidized to tannins (Schwan and Wheals, 2004; Camu et al., 2008). Undesirable flavor characteristics such as astringent, bitter and sour decreased with increasing fermentation durations whilst desirable attributes such as sweet, flowery and fruit generally increased with increasing days of fermentation. However, similar to the results of the GC-MS analysis, the sample produced from the 3 day fermentation did not follow this trend as it was rated less for sweet, fruit and flowery than even the 2 day fermented sample. The latter was also more astringent, bitter and sour than the other three samples (Figs. 24 and 25) (Paper IV).
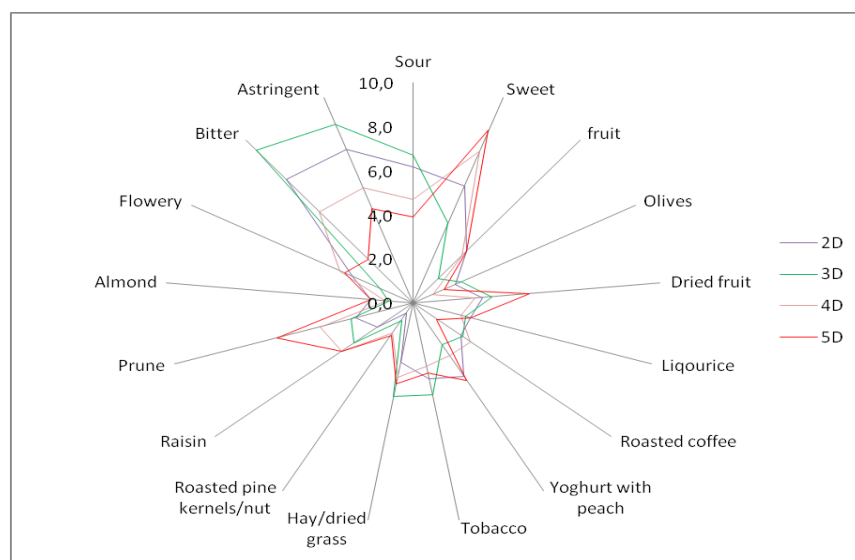
Fig. 24. Intensities of seventeen flavor attributes used to describe four chocolate samples produced from 2, 3, 4, and 5 days tray cocoa fermentation (Paper IV).
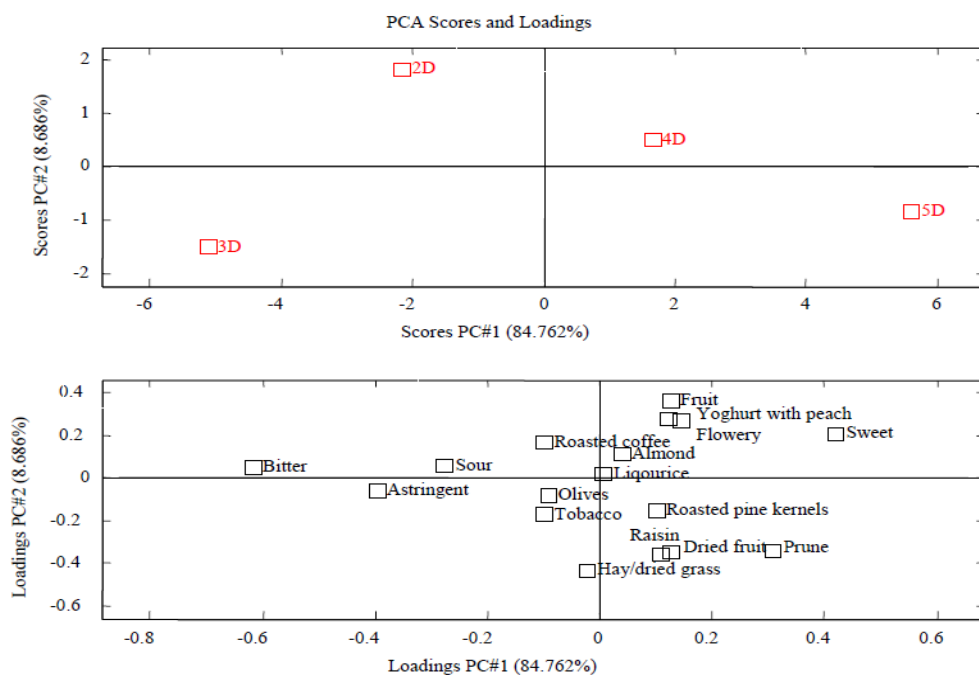

Fig. 25. PCA score and loadings plot of quantitative analysis data of four chocolates produced from tray cocoa fermentation of 2, 3, 4 and 5 days fermentation (Paper VII).

Conclusions of this investigation are only tentative for now as the experiment is being repeated in view of the unexpected results obtained for the 3-day fermented sample which may have been caused by bad quality beans due to weather condition during fermentation/drying or conditions during storage prior to shipment. The repeated experiment will confirm the already obtained results or otherwise and only then can conclusive remarks be made on all samples. However, it is obvious that whilst under-fermented tray cocoa samples will result in sour, bitter and astringent chocolate, proper tray fermentation for 4-5 days will result in the complete development of flavor precursors which give chocolate with a sweet, fruity and flowery flavor. Tray fermentation of at least four days is also required to reduce undesirable flavor characteristics such as bitterness, sourness and astringency in chocolate.

5.5 Influence of fermentation method, roasting and conching conditions on chocolate aroma (Papers V -VII)

The method of cocoa fermentation, roasting and conching are three important processes that determine the flavor of chocolate. Whilst fermentation results in the production of the initial aroma compounds and precursors of those to be formed later, roasting and conching processes involve high temperatures that ensure the development of flavor compounds from precursors. Off-flavors in the chocolate are also excluded through these processes by the elimination of undesirable volatile compounds including acetic acid, which in large amount are detrimental to the flavor quality of the final product (Jinap and Zeslinda, 1995).

It is also known that along with undesirables compounds, some desirable volatiles are also lost through evaporation especially during conching resulting in a decrease in overall flavor (Hoskin and Dimick, 1983; Plumas et al., 1996; Counet et al., 2002). Until now, these three important chocolate aroma-determining processes have been studied individually but production of chocolate typically involves a combination of all three processes. Therefore an investigation of the combined effect of these processes on the aroma of chocolate is important in a bid to optimize them.

While researchers tend to agree on the effect of roasting on the aroma compounds of chocolate, there are conflicting reports on the influence of conching on flavor of chocolate. Counet et al., 2002 showed that although no key aroma compounds were formed during conching, the levels of others such as branched pyrazines increased significantly whilst most Strecker aldehydes were lost through evaporation. Hoskin and Dimick, 1983, reported that the conching temperature, and the concentration of precursors after roasting will be too low for the Maillard reaction. Reports by Heinzler and Eichner, 1991; Plumas et al., 1996 and Counet et. al., 2002, Fischer et al., 2008 however, indicate decrease in concentration of aroma compounds and overall flavor during conching of chocolate. According to Ziegleder, 2005, about 40% of the acetic acid and volatiles compounds of chocolate are removed during conching.

5.5.1   Influence on aroma volatiles (Paper V and VI)

All three processing factors, fermentation method, roasting and conching conditions had an effect on the type and levels of aroma compounds identified, roasting however had a more pronounced effect (Paper V and VI). Although roasting at a higher temperature (150°C for 30min) resulted in the production of the most aroma compounds in both 'heap' and 'tray' chocolates, their levels were generally significantly reduced through long conching duration of 10 h. The temperature as well as the duration of roasting is important in determining the type and level of aroma volatiles formed. The optimum roasting temperature and duration for production of flavor compounds may however be different from that for sensory quality. Ramli et al., 2006 reported that an optimum production of major flavoring compounds occurred at $160\ ^{\circ}$C for 30 min of roasting but roasting at $150\ ^{\circ}$C for 30 min gave the best sensory qualities of lowest astringency and bitterness, as well as low levels of sourness and bitter taste. Other parameters of thermal processing of cocoa beans, such as humidity and flow rate of air, however, are also known to affect the quality of the final product (Ramli et al., 2006).

Long conching duration was almost synonymous with low temperature roasting as far as the total number of aroma volatiles was concerned. Conching generally reduced the concentration of most aroma compounds formed during fermentation and roasting (Paper V and VI). Fischer et al.,

2008 observed an overall reduction in most aroma compounds during chocolate conching and reported that the total aroma contained in chocolate is distributed over three different phases; fat, water-soluble material (sugar and protein) and insoluble material (cocoa solids) Conching primarily affects the aroma content of the fat phase whilst that in the other two phases remain constant. Perception of aroma is however driven by the content of the fat phase (Fischer et al. (2008).

5.5.2   Influence on most important aroma compounds (Paper VI)

PCA based on a reduced GC-MS dataset involving important compounds perceived during GC-O resulted in an increase in the variation explained by two PCs from 60% to 81%. The sixteen compounds were considered the most sensorially important to the aroma of the chocolates since they were detected by all judges in at least one sample. They included 2,5-dimethylpyrazine (popcorn) and 2-ethyl-5-methylpyrazine (roasted, coffee), and 2,3,5-trimethylpyrazine (fried potato) all of which are known to be products of the Maillard reaction. An inseparable peak of two Strecker aldehydes, 2- and 3-methylbutanal (cocoa, roasted); 5-methyl-2-phenyl-2-hexenal (sweet/cocoa/roasted) as well as 1,2/3-butanediol and benzyl acetate (sweet/flowery) from the fermentation process were also identified as important compounds. The two latter odorants remained significantly higher at all conching durations in the tray than in the heap samples, probably contributing to the reported fruity/flowery flavor of chocolates produced from tray-fermented cocoa (Paper VI).
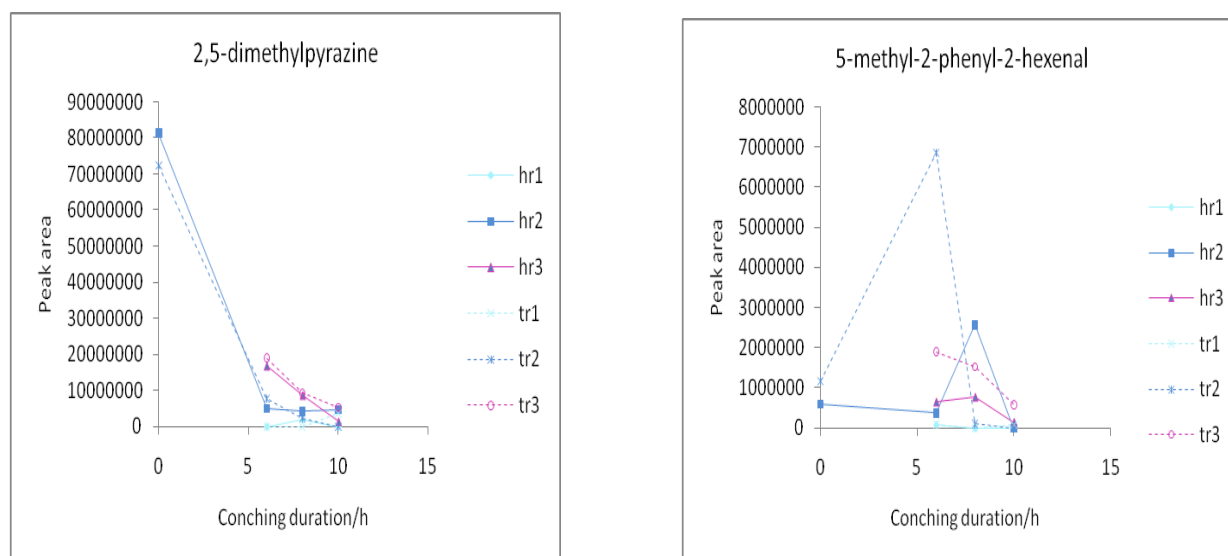
Fig. 26. GC-MS peak areas of two key odorants as influenced by fermentation method, roasting and conching conditions. h: heap; t: tray; roasting at r1:100°C/100 min; r2: 120°C/45 min; r3: 150°C/30 min (Paper VI).

Most of these important odorants generally increased with high roasting at 150°C but decreased with long conching duration from 6-10 h. On the other hand, 5-methyl-2-phenyl-2-hexenal, a known dehydrated aldol condensation product between phenylacetaldehyde and other Strecker aldehydes, has been identified as an important constituent of roasted cocoa aroma (van Praag, 1968; Counet et al., 2002; Bonvehi, 2005; Ramli et al., 2006; Afoakwa, 2009). Medium roast (120°C) resulted in a sharp increase in the peak area from 117 x 10^{4} (for 0 h of conching) to 685 x 10^{4} (in 6 h of conching) but from then on there was a sharp drop up to 10 x 10^{4} (in 8 h of conching) and then a leveling off until 10 h of conching when it was not detected at all. A significant increase in the level of 5-methyl-2-phenyl-2-hexenal from 307 ppm to 546 ppm after conching of dark chocolate has been reported by Counet et al., 2002.

Since the GC-MS dataset was reduced to only represent the volatiles that are most important from a sensory point of view, it can be expected that the heap and tray fermented samples in general will have different sensory quality and that this difference becomes more pronounced

when samples are roasted at the higher temperatures (120 or 150 °C) and conched for rather short time (6-8 hours) or not conched at all (Paper VI).

5.5.3   Influence on sensory flavor attributes (Paper VII)

Differences in the types and concentrations of aroma compounds related to chocolate samples that differ in processing conditions are expected to be reflected in the flavor (taste and smell) of chocolates. Roasting was more effective in reducing astringent flavor in chocolate than conching (Paper VII). It is known that both fermentation and drying reduce the levels of polyphenols which are responsible for this attribute.

Roasting oxidizes the residual polyphenols to quinones which are known to be very reactive agents. Polyphenols are also known to react with proteins and amino acids or polymerize with each other to form tannins most of which also have bitter and astringent sensations (Ziegleder, 1991a; Heinzler and Eichner, 1991b; Kattenberg and Kemmink, 1993; Misnawi et al., 2005). However, roasting reduces the ability of polyphenols to react with proteins, thereby reducing astringency (Misnawi et al., 2005). Misnawi et al. (2005) also mentioned the possible alteration of polyphenols during chocolate manufacturing processes that involve high temperatures and the presence of oxygen such as conching. Although tray chocolate roasted at 150°C for 30 min and conched for 6 h was scored less for astringency than the unconched tray sample (roasted at 120°C for 45 min), the difference was not significant and the observed difference may have been due to the higher roasting temperature the latter was subjected to (Paper VI).

Table. 10. Analysis of variance on flavor attributes with significantly different intensities for chocolate samples (Paper VI).

Attribute	hr0c2	tr0c2	hr2c0	tr2c0	hr3c1	tr3c1
Yoghurt a	9.5^{a*}	6.0^{ab}	4.7^{b}	5.8^{ab}	7.8^{ab}	6.7^{ab}
Fruit	3.6^{bc}	3.1^{c}	6.6^{ab}	6.9^{a}	4.8^{abc}	4.0^{abc}
Banana	3.7^{b}	4.2^{b}	8.6^{a}	6.1^{a}	3.4^{b}	3.6^{b}
Musty t	3.8^{b}	8.2^{a}	4.4^{ab}	5.1^{ab}	2.8^{b}	5.7^{ab}
Prune/raisin	7.8^{ab}	9.6^{a}	7.1^{ab}	5.7^{ab}	9.5^{a}	4.4^{b}
Sweet	6.6^{ab}	6.5^{ab}	8.5^{ab}	5.3^{b}	10.3^{a}	5.5^{b}
Smoked t	5.8^{bc}	8.5^{b}	4.6^{bc}	12.8^{a}	2.6^{c}	8.3^{b}
Astringent	10.9^{a}	11.2^{a}	7.9^{b}	9.5^{ab}	5.6^{c}	9.8^{ab}

*Values with different letters across a row are significantly different ($p < 0.05$).

Chocolate conched for a long duration is generally perceived to be richer in overall chocolate flavor. This perception has been attributed to an improvement in the texture of the chocolate with long conching duration (Fischer et al., 2008). A reduction in undesirable characteristics such as sourness, bitterness and astringency during conching obviously plays a role in this perception.

A PCA bi-plot (Fig 27) used to visualize the relationship between the sensory attributes and chocolate samples accounted for a total explained variance from three PCs of 53% (Fig. 23). Conching reduced the perception of both banana and fruity flavor significantly in both heap and tray chocolates. This was reflected in the close relationship of these two attributes with the two unconched samples in the PCA bi-plot.
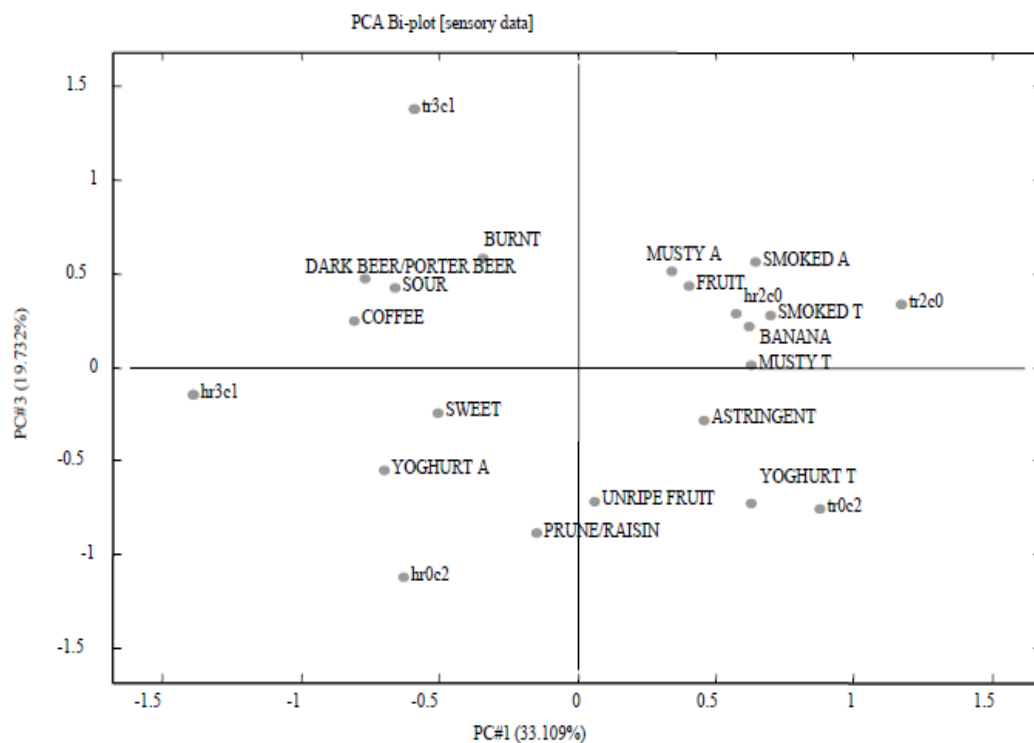
Fig. 27. PCA bi-plot of sensory data (Paper VI).

The relatively high level of smoked taste perceived in the tray samples may have been a contamination from the source of the cocoa beans rather than from the roasting during the chocolate production as both roasted and unroasted samples were rated significantly higher for this off-flavor. Conching, however, seemed to have reduced the perception of this off-flavor (Paper VI).

Tray-fermented cocoa beans used in producing samples in an earlier investigation (Paper I and II) seemed to be of a higher quality than that used in the present investigation (VI). The former were obtained from the Cocoa Research Institute of Ghana (CRIG) and the fermentation seemed to have been well-carried out as compared to the latter samples which were obtained from cocoa

farmers. The tray fermentation technology is still new to a lot of farmers and obviously, cocoa farmers need more training in the method to produce good quality beans.

5.5.4   Correlation of instrumental and sensory flavor measurements (Paper VII)

The use of headspace sampling and Gas chromatography enables the isolation and identification of volatiles in a food matrix closer to what happens in inhaled or sniffed aroma than is obtained from solvent extraction (Hachenberg and Schmidt, 1977; Charalambous, 1978). Sensory evaluation is however important for predicting the actual flavor/aroma of food as perceived by a trained panel. Reports of the influence of flavor perception by taste-aroma interactions that occur cognitively (Noble, 1996; Prescott, 1999) and the possibility of volatiles masking each other or interacting to produce aromas with different characteristics than the compound alone (Noble and Ebeler, 2002) makes the use of both instrumental and sensory measurements essential in the study of flavor in a food matrix. A good understanding of the flavor of any food partly involves relating the sensory perception of its flavor with its volatile chemical components (Drake et al., 2007). Multivariate statistical methods such as PLS do not determine whether any of the compounds associated with specific sensory attributes are responsible for those aroma notes, they however give an indication of compounds on which subsequent sensory evaluations should focus (Nobel and Ebeler, 2002).

PLS enabled the modelling of flavor attributes from important aroma compounds detected during GC-O (Paper V). The sensory attribute fruit was highly explained by the aroma compounds than the other attributes. Six important aroma compounds were highly correlated with fruit, and these were ethyl-3-methylbutanoate (fruity, flowery); 2,5-dimethylpyrazine (popcorn); 2-dihydro-(3H)-furanone (sweet); linalool oxide (sweet, flowery); benzaldehyde (earthy, nutty) and 2/3-methylbutanal (cocoa, roasted). Although only ethyl-3-methylbutanoate was described as having a fruity note, according to Nobel and Ebeler, 2002, subsequent sensory evaluations on fruitiness in dark chocolate samples can focus on these six important compounds (Paper VII).

A PLS model based on these six compounds gave a better model than one based on all sixteen important aroma compounds. Fig. 28 shows the actual versus predicted plots of the two models.

The first model had a correlation coefficient (correlation between measured and predicted values) of 0.55 and an error (Root Mean Square Error of Cross Validation, RMSECV) of 1.62, whilst the second had a correlation coefficient of 0.83 and a reduced error of 0.84 with two components (which gave the least error).

Since fruit was found to be higher in unconched samples (see 5.5.3), it may be possible to use the concentrations of the important compounds with high correlation coefficients with respect to this attribute to predict conching in dark chocolate.

Additionally, 5-methyl-2-phenyl-2-hexenal (sweet, roasted) which was positively correlated with astringent, as well as ethyl-3-methylbutanoate and pentyl acetate both with negative correlations to this attribute, successfully modelled astringent. Although astringent mouth-feel in chocolate is a taste perception caused by polphenols which are not aroma compounds, the use of multivariate analytical tools may allow a means of predicting this sensory attribute in dark chocolate based on the concentrations of some important aroma compounds.
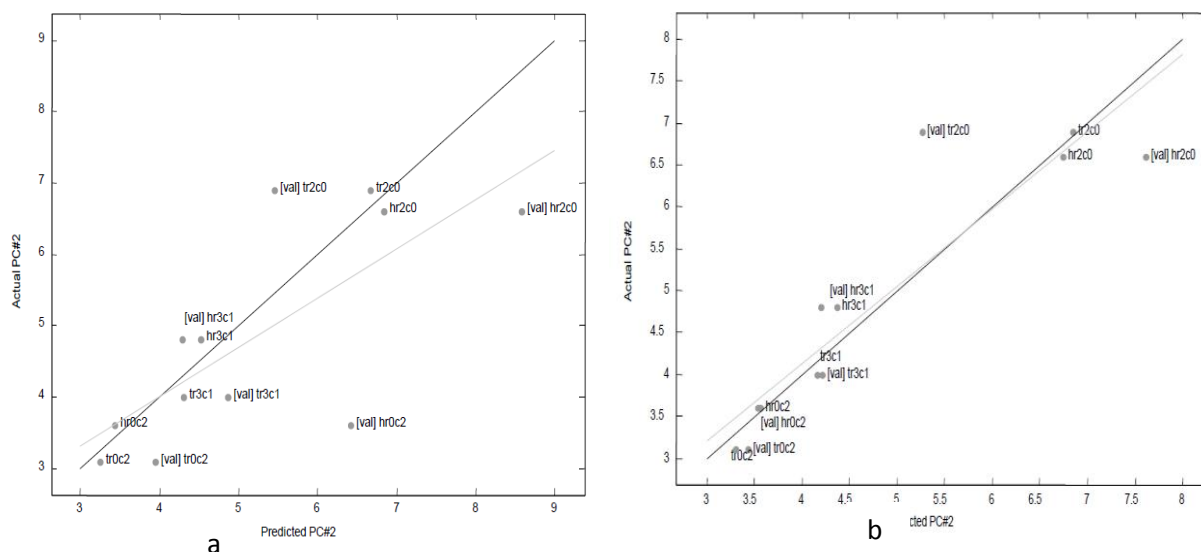
Fig. 28. Actual compared with predicted plots of PLS models based on; a: sixteen important aroma compounds and the sensory attribute with highest explained variance, fruit; b: six important aroma compounds with high correlation with respect to the sensory attribute, fruit (Paper VII).

This is especially important since the concentration of polyphenols in cocoa and chocolate is known to be related to the fermentation and roasting processes (Ramli et al., 2006; Misnawi et al., 2004). Unroasted samples were found to be higher in astringency than roasted samples. The three important aroma compounds highly correlated with astringency can also therefore be used as indicators for roasting in dark chocolate (Paper VII).

6. Conclusion

- This study has contributed to the already existing knowledge on the factors determining aroma in chocolate. In particular it has shown that there are differences in the type and concentrations of aroma compounds between chocolates produced from heap cocoa fermentations and tray cocoa fermentations and these differences were reflected in the flavor characteristics of the two types of chocolate. An optimized dynamic headspace method enabled the efficient isolation of volatile compounds in chocolate.

- The study has for the first time investigated the combined effect of the three most important processes – fermentation method, roasting and conching conditions - that determine aroma in chocolate on the aroma compounds.

- Roasting and conching affected mostly the levels of these compounds. Whilst roasting at a high temperature increased the levels of most aroma compounds, conching for longer duration reduced the levels. The effect of fermentation method was more pronounced with roasting at a high temperature coupled with short conching duration or no conching at all, an indication that long conching duration levels out this effect.

- The effect of fermentation method as well as roasting and conching conditions were more pronounced in compounds that were most sensorially important.

- Conching reduced the perception of fruitiness as well as musty and smoky off-flavors in chocolate.

- Roasting was more effective in reducing astringency than conching.

## 6.	Conclusion

Using multivariate analytical tools (Principal Component Analysis, PCA and Partial Least Squares Regression, PLS-R) models, it was possible to find relationships between important aroma compounds and sensory flavor attributes. Six important odorants modelled fruitiness and since these were more in unconched samples, it may be possible to use the concentrations of these important compounds to predict whether a sample is conched or unconched in dark chocolate. Furthermore, three important odorants modelled astringency in chocolate which was higher in unroasted samples. The latter can also therefore be used as indicators to determine whether a sample is roasted or not.

- Additionally, this study showed that 4-5 days of tray cocoa fermentation is required to develop aroma/flavor precursors that ensures the formation of some important aroma compounds during chocolate processing. Fermenting cocoa by the tray method for less than four days resulted in chocolate with a sour, bitter and astringent flavor whilst 4-5 days fermentation resulted in a sweet, fruity and flowery chocolate.

- Finally, the study has shown that it is possible to obtain desired good quality chocolate from Ghanaian cocoa beans (close to that obtained from fine-flavored beans like Criollo) with improved fermentation method coupled with proper fermentation of the beans and optimization of the chocolate production processes of roasting and conching.

7.   Persepectives – Recommendations for further studies

Chocolate is fast becoming not only a luxury food but also a health food in view of the many acclaimed benefits from consuming especially dark chocolate. In spite of this, consumers' desire for good quality chocolates, especially with regard to flavor/aroma has not dwindled. If good quality chocolate is to be obtained, especially from Ghanaian cocoa, which constitutes more than 20% of the world's cocoa, farmers should be trained to strictly adhere to fermentation practices and adopt new improved methods. In this wise, the tray method offers good prospects of obtaining good quality raw material with a shorter fermentation period and a more uniform quality for the chocolate industry. Proper training of farmers in the method is a pre-requisite in this regard.

Although this study has been limited to Ghanaian cocoa beans, the results may largely be applicable to cocoa from other origins/genotypes and the prospects of this should be pursued.

Results by the trained sensory panel used in this study showed that chocolates produced from heap-fermented and tray-fermented Ghanaian cocoa beans differ in flavor characteristics. It will be interesting to know the results of a preference test involving 'heap' and 'tray' chocolates produced with different roasting and conching conditions. Such a study should involve actual consumers of chocolate.

This study showed a reduction in the levels of most aroma compounds during conching but it will also be interesting to trap the headspace of a conche to identify exactly which compounds and how much are lost through evaporation since there is the possibility of some of the aroma compounds in the chocolate prior to conching being involved in recombination reactions during the process that involves a high temperature. Furthermore, desirable compounds 'wasted' to evaporation could be harnessed and a process found to incorporate them back into the chocolate after conching to improve the aroma/flavor of the final product.

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