Variation of Flavor Quality of Chocolate with Conching Conditions and Composition of Raw Materials

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Fatma ALBAK

ABSTRACT

VARIATION OF FLAVOR QUALITY OF CHOCOLATE WITH CONCHING CONDITIONS AND COMPOSITION OF RAW MATERIALS

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The present study was carried out to develop aromatic chocolates with spices (ginger, cinnamon, and aniseed), lemon peel powder and pistachio nut paste without causing a major modification in their traditional structures. The spices were added as fine powders before conching consisting of three phases: dry, pasty, and liquid phases. The consequences of their interaction with cocoa mass were studied as far as the whole chocolate flavor is concerned. Qualitative and quantitative identification of volatiles were carried out by GC/MS/SPME and GC/MS/O before and in the course of conching, while texture analyses were accomplished by a texture analyzer. Variation in the quantities of total polyphenols, theobromine and caffeine was also carried out using HPLC, and UV-visible spectrophotometry. 13, 23, 25, and 35 principal compounds were identified and quantified for aniseed cinnamon, ginger and lemon peel powder chocolates respectively. Ginger and lemon peel powder chocolates were evaluated as the most aromatic both sensorily and instrumentally. Except pistachio nut chocolate, ginger, aniseed, cinnamon and lemon peel chocolates were observed to have all acceptable textures and aroma profiles. The conching time was optimized according to the most acceptable aroma profile of volatiles using Design Expert software. The results showed that- except the acetate esters- the quantities of the initial volatile components increased up to 270 minutes and decreased after that time.

Key words: Chocolate, aniseed, ginger, cinnamon, lemon peel powder, pistachio nut paste, and conching.

ÖZET

KONÇLAMA ŞARTALARINA VE ÇİKOLATANIN KOMPOZİSYONUNA BAĞLI OLARAK ÇİKOLATANIN AROMA KALİTESİNDEKİ DEĞİŞİM

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176 sayfa

Bu çalışmayla, geleneksel yapılarında önemli bir değişiklik yapmadan, baharat (zencefil, tarçın, anason), limon kabuğu tozu ve antepfıstığı ezmesi ile aromatik çikolataların geliştirilmesi amaçlandı. Katkı maddeleri, üç aşamalı (kuru, macun ve sıvı) fazdan oluşan konçlama öncesinde ince toz olarak eklendi. Bu maddelerin, konçlama sırasında kakao kitlesiyle etkileşiminin çikolatanın tüm lezzetine olan etkisi araştırıldı. Uçucuların nitel ve nicel tespiti, konçlama öncesi ve konçlama sırasında, GC/MS/SPME ve GC/MS/O cihazları, kıvam (tekstür) analizleri tekstür analizörü, toplam polifenol, teobromin ve kafein miktarlarındaki değişim ise HPLC ve UV-visiblespektrofotometre ile gerçekleştirildi. Anason, tarçın, zencefil ve limon kabuklu çikolatalar için sırasıyla 13, 23, 25, ve 35 ana bileşiğin nitel ve nicel analizi yapıldı. Zencefil ve limon kabuklu çikolatalar hem duyusal hem de aletsel olarak en aromatik numuneler olarak değerlendirildi. Antep-fıstıklı çikolata hariç zencefil, anason, tarçın ve limon kabuklu çikolataların kabul edilebilir kıvam ve aroma profillerinin olduğu gözlendi. Konçlama zamanı optimizasyonu, uçucu maddelerin en kabul edilebilir aroma profiline göre, Design Expert yazılımı yardımıyla yapıldı. Sonuçlar, asetat esterleri haricinde başlangıç uçucu bileşen miktarlarının 270 dakikaya kadar arttığını, bundan sonra ise azaldığını gösterdi.

Anahtar Kelimeler: Çikolata, anason, zencefil, tarçın, limon kabuğu tozu, antepfıstığı ezmesi ve konçlama.

TO MY FAMILY

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ABBREVIATIONS

ANOVA: Analysis of varience

AOCS: American oil chemists'society

CAR/PDMS :Carboxen/Polydimethylsiloxane

df: Degree of freedom

DI-SPME: Direct immersion solid phase microextraction

DRC: Vertically stirred rotary conches

DSC: Differential scanning calorimetry

DVB/CAR/PDMS : Divinylbenzene/Carboxen/Polydimethylsiloxane

GC/MS/O: Gas Chromatography/mass spectrometry/olfactometry

GC: Gas chromatography

HCC: Heidenauer continuous conche

HPLC-MS: High performance liquid chromatography-mass spectrometry

HS-SPME: Headspace solid phase microextraction

LLE: Liquid- liquid extraction

Ln: Natural log

MS: Mass spectrometry

NFCS : Polyphenol-rich nonfat cocoa solid content

OAV: Odor activity value

OICC :Office International du Cacao et du Chocolat

PA :Polyacrylate

PDMS/DVB :Polydimethylsiloxane/Divinylbenzene

RF: Response factor

SPME: Solid phase microextraction

TAGs: Triacylglycerols

UV:Ultraviolet

CHAPTER I
INTRODUCTION

The conching process is of paramount importance in terms of flavour formation of dark chocolate. During this process chemical and physical processes are taking place currently and should not be separated from each other. These include the development of fully desirable chocolate flavour and also the conversion of powdery, crumbly refined product into a flowable suspension of sugar, cocoa, and milk powder particles in a liquid phase of cocoa butter. Conching consists of thrree phases: dry phase, pasty phase and liquid phase. Flavor formation, texture and viscosity development of dark chocolate take place in these phases during conching. GC/MS and GC/MS/O are used for characterization of chocolate aroma. In this study, volatiles, affecting the aroma of dark chocolate, and any change in these volatiles during the whole three phases of conching were investigated with GC/MS and GC/MS/O.

Food product development initiated the preservation and it prolongs the shelf life of foods. Thus food became avaliable for people outside the harvesting season. Afterwards, traditional food has been produced in large factories and mechanization starts for food production. Nowadays, new product development becomes obligatory for the company in competitive global world market to strengthen their position in the market. Also food product development is necessity to provide food for growing world population. In this study, spicy chocolate and chocolate with pistachio nut paste were developed and these products were investigated in tems of quality. GC/MS, GC/MS/O, DSC, texture analyser, polorized light microscobe, rheometer, and UV-Visible spectrometer were used for the determination of the quality parameters of these chocolates, which were also assessed sensorily.

CHAPTER II
LITERATURE SUMMARY

2.1 History of Chocolate

Aztecs of Mexico are the first cultivators of cocoa trees. They used the cocoa bean for obtaining a spiced drink called as 'Chocolatl' (Whymper, 1912). Columbus introduced the Europe with cocoa as a result of curiosity. However, afterwards Don Cortez explored cocoa as a new drink (Minifie, 1980). The Spaniards sweteened this drink and the sweetened cocoa quickly became popular in Europeans. Nicholas Sanders is the first person who introduced the chocolate with milk in 1727 (Cook, 1984).

2.2 Cocoa Tree

There are three types of cocoa trees. The first one is Criollo. It has a mild flavour but its yield is low. The second one is Forastero. It is wild and generally cultivated in West Africa in small farms. Third one is Trinitario. It is hybrid of Criollo and Forastero (Beckett, 2006).

2.3 Cocoa Pod

Cocoa pod is the fruit of cocoa trees. Color of cocoa pod changes from yellow to red when they are mature. Its shape and weight are various depending on cocoa tree variety. Generally, the weight of a mature cocoa pod changes between 200 g and 1 kg. There are 30 to 50 beans in the each cocoa pod. Cocoa pods are shown in Figure 2.1 (Fowler, 1999; Whitefield, 2005).

Figure 2.1 Cocoa pods

2.4 Cocoa Bean

Cocoa beans are seeds of cocoa trees as shown in Figure 2.2. Cocoa beans are covered by a white pulp. This pulp and cocoa pod are removed during cocoa processing. (Roelofsen, 1958; Lehrian & Patterson, 1983; Lopez & Dimick, 1995; Thompson et al., 2001; Schwan & Wheals, 2002). The beans comprise of two main parts: cotyledons (called nibs) and a small germ (the embryo plant). Chocolate aroma originates from the cotyledons (Rohan & Stewart, 1965).

Figure 2.2 Cocoa beans in the pod.

The shell which covers the cotyledon is generally used as fuel after drying. Broken fragments of cotyledon are called “nibs”. The roasated nib is directly used in chocolate industry or pressed for cocoa butter production. The remaining part of the nib is called cocoa powder after cocoa butter extraction. It is generally used in the preparation of desserts, baking goods and beverages. The composition of fresh cocoa bean is shown in Table 2.1 (Lopez, 1986).

Table 2.1 Composition of fresh cocoa bean cotyledon (Lopez, 1986)

Component	Composition (/% (w/w) w.b.)
Water	32-39
Cellulose	2-3
Starch	4-6
Pentosans	4-6
Sucrose	2-3
Fat	30-32
Protein	8-10
Theobromine	2-3
Caffeine	1
Polyphenols	3
Acids	1
Salts	2-3

2.5 Major Cocoa-Producing Countries

2.5.1 Ivory Coast

Ivory Coast is one of the biggest producers of cocoa of the world. Cocoa production began in 1970 in this country increased year by year. It provides 40% of world cocoa requirement now. Cocoa cultivators are generally smallholders, many of whom are immigrants (Beckett, 1999).

2.5.2 Ghana

Ghana began cocoa cultivation at the beginning of 19th century and it reached a maximum in the 20th century. Now, it is the second largest cocoa producer country and provides the 15 % of world production (Beckett, 1999).

2.5.3 Nigeria

The production capacity of Nigeria was around 170.000 tones in last decade. Cocoa are cultivated by smallholders but their trees are old (many are 25-75 years old) (Beckett, 1999).

2.5.4 Brazil

Brazil has developed a new system for cocoa production. Cocoa cultivation is made in big plantation and in medium size farms. Bahia is the largest cocoa production area in Brazil whose capacity reached 400.000 tons in 1985. However, this capacity fallen down to 200.000 tons by now (Beckett, 1999).

2.5.5 Indonesia

Indonesian cocoa production increased gradually. The production capacity was 100.000 tons in 1980s increased to 150.000 tons in 1990 and then raised to 325.000 tons in 1998. Cocoa is cultivated everywhere in the Indonesia but the most important production areas are Sulawesi, Sumatra and Java (Beckett, 1999).

2.5.6 Malaysia

Malaysia cocoa production capacity increased rapidly in 1980s and reached about 225.000 tons a year. However, this value decreased to 100.000 tones in 1990s. The decline was due to the higher profitability of oil palm per hectare of the plantation sector. Now cocoa is cultivated only by big plants in large areas (Beckett, 1999).

2.6 Cocoa Processing

Cocoa processing consists of three steps: fermentation, drying and roasting.

2.6.1 Fermentation

Fermentation is the first step in the chocolate flavor development. (Lehrian & Patterson, 1984; Lanaud et al., 1999). Two basic methods are used for cocoa fermentation: heap and box fermentation. Heap fermentation is used in West Africa while box fermentation is used in Asia. A heap is formed with certain amount of cocoa beans, which varies between 25 and 2500 kg, and then banana leaves are placed over the cocoa beans. Heap fermentation lasts for 5 to 6 days. Figure 2.3 shows the heap fermentation in Africa.

Figure 2.3 Heap fermentation

A special type of box is used for box fermentation. These boxes have holes for air ventilation and they allow the water present in beans and pulp to diffuse from these holes. The capacity of the boxes changes between 1 to 2 tons of beans (Beckett ,2006).

Cocoa fermentation is an enzymatic process which gives the specific flavour to cocoa beans. Fermentation consists of four main steps. The first step is the production of volatile and non-volatile organic acids as a result of metabolizing of pulp sugar. Second is formation of peptides and free amino acids as a consequence of degradation of proteins. Third step is the production of insoluble compounds, mainly o-quinones, as a result of oxidation of polyphenols. Fourth is the hydrolysis of glycosides (mainly anthocyanins) (Ziegleder, 1982; Serra & Ventura, 1997; Bonveh&Coll, 1998). Important flavour active components produced during

fermentation include: ethyl-2-methylbutanoate, tetramethylpyrazine and certain pyrazines. Other flavour precursor compounds derived from amino acids released during fermentation include 3-methylbutanol, phenylactaldehyde, 2-methyl-3-(methyldithio) furan, 2-ethyl-3,5-dimethyl-and 2,3-diethyl-5-methylpyrazine (Taylor, 2002).

2.6.2 Microbial Aspect of Fermentation

Fermentation is one of the essential steps giving the characteristic flavor of cocoa beans. Cocoa pods contain lactic acid bacteria (species of Lactobacillus, Leuconostoc, Streptococcus, Pediococcus, etc.), various spices of Bacillus (heat resistant flora), some yeasts and molds. The moisture content and temperature determine the types of mold spices to grow. Lactic acid produced during fermentation reduces the pH and this does not affect the growth of molds because they are acid-resistant. Molds reach the cotyledon (nib) during fermentation and spoil its structure. The major mold growing in the bean is the *Aspergillus* family which causes off-flavors and odors. Some types of molds can produce lipase enzyme and this causes some problems in the beans later. The source of yeast during fermentation is the surrounding environment. The major types present in the beans are Sacchoromyces spp. Bacillus spp. comes from handling and airborne contamination. The number of this spp. is small at the beginning of fermentation. Their growth is influenced by sugar content, oxygen and temperature. Finally, lactic acid bacteria are important in terms of fermentation of hexoses, pentoses, organic acid and phenolic compounds.

As seen in Figure 2.4, growth of lactic acid bacteria during fermentation depends on their optimum growth temperatures. Acetobacter and Gluconobacter oxidize ethanol during the fermentation to produce acetic acid, carbon dioxide and water (Beckett, 1999).

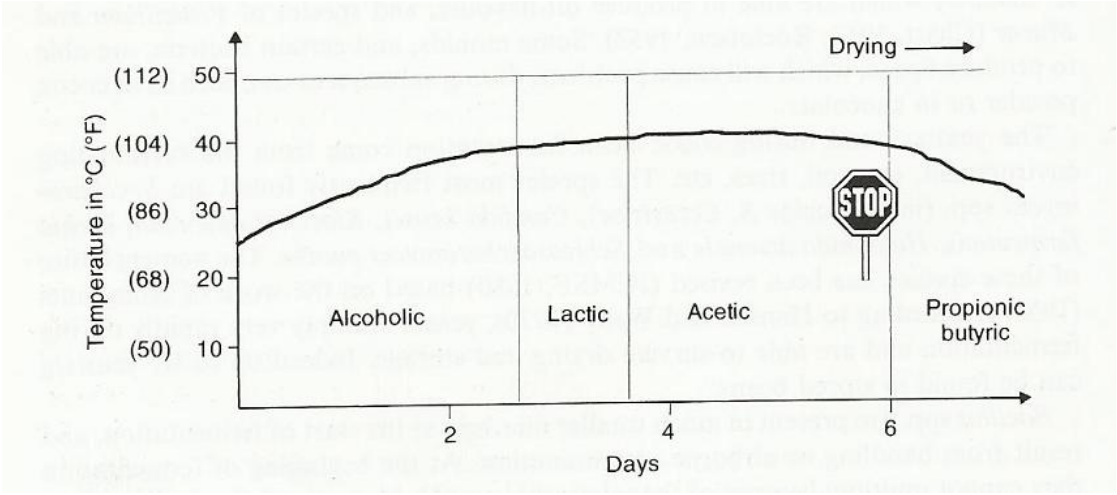


Figure 2.4 A general fermentation process (Beckett, 1999).

2.6.3 Development of Cocoa Flavor Precursors

Cocoa flavour precursors start in the cotyledon during fermentation and drying. There are two important types of cells within the cotyledons: storage cell containing fat and proteins, and the pigment cells containing phenolic compounds and xanthenes. Fermentation is initiated with germination of seed. This causes the uptake of water by protein vacuoles within cells. Later, after bean death has occurred, the cell walls and membrane break down and allow the various compound and enzyme together. These reactions produce the cocoa flavour precursors. Figure 2.5 shows how the cocoa flavour precursors occur.

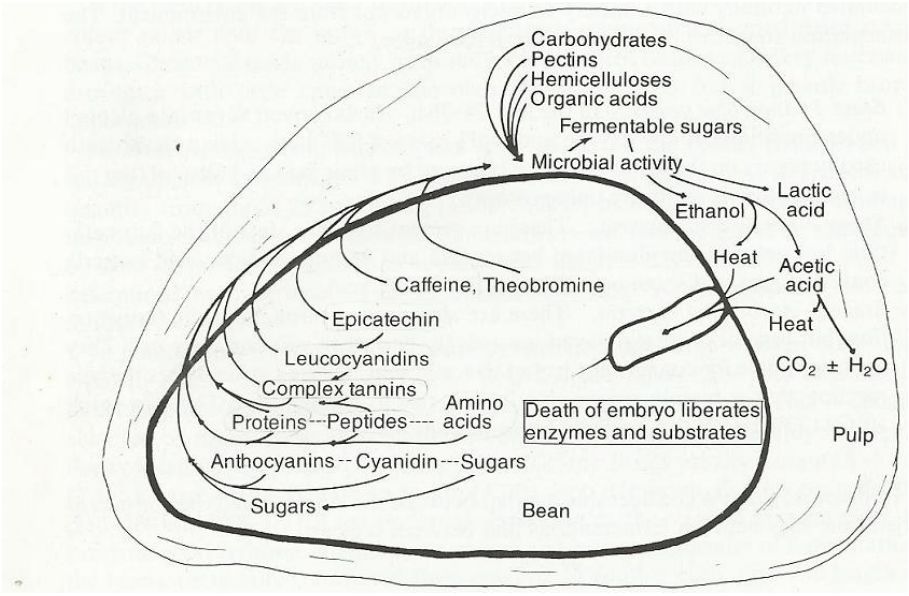


Figure 2.5 Chemical changes within a cocoa bean during fermentation (Lopez, 1986).

The temperature and level of acidity are the main parameters which determine reaction rate. There are several groups of compounds responsible for flavour. The methylxanthines, polyphenolic compounds and anthocyanins are some of them. The methyxanthines gives bitterness. The level of these substances falls to around 30% during fermentation as result of diffusion from cotyledons. Polyphenolic compounds give astringency and their levels are reduced during fermentation. Glycosidase enzymes break down the anthocyanins to cyanidins and sugar. This causes the purple cotyledons to bleach. Other enzymes convert some polyphenols to quinines. Protein complex and polyphenol combines and give brown color which is the typical color of fermentation. Some compounds formed during the Maillard reaction may be important flavor precursors (Beckett, 1999).

2.6.4 Drying

Fermented cocoa beans are immediately dried as soon as the fermentation process ends up in order to prevent seed deteoriation due to over fermentation. Two methods are used for cocoa drying: sun drying and artificial hot air drying. Sun drying is used by smallholders whereas hot air drying is used by big plants (Akpınar et al., 2003; Jain & Pathare, 2007; Jayas et al., 1991; Karathanos & Belessiotis, 1999; Midilli et al., 2002; Thuwapanichayanan et al., 2008; Yaldiz et al., 2001). Africa prefers sun drying whereas Asia prefers artificial hot air drying. Beans are spread out in the form of 100 mm thick layers on mats, trays or terraces for sun drying. Environmental contamination risk is the main problem of sun drying (Beckett, 2006). Drying is very important in terms of bean quality because chemical change during fermentation continue until moisture of beans fall down to 7%. Bean enzymes are inactive at this level. Polyphenol degradation is also related with drying**.** Enzymatic browning involves the transformation of phenolic compounds to a brown or black polymer due to the influence of polyphenols oxidase enzyme that accelerates the polyphenols oxidation reaction. The moisture content of beans decreases from about 55 to 7 % after drying. (Faborode et al., 1995; Jaquect et al., 1980; Thien et al.,1994).

2.6.5 Roasting

Roasting of cocoa beans is one of the major steps for flavour development. Aroma of beans is acidic and nutty before roasting. This step reduces the acidity of beans. For instance, concentration of acetic acid decreases during roasting however non volatile acids like oxalic acid are not affected (Beckett, 2000; Ramli et al., 2006; Granvogl et al., 2006). There are two methods for cocoa roasting: low temperature roasting at 120°C and high temperature roasting at 150°C. Low temperature roasting is used for the production of milk chocolates and certain dark chocolates. High temperature roasting, however, is used for certain dark chocolates (Kim & Keeney, 1984; Kealey et al., 2001; Awua, 2002).

Rosating is a time-tempetature dependent process. The most commonly used temperature for roasting is 135°C and a time ranging from 15 to 45 minutes. Three important changes occur during roasting: colour, flavour and texture. Humidity and air flow rate are other factors affecting the final product quality. For example, when air with high humidity is used for roasting the removal of husks from kernels is becomes easier (Benz, 2002; Finken, 1996; Minifie, 1981; Nebesny & Rutkowski, 1998; S ´Wiechowski, 1994).

Generally, the roasting process causes: (1) the synthesis of heterocyclic aromatic compounds and the possible formation of diketopiperazines (DKPs) (Ziegleder ,1991; Pino et al., 1992; Serra et al.,2000), (2) effective inhibition of polyphenoloxidase activity and (3) decreasing free acetic acidity, thus eliminating some undesirable volatile compounds of cocoa (Jinap, & Dimick,1991)

2.6.6 Winnowing

Winnowing is a process which separates the husk from the beans. The aim of winnowing is to separate the nibs in such a way as to have as large particles as possible (Beckett, 2006).

2.6.7 Grinding

Two different methods are used for the grinding process: Fine ingredient milling and combined milling. Cocoa mass and other ingredients for chocolate making are seperately milled and then mixed in the fine ingredient milling in contrast to

combined milling in which all ingredients are milled together. The two processes are likely to give a different flavour, as the sugar will pick up many of the aromas in the mill where it is being ground and in the latter case there is cocoa in close proximity (Beckett, 2006).

2.7 Chocolate Processing

The chocolate production basically consists of five stages: mixing of ingredients, refining, conching, tempering and final crystallisation and moulding. (Cidell & Alberts, 2006). The chocolate process diagram is shown in Figure 2.6

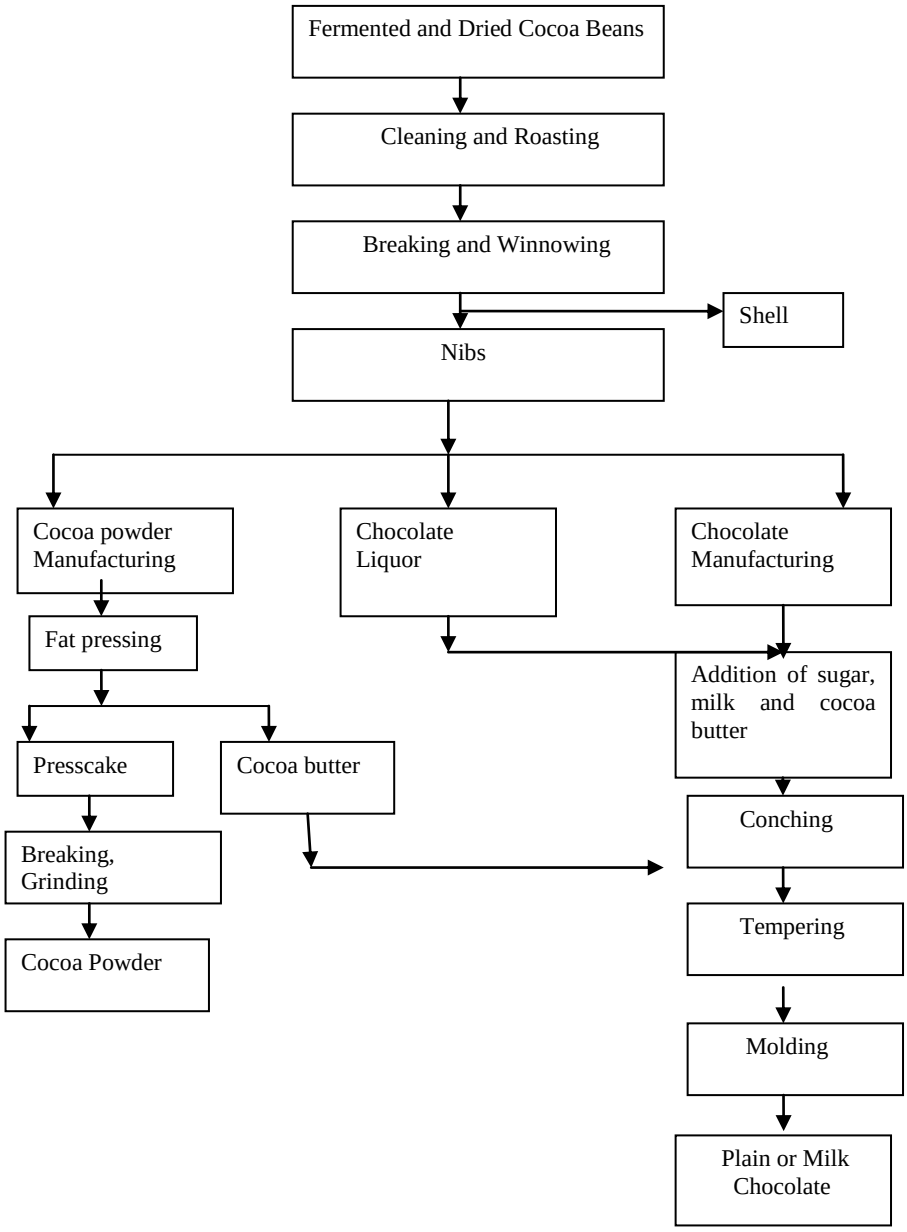


Figure 2.6 Chocolate production process (www.horiba.com)

2.7.1 Mixing and Refining

Mixing is one of the important steps in chocolate processing. Finely ground powders are mixed with each other in a fat phase. They are firstly blended with certain amount of cocoa butter in order to obtain the desired consistency for refining and then the particle size is reduced. The aim of refining is to obtain a product whose particle size cannot be detected in the mouth (Lucisano et al., 2006). There are three parameters affecting the choice of equipment for the size reduction: type of material

used, feed rate and final particle size. As shown in Figure 2.7, a five roller refiner is the most commonly used machine for particle size reduction.

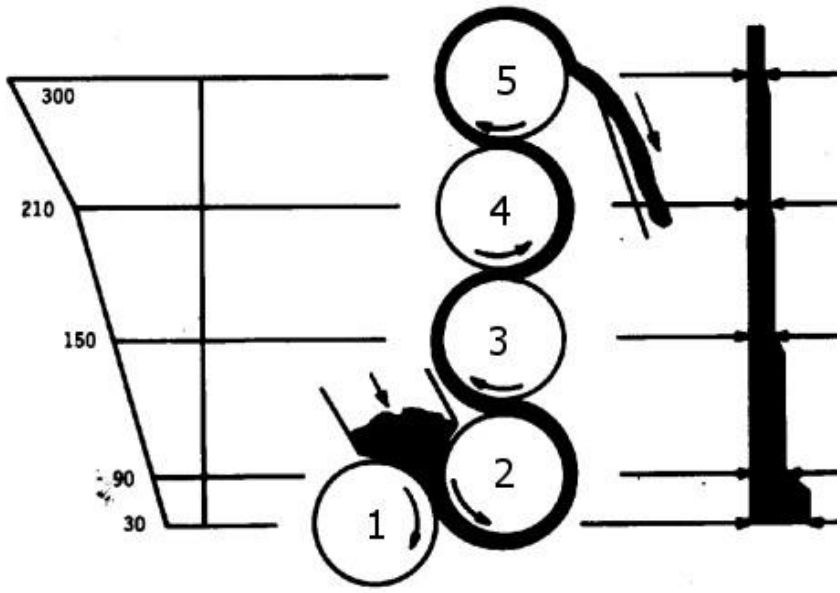


Figure 2.7 Schematic view of a 5-roller refiner (www.horiba.com)

This machine consists of five horizontal cylinders, with four of the cylinders placed one above other. The first cylinder is known as the feed cylinder which is placed below the others but on the side there is a gap between the two cylinders. This gap is known as the crushing gap and there are four crushing gaps in a five roller machine. The gape is filled with the material to be refined. Water circulated inside the cylinders provides the heating or cooling operation. Chocolate paste is removed from the cylinders with a knife and thus the chocolate mass is transferred between cylinders (Beckett, 2006).

2.7.2 Conching

2.7.2.1 History of Conching Machine

Before conching machine was invented, a stone was used to mix the chocolate. The first conching machine was invented by Rudi Lindt in 1878. This machine takes its name from the shape which looks like a conche shell (Figure 2.8).

Lindt stated that chocolate has a smoother structure with a modified flavour at the end of conching process. Granite rolls act forward and backward and thus provide a

smother chocolate. The function of the current conching machine is different from that of the past. The current conching machine changes the chemical structure of chocolate in addition to breaking the agglomerates.

Longitudinal conching machine consists of two granite stones. Some manufacturers avoided the use the metal conching machine due to the possibility of flavour modification by the metal in contact with chocolate. Even today, some producers still use the longitudinal conches instead of the modern ones. There are several disadvantages of longitudinal conches. Some of them have high energy consumption, small capacity and poor temperature control (Beckett, 1999).

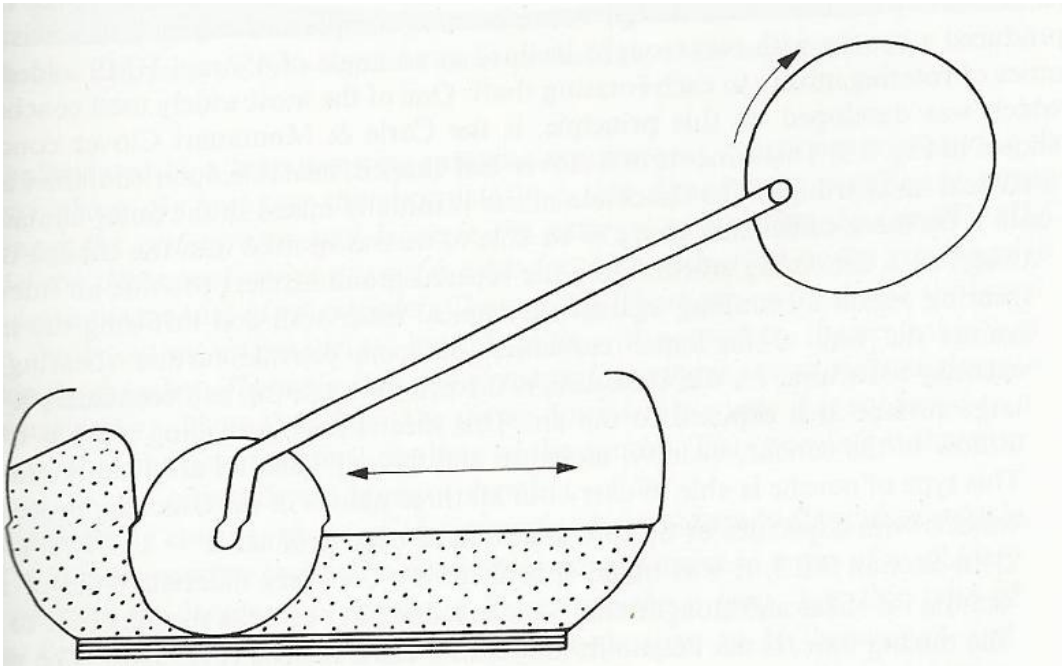


Figure 2.8 Diagram showing a longitudinal conche (Beckett, 1999).

2.7.2.2 Horizontally Stirred Rotary Conches

Horizontally stirred rotary conches are commonly used for conching as shown in Figure 2.9. This machine consists of three main parts: a clover-leaf-shaped, heatable outer container and a conical innner trough. Conching takes place in two steps: Firstly, the cocoa mass is stirred in the outer container until it becomes pasty and then it is transferred into the conical inner part (Beckett, 1999).

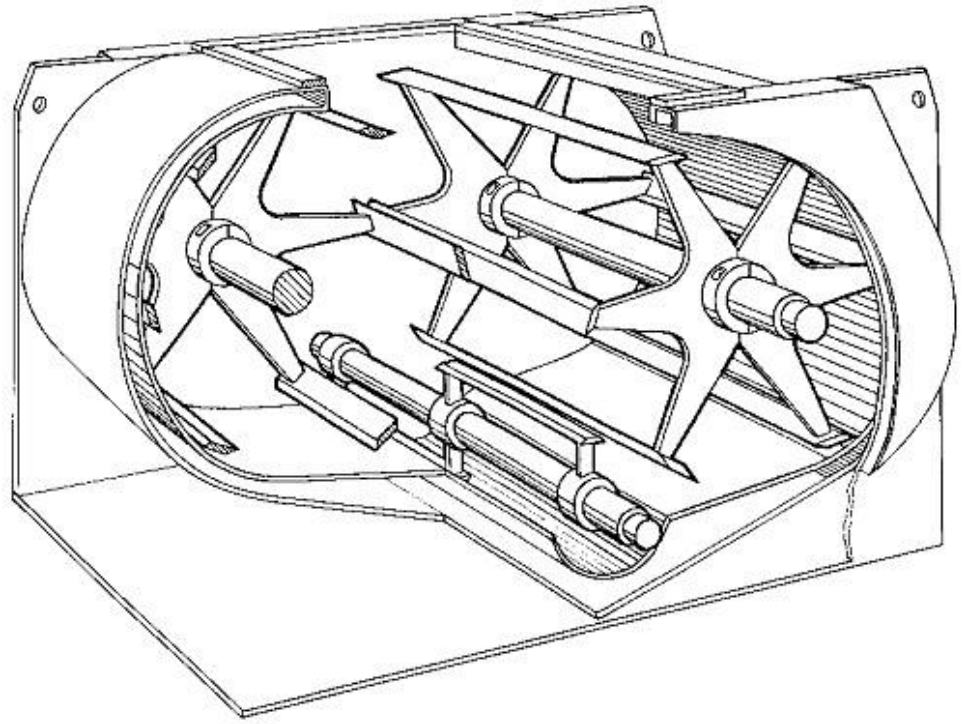


Figure 2.9 Diagram showing a Carle-Montanari Clover conche (Beckett, 1999).

2.7.2.3 Vertically Stirred Rotary Conches

There are many types of vertically stirred rotary conches. However, the most suitable machine for conching is the one with two or three chambers containing mixer/scraper arms rotating about horizantal shafts as seen in Figure 2.10. These arms may have different or identical size and can be overlapped or apart from each other. As the arm turns, the cocoa mass is raised into air. Then the arms act towards the bottom of the conch as a result of combination of gravity and centrifugal forces. Thus aeration of cocoa mass is performed and the undesired volatiles and moisture are removed from cocoa mass (Beckett, 1999).

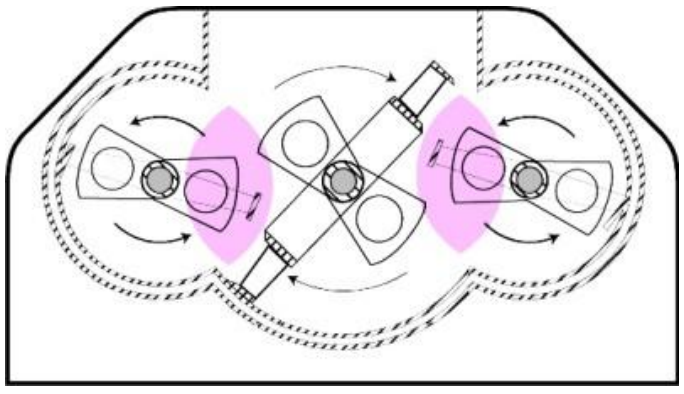


Figure 2.10 Diagram showing the Thouet DRC conch (Beckett, 1999).

2.7.2.4 Continuous Conches

Two types of continuous conching machines exist. One consists of a serial containers. Cocoa mass is passed from one container to the other with increased mixing/shearing. This type of conching requires long time and low fat. Second consists of one mixing tank. It is used for small amount of cocoa mass and short conching time. This type is suitable for chocolate plants which work with many formulations. A continuous conching machine, known as Heidenauer HCC conche is shown in Figure 2.11 (Beckett, 1999).

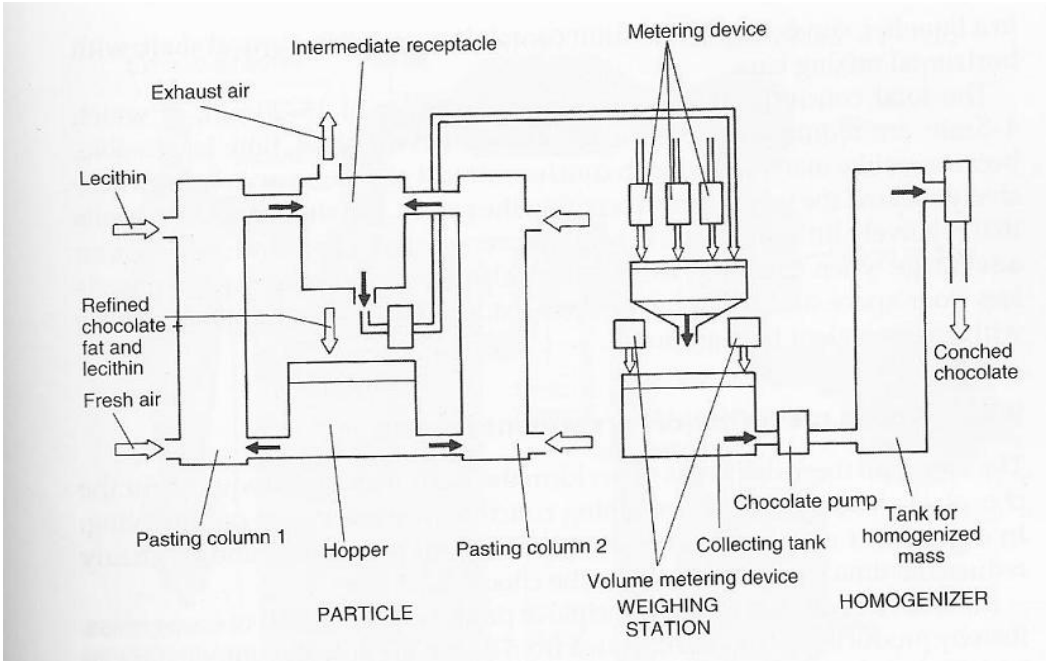


Figure 2.11 Diagram showing the Heidenauer HCC continuous conche (Beckett, 1999).

2.7.3 Conching

Conching consists of two different processes: flavor and texture development. Flavour development starts with fermentation and continues with roasting and conching. The volatiles with bad odors are in general removed during conching. Some modifications in the viscosity and texture of chocolate also take place. The dry thick paste transforms into a free flowing liquid. Particles in the chocolate mix are covered with cocoa butter and they slide over each other. Thus the cocoa mix is fluidized (Beckett, 2006).

Two methods are used for coating particles with fat: two-fold shear mixing and elongational mixing. In shear mixing, chocolate mass is transferred between two surfaces driven with motor power. Shear is increased with increasing motor power or decreasing the gap between rotors in order to decrease the viscosity of chocolate. In the elongational mixing, a high shear region between the rotor and conch surface is formed. Chocolate mass is first spread and then scraped from the conch wall. This process causes a decrease in the viscosity of chocolate (Chevalley, 1999; Windhab, 1995).

There is an inverse relationship between time and temperature of conching process. High temperatures require less conching time and vice versa. There are three phases in the conching process: dry, pasty and liquid phases as described below (Kleinert, 1997, Beckett, 1990).

2.7.3.1 Dry Phase Conching

Feed material is in the form of powder at the beginning of conching. Generally, 1% of cocoa butter is added at this stage. Cocoa mass containing 25-26% fat is heated, stirred and aerated during the dry phase as seen in Figure 2.12. The aim of this step is to evaporate the water emerging from the ingredients as this water causes agglomeration of sugar and, this results in a gritty taste in the mouth. The moisture content of chocolate falls below 1 % during the dry phase (Beckett, 1999; Franke et al.,2002).

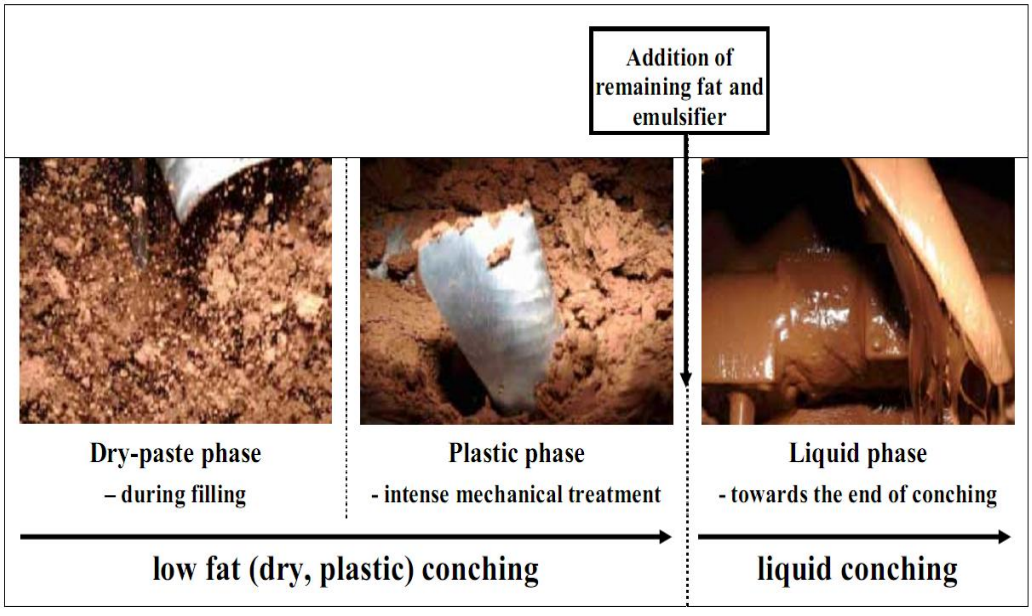


Figure 2.12 Conching stages (Source of photos: Bühler, CH Uzwil, 2004)

2.7.3.2 Pasty Phase Conching

Under permanent kneading, shear, temperature increase, and degassing, the mix enters the second pasty phase. High temperature and mixing action are used in this step to obtain desired quality of chocolate. In this stage, cocoa mix become pasty, viscosity decreases, volatile components evaporate, moisture removal continues and particles are covered with cocoa fat with high shear force and temperature (Beckett, 2009). Typical chocolate flavour forms during this phase due to volatilization of undesired volatiles causing bad odours and flavours.

2.7.3.3 Liquid Phase Conching

Additional cocoa butter and lecithin are added to chocolate paste with accompanying shear force in the liquid phase and chocolate paste is liquefied (Schantz et al., 2001). The shortest stage of conching is the liquid phase. Viscosity reaches the ultimate value and also there is a little flavor change in this stage. Temperature and shear force are gradually decreased to add emulsifier (Beckett, 2009).

2.7.4 Effect of Conching on Volatiles

Conching is the major step in chocolate process to obtain a mellow flavor, appropriate texture and viscosity. Although used for years, there are limited investigations about volatilization during conching. According to a study, there was high reduction in the headspace volatiles of semi-sweet chocolate during 44 hours of conching at 82°C (Maniere & Dimick, 1979). The quantity of low boiling flavor compounds has been reported to decrease by 26% within 6 hours of conching (Mohr, 1959). The volatile acidity is claimed to decrease in chocolate with different roasting temperatures after conching (Nebesny & Rutkowski, 1998). Conched chocolate is regarded as mellower than the un-conched and has less bitterness and acidity. Researchers believe that conching improves the flavor; not only removes the undesired volatile compounds but also retains the desired flavors (Stauffer, 2000).

2.7.5 Effect of Conching on Aroma Development

As stated, conching is the modification in the chocolate flavor, texture and viscosity. Bitterness and acidity is reduced during conching and a mellow flavour is obtained. Compounds with high volatility and short-chain fatty acids with low boiling points

and the moisture are reduced by about 30%, allowing other flavour notes to be more pronounced. Components influencing chocolate flavour such as 3-methyl-butanal, ethyl-2-methylbutaonate, dimethyl disulphide and hexanal diminish during conching. There is a small change in the amino acid and polyphenol content after conching. The flavor compounds are dispersed into cocoa fat and transfered into sugar surface during conching. Uniform aroma perception is obtained with coating of sugar particles. Porosity of sugar increases during conching and this may help the absorption of flavour components. This means that sugar acts as a flavour carrier (Beckett, 2009).

2.7.6 Tempering

Cocoa butter has 6 different crystal forms as shown in Figure 2.13. However seeds should crystallize in the correct forms to obtain a high quality chocolate. $Beta_2$ (β_2) is the only crystal form which gives proper melting point and glossy apperance at room temperature. This form is very resistant to the formation of fat bloom, which gives bad appearance to the chocolate. Another advantage of this form is to facilitate the moulding (Dhonsi & Stapley, 2005).

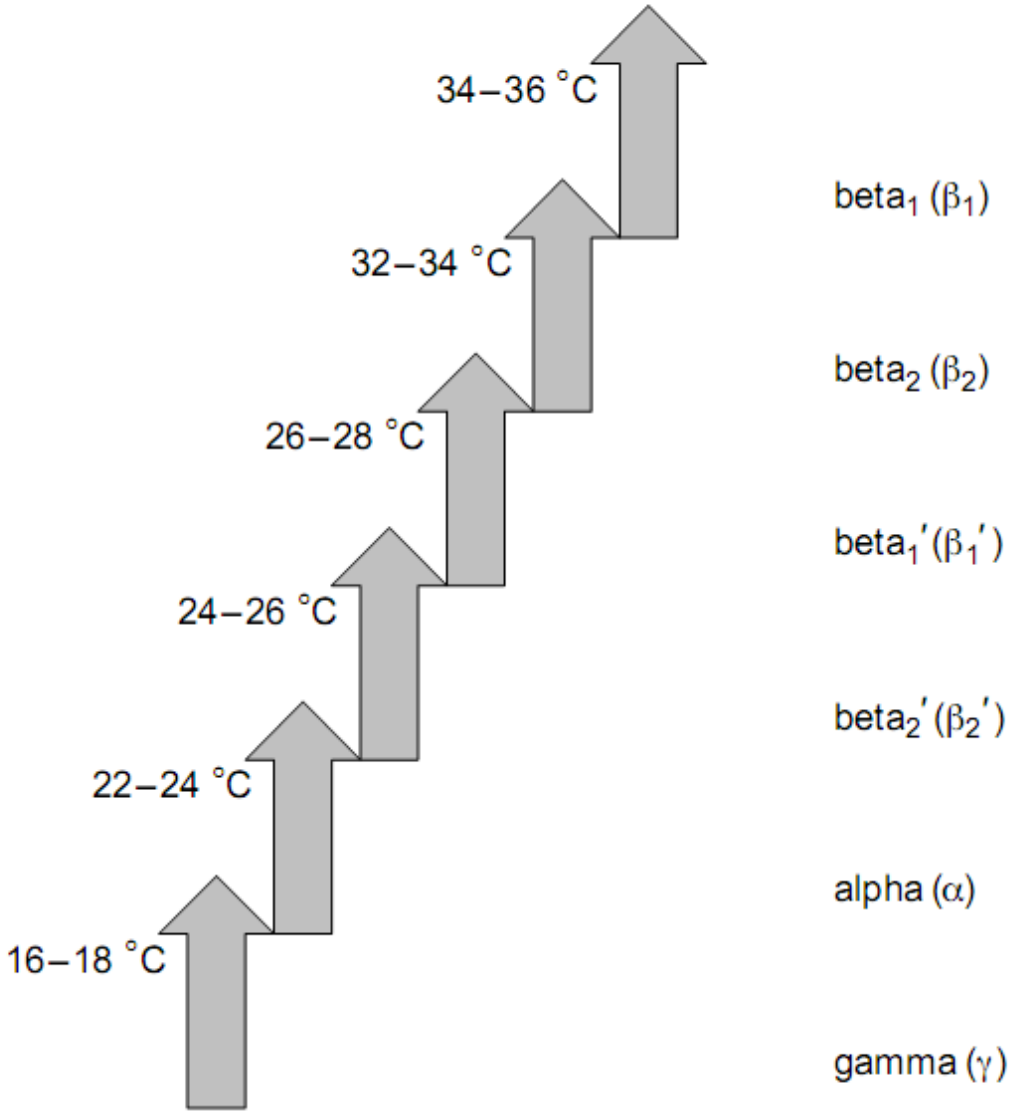


Figure 2.13 Various polymorphs of cocoa butter (Tannenbaum, 1993)

The aim of tempering process is to crystallize the cocoa butter in the right form so as to minimize fat bloom. Cocoa mass is sheared at controlled temperature in order to form triacylglycerols (TAGs) in cocoa butter to give good setting characteristics, foam stability, remoulding properties, product snap, contraction, gloss and shelf-life characteristics. Chocolate tempering consists of three steps: The first one is melting at 45°C, second is cooling to 28-29°C to start the crystallization and being maintained at this temperature. Third is to heat to 30-32°C to melt the unstable crystals. Then chocolate is moulded and cooled to 18°C (Wainwright, 1996).

2.7.7 Moulding

Moulding is a method of producing a precision product. Moulds were initially made of metal however they were very heavy and have now been replaced in most plants by injection- moulded plastic moulds.

2.8 Composition of Chocolate

The basic ingredients for chocolate manufacture are cocoa mass, cocoa butter, butter fat, sugar, milk powder and emulsifiers (Minife, 1989) as shown in Figure 2.14

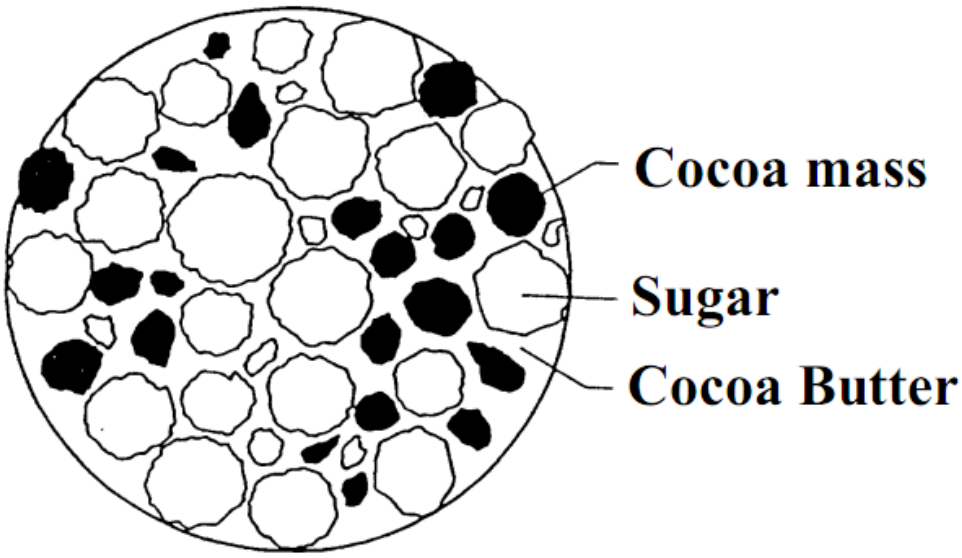


Figure 2.14 Compositon of Dark Chocolate (Serguine,1991)

The relative quantity of ingredients added depends on the type of chocolate to be manufactured as well as the specialty of the plant. A typical recipe for dark, milk, and white chocolates is given in Table 2.2. (Borchers et al., 2000).

Table 2.2 A typical recipe for milk, dark, and white chocolates. All in wt %

	Milk chocolate	Dark chocolate	White chocolate
Sugar	45.0	43.5	45.0
Skimmed milk powder	15.6	-	17.9
Milk fat	5.3	-	4.0
Cocoa mass	10.0	44.0	-
Cocoa butter	23.6	12.0	32.6 (deodorized)
Lecithin	0.5	0.5	0.5
Total fat content	35.0	35.0	36.6

2.9 Chocolate Flavor

Chocolate is a popular food in the whole world due to its highly acceptable flavor. Its aroma originates from the volatiles in the chocolate. Around six hundred compounds including aldehydes, pyrazines, acids, ketones, esters, furans, and phenols were detected in chocolate as shown in Table 2.3.

Table 2.3 Class of Compounds Identified in Chocolate (Pflaumer et al., 1990)

Compound	Aliphatic type	Aromatic type	Heterocyclics	
Hydrocarbons	15	32	Pyrroles	10
Alcohols	23	5	Pyridines	8
Aldehydes	18	6	Quinoline	1
Ketones	25	5	Pyrazines	74
Ester	44	12	Quinoxalines	3
Ethers	8	3	Oxazoles	4
Nitrogen compounds	9	4	Furans	19
Sulfur Compound	12	2	Pyrones	4
Acids	22	15	Lactones	6
Phenols	-	7	Thiazones	3
Total	176	91		132

2- methylbutanal and 3-methylbutanal are important compounds contributing to the odor and taste of the dark chocolate (Counet et al., 2002), while 3-methylbutanal, (E)-2-octenal, 2, 3-diethyl-5-methylpyrazine, (E)-2- nonenal, 2- and 3-methylbutanoicacid, vanillin, R-δ-decalactone, furaneol, and (E, E)-2,4- decadienal are the major volatiles in milk chocolate (Schnermann & Schieberle, 1997). Many factors such as the origin of cocoa, fermentation, roasting and other ingredients besides cocoa affect the chocolate flavor (Guinard & Mazzucchelli, 1999).

2.10 Effect of Ingredients on the Chocolate Flavor

Sugar, added to impart sweetness, is the one of the main ingredients of chocolate. Sugar contributes to counteract the bitterness (Guinard & Mazzucchelli, 1999). More sucrose provides more perception of chocolate taste in the mouth (Geiselman et al., 1998). Cocoa butter also decreases the chocolate bitterness because it coats the bitter compounds and thus perception of bitterness decreases in the mouth. Cocoa butter is solid at room temperature but liquid in the mouth. This property increases the perception of chocolate flavor (Guinard & Mazzucchelli, 1999). Milk solids give the chocolate a milky and a caramel flavor (Patton, 1955).

2.11 Method of Analysis

2.11.1 Gas Chromatography (GC)

Gas chromatography is an analytical method that has been studied and used intensively for more than 40 years in solving a wide variety of analytical problems (Rubinson, 2000). GC separates a mixture into its constituents by passing a moving gas phase over a stationary sorbent. Only two possibilities exist for stationary phase ; it can be a solid or a liquid. This immediately limits the separation mechanisms to absorption or partition, both of which are extensively employed in GC. Gas chromatography has been refined so that it is possible to separate very complex mixtures containing up to 200 related compounds but it does have inherent limitations. The sample must be able to exist in the gas phase, so it may only be applied to volatile materials (Mendham & Denney, 2000).

2.12 Description of GC components

Gas chromotography instrument comprises of six parts as shown in Figure 2.15: carrier gas, sample injection, column, oven, detector and computer. A carrier gas, which is normally inert, is used to carry the sample through column. Generally, helium, hydrogen or nitrogen are used as carrier gases. The carrier gas choice depends on detector type, application, separation efficiency and safety. Many devices are used for introduction the sample but the major applications involve liquid sample that are introduced using a microsyringe with hypodermic needle. There are five injection systems used in capillary gas chromatography: Split, splitless, cold on column, programmable temperature vaporizing, thermal desorption. The oven is used to keep the column at certain temperature. The actual separation of sample components is effected in the column: the nature of the solid support, the type and amount of the liquid phase, the methods of packing, column length and the temperature are important factor in obtaining the desired resolution. There are two type of columns in GC: packed and capillary column. The function of detector , which is situated at the exit of the separation column, is to sense and measure the small amounts of the separated components present in the gas stream leaving the column. Flame ionization detector, thermal conductivity detector, mass spectrometric detector and electron capture detector are used detectors in GC (Mendham et al., 2000).

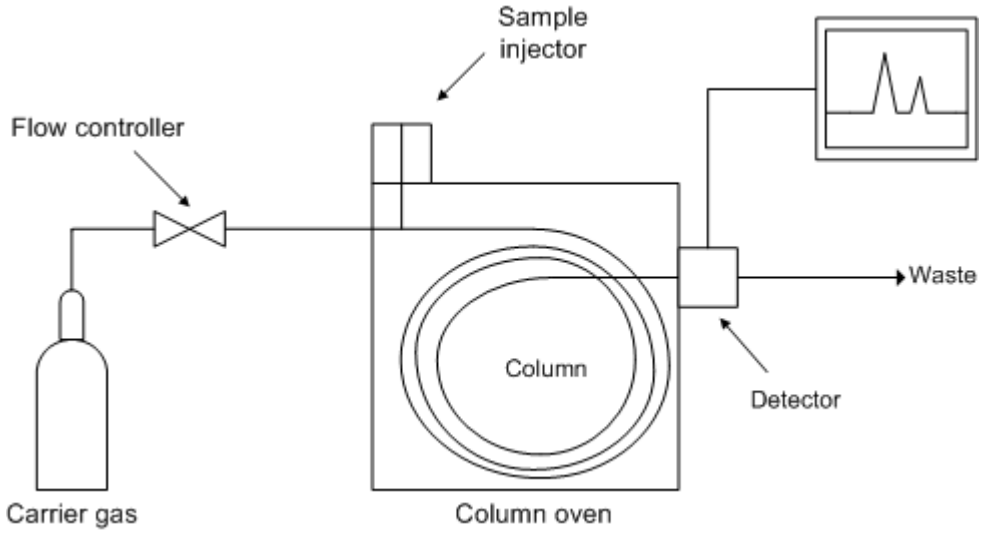


Figure 2.15 Diagram showing a gas chromotograp
(www.chromatographer.com/gas-chromatograhy)

2.13 Mass Spectrometry

A Mass Spectrometer (MS) is a device that characterizes the substance in the magnetic field. MS produces charge fragments from molecules in the magnetic field and display a spectral plot including the mass of each fragment. Mass spectrometry is used to identify composition of substance, structure of organic and inorganic molecules, analysis of complex mixtures, stucture and composition of solid surface (Mendham et al., 2000).

2.14 Sample Preparation Methods for GC/MS

All of analytical techniques require sample preparation because some sample matrices cannot be analyzed directly. It is a time consuming process for many analysis. However, it affects the reliability and accuracy of results. There are four common sample preparation methods used in GC: Static Headspace, (liquid- liquid extraction (LLE)), Solis Phase Microextraction (SPME) and purge and trap methods. Headspace is relatively simple method for concentration of volatile organic compounds. The sample is placed in a vial and volatiles are concentrated in headspace as shown in Figure 2.16

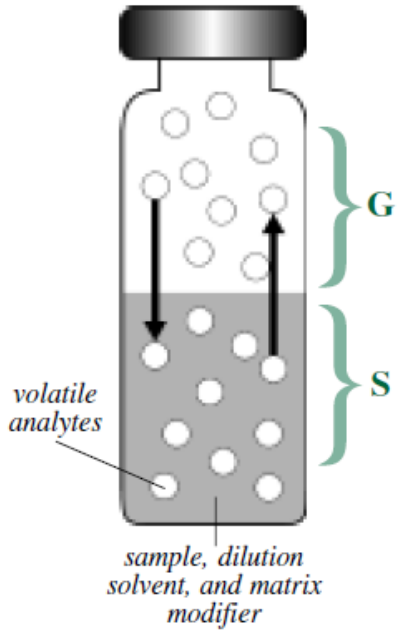


Figure 2.16 Diagram showing headspace methods. G: gas phase (headspace,) S: The sample phase (Vas & Vekey, 2004).

After concentration, headspace above sample is transferred into GC. There are some techniques for transferring: gas tight syringe, balanced pressure sampling instrument, pressure loop sampling instrument. LLE is a separation method based on the difference in solubility of a compound in two immiscible solvents at an appropriate pH. There are some disadvantages of using solvent as a sample preparation. For instance, concentration can cause to loss of analyzing volatile compounds. Disposal of solvent is another problem. It causes extra cost, and health hazards to laboratory personnel.

SPME (solid phase microextraction) is a new approach for sample preparation. Pawsliszyn and co-woeker invented it in 1989 in order to readress limitations inherent in LLE. Sampling, extraction, concentration and sample introduction are combined by SPME in a single solvent-free step. Extraction fiber is used for extraction and concentration of analytes in the sample. This method has advantage in terms of time and disposal cost. In addition, it improves the detection limits. It is successfully applied in extraction of volatiles and semi-volatile organic compounds from environmental, biological and food samples. At the same time SPME is used for HPLC-MS for detection of weakly volatile or thermally labile compounds. The main advantage of SPME is the combination of good analytical performance with simplicity and low cost. SPME produces relatively clean and concentrated extracts and is ideal for MS applications.

Dynamic headspace continually sweeps the headspace of the sample concentrating the analytes onto a trap. They are retained on the trap until desorption to the GC/MS for separation and detection. (Vas & Vekey, 2004).

2.15 SPME

As seen in Figure 2.17, SPME is a modified syringe. It comprises of two parts; fiber and fiber holder.

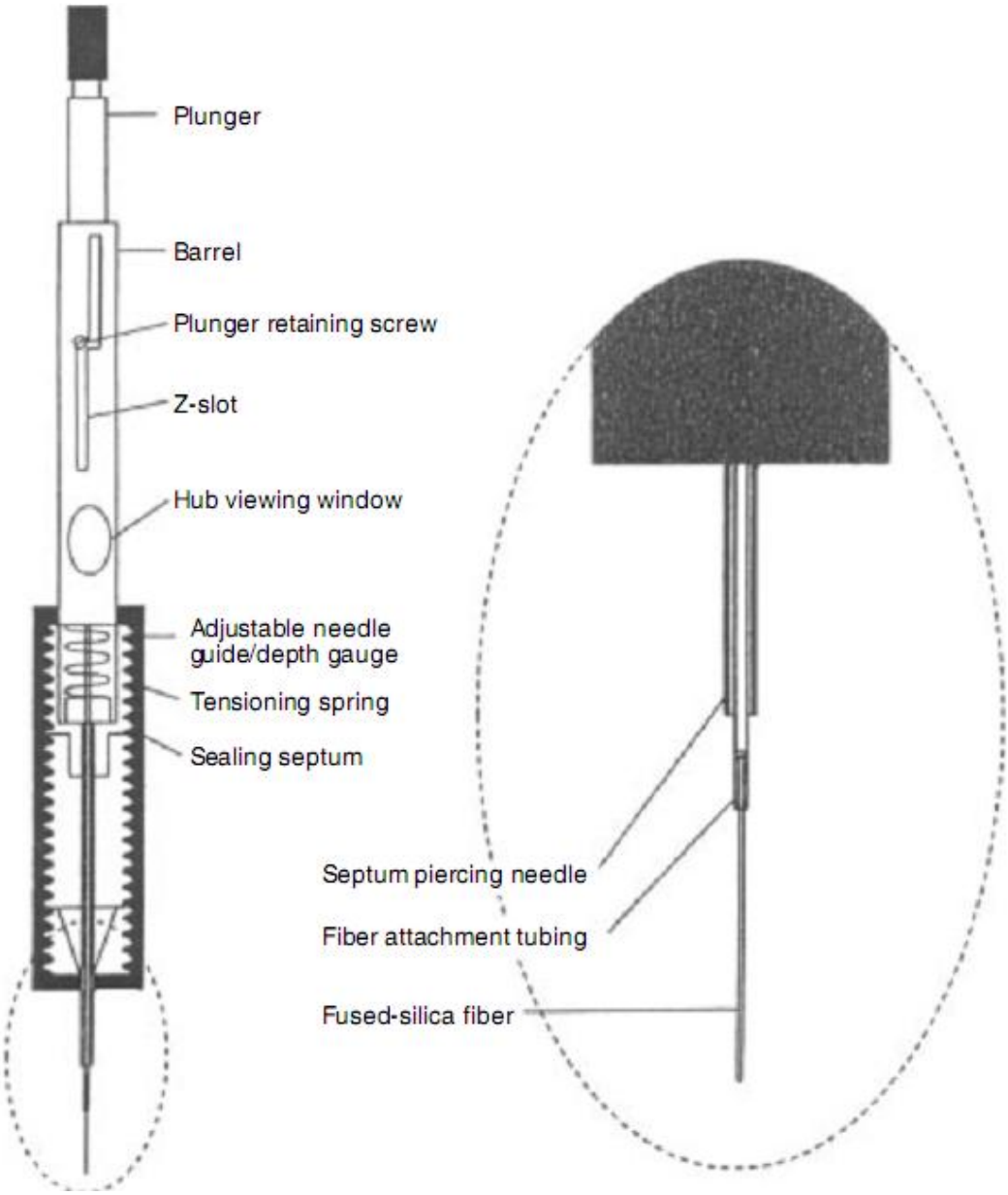


Figure 2.17 Schematic diagrams showing a commercial SPME device (Vas & Vekey, 2004).

The SPME fiber consists of a thin fused-silica optical fiber and a thin polymer film. There are two SPME applications: first is sampling gases (headspace) and second is sampling solutions. SPME needle is dipped into sample solution or environment around sample and thus fiber in the needle is subjected to analyte in the sample. The polymer acts like a sponge, concentrating the analytes by absorption/adsorption process. Principle of extraction is similar to that of chromatography; film thickness,

agitation of sample, and sampling time are factors which influence SPME kinetics. SPME producure consists of two steps; adsorption and desorption of sample. After sampling, the next step is transfer of analyte from fiber into the chromatograph (Vas & Vekey, 2004).

2.15.1 Coating Material

A thin polymeric film is used to coat the fiber. It provides concentrating of organic volatile from sample matrix. The extraction is similar to gas-liquid or liquid-liquid system. The extraction principle depends on general rules of equilibrium. It depends on some parameters such as geometry, sample size and type of fiber. Equilibration time is proportional to the fiber thickness whereas it is inversely proportional to agitation. The most important parameter affecting analyte extraction is thickness of coating material (Vas & Vekey, 2004).

2.15.2 Fiber Extraction

Figure 2.18 shows the sampling process with SPME. First sample is placed in a small bottle, called vial, which is closed with a septum-type cap. Before analysis, fiber has to be cleaned from the contaminants to prevent its effect on the chromatogram. When the SPME needle pierces the septum, volatile partition between the sample matrix and the stationary phase takes place**.**

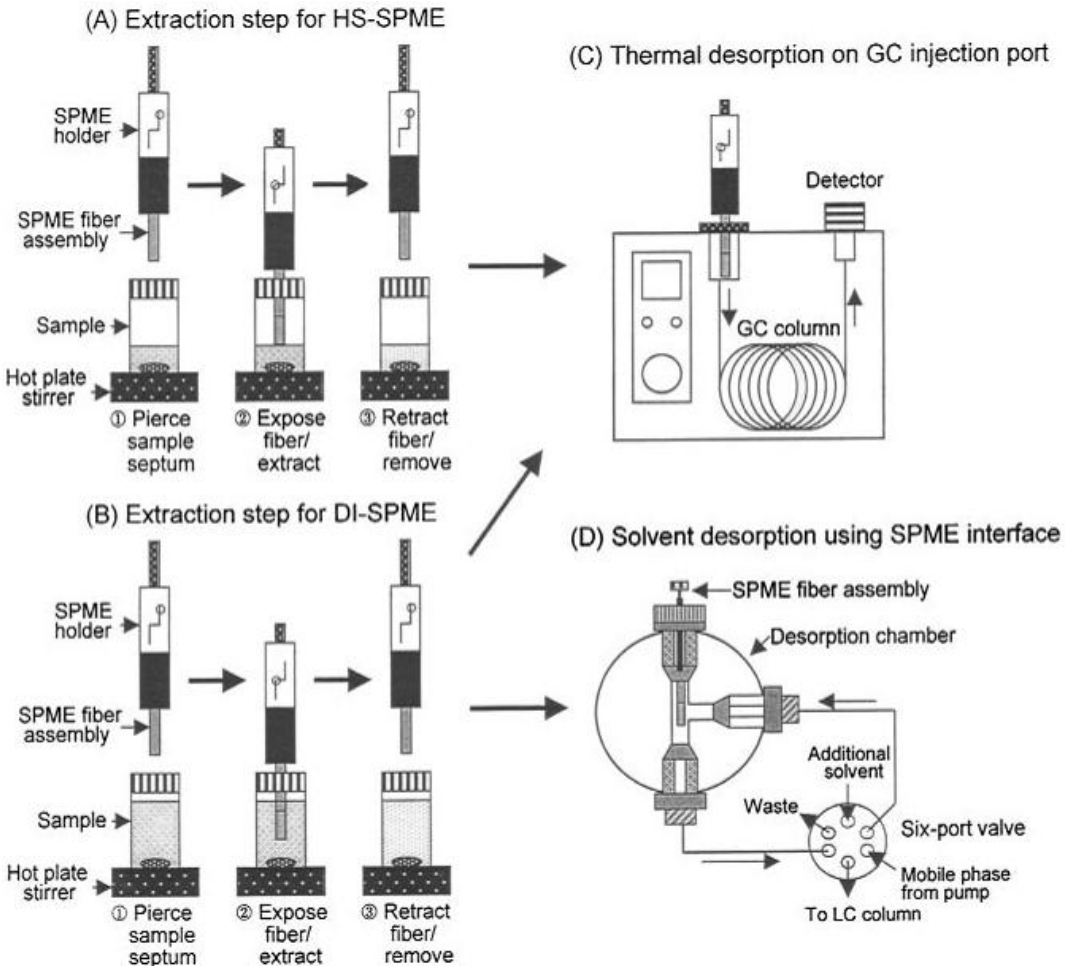


Figure 2.18 SPME procedure for GC and for LC. *Reprinted from Journal of Chromatography A, 880, Kataoka H, Lord LH, Pawliszyn Applications of solid-phase microextraction in food analysis, page 40, Copyright (2000), with permission of Elsevier.*

Two methods are used for extraction: headspace (HS-SPME) and direct immersion (DI-SPME). Fiber is subjected to environment around the sample in the HS-SPME whereas fiber is directly dipped in the ID-SPME. Sometimes agitation is carried out for shortening the equilibration time. After volatile extraction from sample, needle is taken from the vial and needle is placed into injection port for desorption of volatiles in the fiber. Both methods can be utilized together for extraction. Heating is used for desorption of volatiles in the fiber in GC/MS system shown in Figure 2.18 (Vas & Vekey, 2004).

2.16 Applications of SPME in Food Analysis

Food analysis is critical to the assessment of nutritional value, food freshness and controlling of food additives and other toxic contaminants. Food composition is important for flavor. Any change in the food composition may cause a change in the food flavor. Aromatic compounds causing food flavor are generated by metabolic pathways during ripening and storage. Many factors influence their production. Therefore it is critical to know typical chromatographic patterns of a given food and the modified patterns during processing in order to identify any change in the volatile composition. Infection during the storage of foods can be avoided by early detection of vapors from the food by SPME which is the method of choice to analyze many kind of components and impurites. Qualitative and quantitative flavor and aroma determination are vital for determination of food freshness. Concentration of aroma and flavor components in food is very low and comprise of wide variety of organic components. Many flavor components are volatile and this is advantage for isolation from sample matrices. Steam distillation, solvent extraction is general method used before GC analysis. However there is a disadvantage of these methods like high labor intensive. Using SPME combined with GC/MS, the disadvantage of these commonly used sample preparation methods can be avoided. Characterization and differentiation is vital for aroma analysis. various kinds of fibers are used for this purpose (Vas & Vekey, 2004).

Augusto et al., (2000) utilized SPME/GC/MS in order to identify several alcohol ester carbonyl compounds of tropical Brazian fruits. Carboxen fiber is used for this aim and they found that it is effective for isolation of aroma for HS sampling.

Pinho et al., (2002) studied volatile compound of Terrincho cheese. They developed a new HS-SPME/GC/MS method.

Kim and Lee, (2002) used SPME for the volatile analysis of Lavandula species. They applied different extraction methods and they compared the result of these methods. They found that 100 μm PDMS-coated extraction fiber is the best fiber for volatile extraction.

Another SPME application was performed by Paliyath et al., (1997). They evaluated the potential differences between healthy and infected fruits. They used apples for

this aim. They stated that 100 μm PDMS filter was most affective fiber for the HS-GC/MS method.

2.17 New Product Development

Food product development was initiated with preservation and prolongs shelf life of food. Thus food is available for the people outside of the harvesting season. Afterwards, traditional foods have been produced in the large factory and mechanization started for food production (Linneman, 2006). Nowadays, new product development becomes obligatory for the company in competitive global world market to strengthen their position in the market. Also food product development is a necessity to provide food for the growing world population (Barrena & Sancez, 2012). New food product development is divided into some categories described below (Anon 1999, Fuller, 1994):

Me too product: is a product which exists in the markets but reproduced by different companies under different names.

Line extensions: is a product which is produced by changing the well known product like adding some flavoring into a food product.

Repositioned existing product: is a product which is produced by increasing the healthy properties of it. For example, margarine production enriched in terms of vitamin E.

New form of existing product: is a product which is produced in different physical form. For example, production of dried soups.

Reformulation of existing product: is a known product with new formula.

New packaging of existing product: is a existing product with new packaging system like modified atmosphere packaging.

Innovative product: is obtained by changing the existing product with some process methods. For example ready to cook products.

Creative product: is a new product which did not exist in the market before.

2.18 Additives Used

2.18.1 Cinnamon

Cinnamon is the shell of trees which belong to a *Cinnamomum verum* family and used as spice for flavoring most of sweet and savory foods. Cinnamon contains 0.5 to

1 % essential oil which have characteristic odor of cinnamon. Mexican is the major importer of it use especially the cinnamon in chocolate. Middle eastern use the cinnamon in lamb and chicken whereas Americans use the cinnamon only cereal product like cake. Turkish people use cinnamon for both sweet and savory foods (Raghavan, 2007).

2.18.2 Ginger

Ginger belongs to *Zingiberaceae* family and is used as medicine, or spice. Motherland of ginger is southern of China. It was expanded to Asia, West Africa and other places from there. Western people use ginger for sweet food like ginger biscuits whereas Americans use the ginger for flavoring of their tea and coffee. India and Pakistan use the ginger for flavoring the lentil, pulse and vegetables (Raghavan, 2007).

2.18.3 Aniseed

Aniseed belongs to *Apiaceae* family and origin of it is eastern Mediterranean region and Southwest Asia. It is very aromatic spice. British use the aniseed in jelly bean Mexican used for flavoring the hot chocolate. Ancient Romans used aniseed in the cake. It is used as flavoring agent for arak in the Middle East (Raghavan, 2007).

2.18.4 Lemon Peel

Lemon belongs to *Rutaceae* family and its motherland is Asia. It is used in most kind of foods everywhere also for medicine and cosmetics. Lemon peel contains more vitamins than lemon juice. It is also the major source of minerals like magnesium, potassium, and calcium (Raghavan, 2007).

2.18.5 Pistachio Nut

Pistachio nut belongs to *Anacardiaceae* family and the origin of its trees is Central Asia and Middle East. Pistachio trees can be found in regions of Iran, Syria, Lebanon, Turkey, Greece, Xinjiang (China), Tunisia, Kyrgyzstan, Tajikistan, Turkmenistan, India, Pakistan, Egypt, Italy (Sicily), Uzbekistan, Afghanistan (especially in the provinces of Samangan and Badghis), and the United States, specifically in California. The fruit of this tree is the kernel and it is eaten in the form

of fresh, roasted or salted. It is used as a constituent in many desserts or confectionary products everywhere (Raghavan, 2007).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Refined chocolate mix, containing sugar and cocoa mass, were supplied from Şölen Chocolate, Food Indusrty and Trade (Gaziantep, Turkey) together with lecithin and cocoa butter. The spice additives comprising of aniseed, ginger, and cinnamon were purchased from a local store in Gaziantep. The lemon peel powder on the other hand was prepared in our laboratory by peeling fresh lemons, drying and grinding the peels. Pistachio nut paste was purchased from a local factory in Gaziantep.

All other chemicals (such as catechin, theobrimine, caffeine) were purchased from Sigma Aldrich (St. Louis, MO, USA).

3.2 Determination of Quantity of Spices Added

The range of percentage of spices added to dark chocolate was determined firstly by a sensory panel then the data obtained were evaluated through the Response Surface Methodology to decide on the final percentages. A range of 1 to 5% was then specified for the mentioned spices. The influence of spices on the hardness, viscosity, moisture, sensory quality, and total color change was investigated. Hardness, viscosity, moisture and color of dark chocolate were used as target parameters to determine the optimum spice concentration, keeping the traditional structure of dark chocolate.

3.3 Determination of Addition Time of Citral and Cinnemaldeyde

The time to add the cinnamaldeyde, aniseed essential oil and citral to chocolate during conching was determined with an experimental design. Cinnemaldeyde in the cocoa butter was added at the beginning of conching and the change in its quantity was followed for each hour interval. This is illustrated in Figure 3.1.

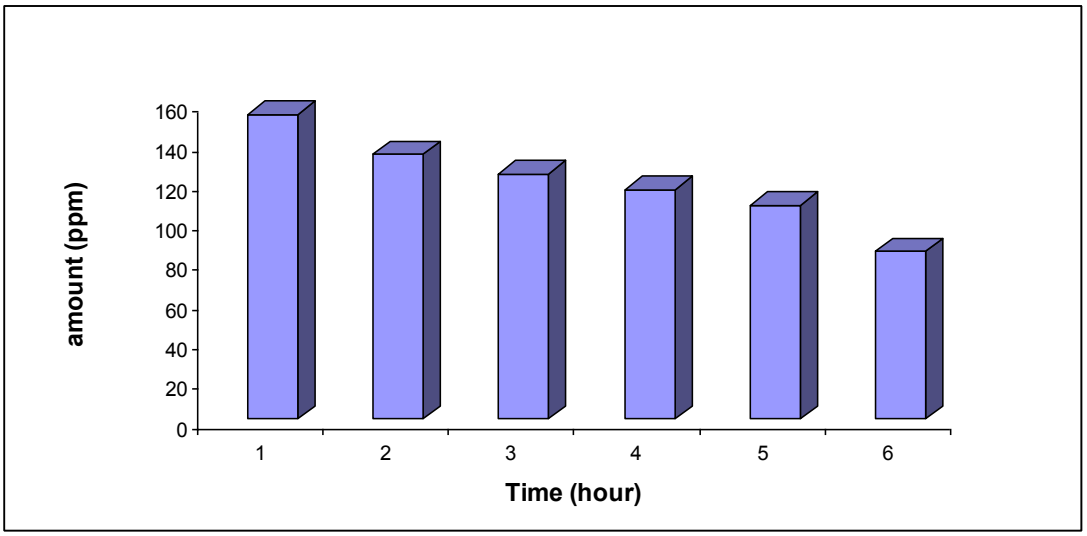


Figure 3.1 Change in the amount of cinnemaldehyde during conching

As shown in the figure, the amount of cinnemaldehyde decreased from 154 ppm to 85 ppm within approximately 6 hours of conching. Cinnemaldeyde and citral were therefore added at the end of conching to prevent their loss.

3.4 Conching Process of Dark Chocolate

Refined chocolate mix, containing sugar and cocoa mass, was supplied from a local plant together with lecithin and cocoa butter. A 5 kg-capacity, laboratory-scale conching machine (ELK'olino single-shaft conche, Bühler, AG, Switzerland) with temperature and speed control was utilized for the conching process, which is made up of three phases; dry phase: 1 hour at 50°C, pasty phase: 4 hours at 80°C and liquid phase: 1 hour with linearly decrease in temperature from 80 to 45°C. The rotation speed of the conching machine was in the range of 600-1500 rpm. The formulation of dark chocolate used in this study is shown in Table 3.1.

Table 3.1 Formulation of dark chocolate, all figures are in % (w/w)

Ingredients	% (w/w)
Cocoa mass	45.07
Sugar	39.14
Cocoa butter added during conching	15.45
Lecithin	0.34

3.5 Conching Process of Dark Chocolate With Additives

The spice additives comprising of aniseed, ginger, and cinnamon were purchased from a local store in Gaziantep. The lemon peel powder on the other hand was prepared in our laboratory by peeling fresh lemons, drying and grinding the peels. After screening to 20 μm mesh, the mentioned additives were added to the chocolate mix prior conching.

The conching process was carried out as stated above. The formulation of dark chocolate with spices is given in Table 3.2. The amount of additives was determined as a result of an experimental design in Design Expert program and sensory analyses.

Table 3.2 Formulation of dark chocolate with spice additives, all figures are in %(w/w).

Ingredients	Dark chocolate	Aniseed Chocolate	Ginger chocolate	Cinnamon Chocolate	Lemon peel chocolate
Cocoa mass	45.07	43.47	42.57	40.07	43.07
Sugar	39.14	39.14	39.14	39.14	39.14
Added cocoa butter	15.45	15.45	15.45	15.45	15.45
Lecithin	0.34	0.34	0.34	0.34	0.34
Additive	-	1.6	2.5	5	2

3.5.1 Pistachio Nut Chocolate

Pistachio nut paste was purchased from a local factory in Gaziantep. It was added to the chocolate mix prior conching.The conching process was carried out as stated above. The formulation of dark chocolate with pistachio nut paste is given in Table 3.3.

Table 3.3 Formulation of dark chocolate with pistachio nut paste, all figures are in %.(w/w).

Ingredients	**Dark chocolate**	**Pistachio nut chocolate**
Cocoa mass	45.07	35.07
Sugar	39.14	39.14
Added cocoa butter	15.45	15.45
Lecithin	0.34	0.34
Additive	-	10

3.6 Composition Analysis

The moisture, fat, protein and ash contents were analyzed according to the standard methods (AOCS, 1990). The percent carbohydrate was then determined by difference. The analyses were carried out in triplicate and the values were averaged. The composition of dark chocolate with and without additives is shown in Table 3.4

Table 3.4 Composition of dark chocolate with and without additives (w/w).

Chocolate type	Moisture	Fat	Protein	Ash	Carbohydrate
Dark chocolate	0.52±0.09	34.25±0.29	8.42±0.33	1.73±0.07	55.08
Cinnamon chocolate	0.54±0.05	30.70±0.21	8.73±0.4	2.15±0.09	57.88
Aniseed chocolate	0.96±0.34	35.16±0.21	9.47±0.48	1.92±0.005	52.49
Ginger chocolate	0.66±0.18	34.71±0.35	9.14±0.06	1.92±0.02	53.57
Lemon chocolate	0.54±0.02	33.45±0.15	9.16±0.09	1.92±0.09	54.93
Pistachio nut chocolate	0.85±0.07	35.02±0.11	13.07±0.02	1.86±0.02	49.20

3.7. Moisture Content Determination

Moisture content of sample was analysed according to AOCS 931.04.

200 gram dark chocolate was melted by placing in suitable container and partly immersing container in the bath at 50°C. After melting, a 2-gram sample was placed in aconstant weight petri dishes in air at 100°C. Weight loss of water was reported.

3.8 Fat Determination

200 gram dark chocolate was melted by placing in suitable container and partly immersing container in the bath at 50°C. After melting, 3-4 gram chocolate was weighed and placed into 300-500 ml beaker. 45 ml boiling water to give homogeneous suspension was added slowly while stiring. 55 ml 8N HCI was added and stirred. Mixture was covered with watch glassy bringing slowly to boil, and boiled gently 15 minute. Later, mixture was rinsed with 100 ml water. After, mixture was filtered with 15 cm medium fluted paper, and rinsed beaker 3 times with water. And then sample was washed until last partition of filtrate is CI free as detect addition of 0.1 $AgNO_3$. After that sample was transferred wet paper and dried 6 hours at 100°C. Later sample was cooled to room temperature in desiccators and weighed. The dried sample was placed in soxhlet. After soxhlet application, sample was dried in flask at 100-101°C to constant weight (1.5-2 hour). Later sample was cooled in desiccators to room temperature and weighted. Constant weight was attained when successive 1 hour drying periods. % Fat =(g fat X 100)/ g sample.

3.9 Protein Determination

Protein content of sample was analysed according to AOCS 939.02. 10 gram finely divided dark chocolate was weighed into 250 ml flask and extracted twice with 100 ml ether until obtaining uniform and decant ether layer each time. Then sample was placed in bottle 2-hole stopper carrying bent glass tube. Ether was expelled by applying suction to bent tube and draw moderate air current bottle while it is moderately warm place. When the ether expelled, sample was dried at room temperature. 1 gram sample was weighted and placed in tube. 7 gram K_2SO_4,12 ml H_2SO_4 and 1 spatula $CuSO_4$ was added to sample respectively. Later, mixture was

placed in machine for digestion at 400°C for 40 minutes. After digestion, sample was placed in washing unit and then sample was titrated with 0.1 N HCI solution. Pretein was calculated.

3.10 Ash Determination

Protein content of sample was analysed according to AOCS 972.15.

200 gram dark chocolate was melted by placing in suitable container and partly immersing container in the bath at 50°C. After melting, 2-5 gram chocolate was weighed and placed into 25 ml quartz dish previously heated 600°C covered with watch glass, cooled in desicators and weighed. The sample was waited in oven at 600°C for 4 hours. Later, sample was cooled room temperature in desicator and 2 ml of 96% ethyl alcocol was added. After sample was dried in water bath. And then, sample was dried at 600°C for hour. Finally, sample was cooled in desicator and ash content was calculated.

m_2 = emty dish + ash, (g)
m_1 = empty dish , (g)
m = amount of sample , (g)

$$\text{Ash content } (\%) = \frac{m_2 - m_1}{m} \text{ x } 100$$

3.11 SPME Extraction

Volatiles from samples were extracted by using 75µm divinylbenzene/carboxen on polydimethylsiloxane on a Stable /Flex fiber (CAR/PDMS). Extractions were carried out in the vials. A 2g-sample together with 1µL toluene, as internal standard, was placed in a 20-ml vial. After tightly plugging its lid and inserting the SPME fiber, it was equilibrated for 60 minutes at 60 °C. The desorption time was 5 min and the temperature in the GC liner was 250° C (Ducki et al., 2008).

3.12 GC/MS

The volatiles extracted by fibers were thermally desorped and introduced into the capillary column(EQUITY^{TM}-5 FUSED SILICA Capillary Column30 m × 0.32 mm × 0.25 µm film thickness Supelco). The GC (Perkin Elmer Clarus 500)-MS (Clarus 500 MS Perkinelmer) was set up with constant carrier flow of 2ml/min (helium), the oven temperature was programmed starting at 80°C (5 min)-(10°C/min) 150°C-150°C(10min)-(10°C/min) 200°C-200°C(5min). The injector temperature was

250°C. The analysis was carried out by using gas a chromatograph coupled with a mass spectrometer. The ionization voltage was 70eV, mass range m/z 40-300 (Ducki et al., 2008).

3.13 Odour Identification by Using Olfactometry

Selection of panellists was based on their sensivity in distinguishing individual basic taste including sweet, salty, sour, and bitter followed by combination of two or three basic tastes. Two trained panelists sniffed the outflowing gas from the olfactometer's detection port. The sniffing was carried out simultaneously during GC analysis. Panelists described their perception of the sniffed odour (Misnawi, 2011).

3.14 Sensory Evaluation

12 research assistants from Gaziantep University Food Engineering Department were trained before analysis. They were trained to identify, define and scale the chocolate samples with respect to flavour attributes and melting characteristics on a 9-point scale (appendix A35). For the flavour attributes the judges were instructed to place the sample between the teeth and chew once before bringing it to the top of the tongue and melting it by rubbing against the roof of the mouth. The judges were instructed to start their evaluation of the attribute intensity as soon as the sample was placed on the top of the tongue. For the melting characteristics the judges were instructed not to chew on the sample but to placed it on the top of their tongue and melt it by rubbing it against the roof of the mouth. The judges were instructed to start recording their response as soon as the sample was placed on the tongue. The intensity of response was recorded in terms of the amount of effort required to melt, manipulate and swallow the melted sample. Chocolate samples were evaluated by these panelists in terms of taste, odour and texture. The sensory test was carried out under blue light in order to reduce the impact of apperance. Samples were evaluated in duplicate by each judge who was provided with a spit cup and a cup of water at room temperature to rinse his/her mouth prior sample tasting. Results were evaluated statistically by SPSS (Meilgaard, 1999).

3.15 Melting Point Determination

A differential scanning calorimeter (Perkin Elmer FC100 ped2 27603) was used for determination of melting points of the chocolate samples. 9 mg of each sample was placed into a pan, which was sealed with lids using a sample press. The pans were heated from -5°C to 65°C in a N_2 stream. The onset temperature (T_{onset}), peak temperature (T_{peak}), and the end temperature (T_{end}) were calculated automatically by the software (Pyris software for windows version 7) (Afoakwa et al., 2008).

3.16 Texture Measurements

The hardness of solid chocolate samples was measured by a TA-XT plus Texture Analyser (Vienna Court Lammas Road, Godalming Surrey GU 1 YL UK) with a penetration probe (needle P/6) attached to an extension bar. The maximum penetration force through the samples with 4.5 cm diameter and 6 mm thickness was determined as triplicates at a pre-speed of 3 mm/s, a test speed of 1mm/s, post-speed of 10 mm/s, and trigger force of 1g, penetrating 1mm at room temperature. The mean values were converted into hardness data using TEE 32 Exponent Microsystem Version 4.09.0 (2007) Software (Afoakwa et al., 2008).

3.17 Particle Size Determination

Structure of the sample and particle size was observed by Polarized Light Microscopy (Olympus BX51TF Tokyo Japan). The structure of the samples was imaged by using a digital camera (Viewfinder version 2.1.1. Pixera). Slides were prepared by melting the chocolate at 45°C for 10 min. A capillary pipet was used to deposit a small droplet of chocolate onto a glass slide. One drop of vegetable oil was used to solve the chocolate. Glass cover slip was then placed on the surface of the droplet. The samples were observed under the microscope for up to 1 h. Images were captured every 10 min. Counting method was used to determine the dimension of particle by using theBS Image system BS 200 Pro Plus version 3.0 software (Hoskin & Dimick, 1980).

3.18 Cafein and Theobromine Determination

2 grams of chocolate was weighed and placed in a beaker. 25 ml of Petroleum ether was added and mixed for 5 min. The solution was centrifuged at 15Xg (2000 rpm) for 5 min. After decanting the petroleum ether, the procedure was repeated. After second petroleum ether wash, the residue was dried at room temperature overnight. 0.5 gram of residue was wighed and 40 gram of deionized water was added. The solution was heated in boiling water for 30 min and then solution was cooled at room temprature. After cooling, the solution was centrifuged at 2000 rpm for 5 minute. The supernatant (2 ml) was filtered through a 0.45µm nylon filter. Theobromine and caffeine were analyzed by high performance liquid chromatography (LC -2A AB made in Japan). Seperation occurred on a Inertsil ODS-3 5µ 4,6X250 mm (made in Japan) using methanol/water (85/15) mobile phase. Flow rate was 1 ml/min and detection occured at 280 nm with a UV detector. Stock standard solution of theobromine (400 ppm) and cafeine (1000 ppm) were prepared by dissolving the appropriate amount of these compounds in water and subsequently stored at 4°C. Working solution for external standard curve was prepared by diluting of stock standard solution in water (Caudle et al., 2001).

3.19 Viscosity Measurements

Rheological behaviors of the samples were characterized using a RheoStress RS1 (Haake) controlled stress rheometer equipped with a TCP/P peltier temperature controller unit and a thermostat. A cone and plate configuration with 3.5 cm diameter and 2° angle was used. The shear rate range was 0–300 s^{-1}

All samples were incubated at 50°C for 75 minutes for complete melting. Shear stress was measured at 40 °C as a function of shear rate from 5 to 300s^{-1}. 60 measurements were taken in 300 s. The mean value and standard deviation of the duplicate readings were recorded.

Casson plastic viscosity and Casson yield values were calculated from the data by interpolation using ThermoHaake RheoWin Pro Data Manager, Version 2.64 Copyright 1997software. Other rheological parameters (yield stress, and apparent viscosity) were deduced from the data as recommended by ICA (2000) and Servais et al., (2004). According to this method, the value of shear stress at a shear rate of 5

s^{-1} represents the yield stress, while the viscosity at a shear rate 30 s^{-1} represents the apparent viscosity.

3.20 Total Polyphenol Determination

Total polyphenol was determined spectrophotometrically using the modification method of Singleton and Rossi, (1965): 250 milligrams of defatted chocolate was dissolved in 40 ml of 80% acetone solution.The solution was sonicated in a beaker fitted inside an ultrasound bath(Model B-2200 E4 Blason, Power output 60W, Frequency 47 Hz, Danbury, CT) for 30 minutes at 0°C. Sonification was preferred over shearing as an aid in solubilizing polyphenol since shearing promotes browning of the polyphenol extract by oxidation (Shamsuddin and Dimmick, 1986). The solution was then filtered through Whatman no.1 filter paper under vacuum and thus a clear solution was obtanied. The residue and erlenmayer were washed with 80% acetone solution and total volume was made up to 100 ml. 1 ml of the extract was placed in a flask and diluted to 70 ml with distilled water. 5 ml of 2N Folin-Ciocalteau's reagent was added and kept for 2 minutes. 15 ml of saturated Na_2CO_3 solution was then added to stabilize the color within 2 hours. The absorbance was measured at 765 nm spectrophotometrically (Lambda 25 UV/VIS spectrometer Perkin Elmer Shelton USA). Nine known concentrations of catechin (a commonly used polyphenol standard), ranging from 100 to 900 mg/L, were used to prepare the standard curve. The results were expressed as mg of catechin-equivalent per liter solution.

3.21 Color Determination

Variation in color was followed with a Hunter Lab Colorimeter (Colorflex /A60-1010-615 Model Calorimeter, Reston, VA) in terms of L, a, b values as measures of lightness, redness and yellowness, respectively. The equipment was calibrated with a white tile standard (L=93.01, a=-1.10, b=1.29). For each sample, three measurements were taken and averaged. The results were expressed as total color difference (ΔE) between the reference (dark chocolate) and samples according to the following equation:

$$\Delta E = \sqrt{(L_{sample} - L_{dark})^2 + (a_{sample} - a_{dark})^2 + (b_{sample} - b_{dark})^2}$$

* dark: dark chocolate as reference sample

3.22 Quantative Determination of Volatiles

Concentration of volaties was calculated according to the equation given by (Kelly et al.,1999):

$Cv = (A_i/A_{st}) x C_{st} x RF$

Cv: concentration of volatiles

A_i:Peak area of compound

A_{st}: Peak area of internal standard

C_{st}: concentration of internal standard (ppb)

RF: response factor

3.23 Determination of Response Factor

Response factor was calculated for each functional group (such as acids, alcohols, pyrazines, esters, aldehydes and hydrocarbons) according to equation by (Anon, 2002b):

RF=(Peak area of standard/peak area of compound)x (concentration of compound/concentration of internal standard)

3.24 Determination of Odor Activity Value

Odour activity value was calculated according to equation below (Cabaroğlu et al.,2002):

Odour activity value= concentration of compound/threshold value of compound

Dilution factor (threshold value of compound) for odor activity value with SPME was determined by the following procedure: the quantity of the sample was reduced successively by half until its odor was not perceived.

3.25 Statistical Analysis

General factorial design feature of Design-Expert version 6.01.0 (Stat- Ease, Inc Minneapolis, MN) was used to evaluate the experimental results. Factors and their range was shown in appendix A1.

The experimental data obtained from the design were analyzed by the *response surface regression* procedure using the following second-order polynomial equation to describe the response variables,

$$Y_i = b_o + \sum b_i x_i + + \sum b_{ii} X^2_i + + \sum b_{ij} x_i x_j \qquad (8)$$

where, Y_i is the predicted response(dependent variable), x_i, x_j are the independent variables, b_o is the offset term, b_i is the linear coefficient, b_{ii} and b_{ij} are the quadratic coefficients, w_i is the interaction coefficient.

The statistical software package, Design-Expert 6.01.0 (Stat- Ease, Inc Minneapolis, MN) was used for regression analysis of the experimental data. Analysis of variance (ANOVA) was used to estimate the statistical parameters. A second order polynomial equation was employed to fit the experimental data. The significance of the model equation and model terms were evaluated by F-test. The evaluation of the fitting was checked by the coefficient of determination (R^2), F and the p-values (error probabilities). Response surfaces were generated using a final model considering only the influence of significant factors of above equation at a 95% confidence level.

SPSS 16.0 (Copright 1989-2007 Polar Engineering and Consulting) was used to assess the results of the sensory analysis.

Design expert version 6.01.0 (Stat- Ease, Inc Minneapolis, MN) was utilized to evaluate the conching kinetics experimental data which were fitted to a second order polynomial statistically. The related experimental design is shown in Appendix A13. The fit of design model was evaluated by comparing different R values of each method with each other, and the model was verified by comparison the actual value with predicted value obtained by the equation. The related data are given in appendices A13- A20.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Optimization of Fiber Type, Extraction Time, and Temperature

An experimental design shown in appendix A1 was performed in accordance with response surface methodology. The central composite design feature of Design-Expert version 6.01.0 (Stat- Ease, Inc Minneapolis, MN) was used to observe the effect of the process variables on change in the number of organic acids, alcohols, and other compounds (other than acids and alcohols) and total (all volatile components detected by GC-MS) as shown in Table 4.1.

Table 4.1 Design matrix for optimization of extraction parameters using Design Expert software

Study Type			Response Surface		
Initial Design			Central Composite		
Design Model			Quadratic		
Factor	**Name**	**Units**	**Type**	**Low Actual**	**High Actual**
X	Temperature	°C	Numeric	40	70
Y	Time	Minute	Numeric	15	60
Z	Fiber type		Categorical	A*	D*
Response	**Name**	**Units**	**Analysis**	**Minimum**	**Maximum**
Y1	Acids	Number	Polynomial	2.0	10
Y2	Alcohols	Number	Polynomial	0.0	8
Y3	Others	Number	Polynomial	1.0	24
Y4	Total	Number	Polynomial	6.0	41

Types of fiber used in SPME

*range of fiber from A to D

A:DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane)

B: PDMS/DVB (Polydimethylsiloxane/Divinylbenzene)

C: CAR/PDMS (Carboxen/Polydimethylsiloxane)

D: PA (Polyacrylate**)**

The design model was evaluated by considering R values and comparison of actual values with predicted values obtained by the fitted equation. The related data, which were fitted to a second order polynomial, are given in appendix A2, A3, A4 and A5. The significance of terms in the model was found by analysis of variance (ANOVA) for each response. Significance was judged by determining the probability level. In this study, temperature, time and fiber type were selected as independent variables shown in Table 4.2.

Table 4.2 Range of process parameters and responses

Parameter/response	Goal	Lower Limit	Upper limit	Lower Weight	Upper Weight
Temperature	in range	40	60	1.0	1.0
Time	in range	15	60	1.0	1.0
Fiber type	in range	A	D	1.0	1.0
Acids	maximize	2.0	10	1.0	1.0
Alcohols	maximize	0.0	8.0	1.0	1.0
Others	maximize	1.0	24	1.0	1.0
Total	maximize	6.0	41	1.0	1.0

Response surface methodology was used to optimize process parameters time, temperature and SPME fiber for the GC-MS analysis. It was reported that (Pawliszyn, 1997) there are various parameters affecting the precision of the SPME method developed. Parameters like sample volume, exposure time, temperature, and so forth have an influence on the extraction efficiency of the method. The selection of conditions for analysis is very important in terms of correct identification of the compound in sample with GC-MS. Design-Expert software was, therefore, used for the experimental design. The experimental results were statistically evaluated as

shown in Table 4.3. Linear and quadratic regression equations describing the effects of temperature, and time on the number of acids, alcohols, others and total were developed and regression equation coefficients are given in Table 4.3**.** It is evident that the number of acids, alcohols, and others increases with time and temperature as can be seen from Table 4.3.

Table 4.3 Regression equation coefficients for acids, alcohols, others and total

ACIDS

Fiber	Intercept	Temperature	Time
A	0.82193	9.838E-003	7.582E-003
B	0.60313	9.838E-003	7.582E-003
C	0.74148	9.838E-003	7.582E-003
D	0.70638	9.838E-003	7.582E-003

ALCOHOLS

Fiber	Intercept	Temperature	Time	Temp.*Time	Temp.^{2}	Time.^{2}
A	1.2305	0.1203	0.1115	-2.849E-004	-1.175E-003	-1.509E-003
B	7.8935	0.2162	0.1134	-2.849E-004	-1.175E-003	-1.509E-003
C	6.7574	0.2072	0.1321	-2.849E-004	-1.175E-003	-1.509E-003
D	1.9310	0.1406	0.1049	-2.849E-004	-1.175E-003	-1.509E-003

OTHERS

Fiber	Intercept	Temperature	Time	Temp.*Time	Temp.^{2}	Time.^{2}
A	26.7755	0.78690	0.3252	-6.010E-003	6.651E-003	8.287E-004
B	59.5827	0.83076	0.3234	-6.010E-003	6.651E-003	8.287E-004
C	16.6997	0.59560	0.2927	-6.010E-003	6.651E-003	8.287E-004
D	23.7084	0.73880	0.3514	-6.010E-003	6.651E-003	8.287E-004

TOTAL

Fiber	Intercept	Temperature	Time
A	1.2748	0.0205	7.555E-003
B	1.1390	0.0205	7.555E
C	1.2775	0.0205	7.555E
D	1.1399	0.0205	7.555E

An inverse correlation was observed between the process time and temperature. In other words as it is seen in Table 4.3, an increase in the process temperature caused the process time to decrease. Mestres et al., (2000) reported that time and temperatures are parameters closely related to each other e.g., an increase in temperature enables shorter exposure time, thus accelerating the analysis time.

Pawliszyn, (2002) declared that the exposure time is also very significant. A longer time favors the occupation of more sites on the fiber by analyte molecules, but prolonged time when all sites are occupied does not affect the pre-concentration efficiency and sometimes can cause desorption. In another study by Reto et al., (2007) five different exposure times were tested in order to determine the best compromise between time and analyte response and establish extraction profiles of the vitamin K from green tea. They concluded that the HPLC peak areas of vitamin K increased with an increase in exposure time.

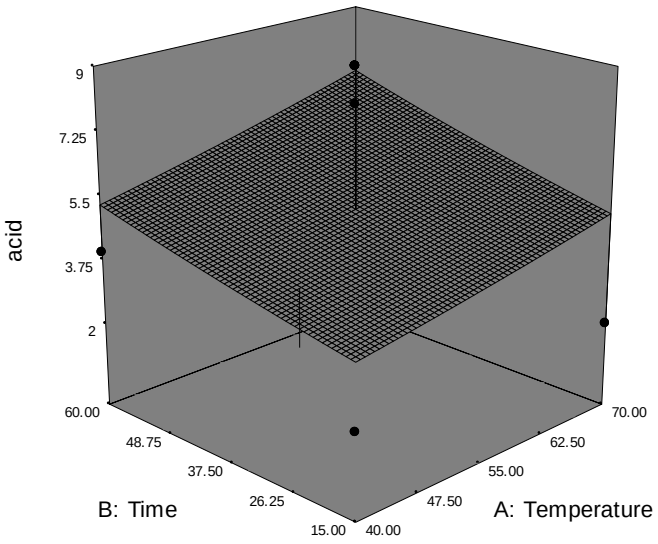
The results given in Table 4.4 show that the number of acids, alcohols, others and total is linearly affected by temperature and time. The influence of temperature was found to be highly significant ($p<0.05$). The significance of each coefficient was statistically determined. The larger F values and small p-values are thought to be significant. The coefficients given in Table 4.4 are significant at 95% level.

Table 4.4 ANOVA for Response Surface Linear Model of Acids

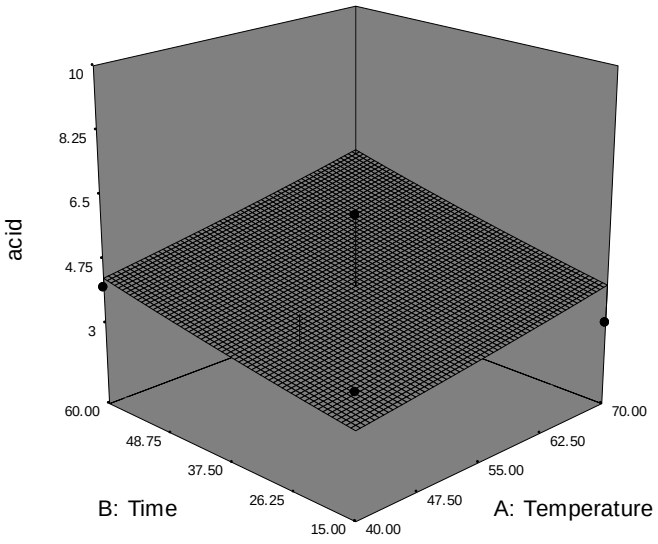
Source	Sum Squares	df	Mean Square	F value	p- value Prob>F	
Model	1.97	5	0.39	3.11	0.0168	significant
A-Temperature	0.69	1	0.69	5.42	0.0244	
B-Time	0.93	1	0.93	7.34	0.0094	
C-Fiber	0.32	3	0.11	0.85	0.0476	significant
Residual	5.83	46	0.13			
Lack of Fit	5.26	29	0.18	5.44	0.0003	significant
Pure Error	0.57	17	0.033			

The number of acids, alcohols, and others absorbed by fiber in each run were evaluated statistically depending on extraction time and temperature. The response surface plot is presented in Figure 4.1 which indicates that an increase in the number of acids absorbed by fiber is directly proportional to extraction time and temperature. Also, fiber C was observed to retain more acids than other fibers. No quadratic effect of temperature and time-temperature interaction on the number of acids was

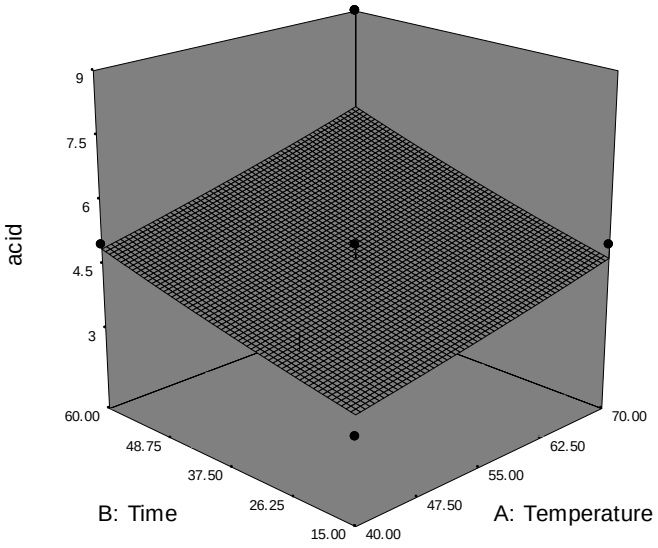
observed. Similar results were obtained for alcohols, others and total compounds (Data are given in appendix A6, A7 and A8).



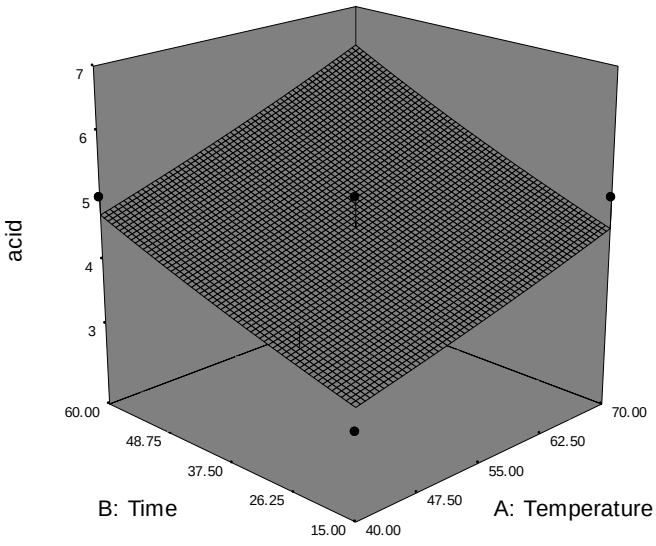
Fiber A



Fiber B



Fiber C



Fiber D

Figure 4.1 Variation in the number of acids as a function of time and temperature and fiber type.

4.1.1 Optimization of Process Variables

Response surface methodology was used to determine optimum conditions that yield maximum number acids, alcohols, others and their total. King et al., (2003) reported that an increase in extraction temperature causes an increase in extraction rate but a decrease in the distribution constant. This conversely causes a decrease in the sensitivity of the extraction process. A well-balanced compromise between sensitivity and extraction rate with regard to the extraction temperature can be obtained by careful optimization.

In this study, temperature, time and fiber type were selected in the range shown in Table 4.2. Applying desirability function method, seven solutions were obtained for optimum criteria. Also desirability value of first solution was greater than others. The desirability figure is shown in appendix A9. The optimum conditions were determined to be 60°C, 60 minutes and fiber C (Figure 4.2). At the optimum point, the number of acids, alcohols, others and their total were found to be 6, 5, 11 and 22 respectively.

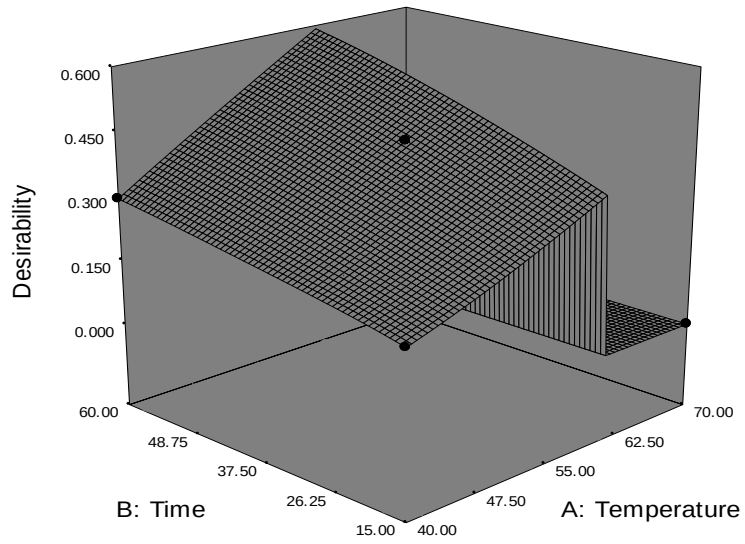


Figure 4.2.Optimum conditions as obtained by the design expert

4.2 Total Aroma and Polyphenol Content of Dark Chocolate During the Three Phases of Conching

Conching is the agitation of chocolate coupled with aeration and heat. This is a time and energy consuming process but it has remarkable effects on the final chocolate flavor. Undesirable volatiles, derived from oxidative and carbonyl-amino browning reaction catalyzed by heat and aeration, are removed in this process. The conched chocolate flavor can be differentiated organoleptically from that of the unconched chocolate but the knowledge related with change in components responsible for the sensory quality is still lacking. The nature of flavor change during conching process is not exactly understood at the chemical level. However, the level of volatile compounds such asacids, pyrazines, phenols, aldehydes and ketons has been shown to decrease (Keeney, 1971).

Different aroma profiles were observed during the three phases of conching as a result of GC/MS/SPME analyses, which showed that there is large difference in number and level of aroma compounds. Calculation showed that the decrease in the quantity of total aroma was about 57 %. Table 4.5 shows this drastic change in the chocolate aroma profile for the three phases of conching.

The main compounds making up the dark chocolate volatiles in the dry phase were observed to be nonanal, acetic acid, tetramethyl- pyrazine, benzaldeyde, 2-methyl-heptadecane, and n-octane. Becket, (2000) stated that the air space surrounding conching machine has acidic odor and acids whose concentration decrease firstly are the short chain volatile acids such as acetic acid known to be end product of fermentation. In the dry phase, the concentration of some of these compounds, such as nonanal and tetramethyl-pyrazine decreases while some of them such as acetic acid, increases. Also some new compounds were observed to form during this phase probably due to increase in temperature and agitation. 2-decen-1-ol, hexadecanoic acid, 2-methyl-heptadecane, and 2-oxo-ethly ester-propanoic acids are some of them. The total number of volatile compounds decreased from 35 to 31 in this phase; increased from 31 to 39 in the pasty phase and decreased again from 39 to 31 in liquid phase. The amount of volatiles was observed to increase mostly in the pasty phase of conching. This increase may be attributed to the relatively higher temperature (80°C) and longer time (4 hours).

Table 4.5 Change in the amount of dark chocolate volatiles during three phases of conching

Compound	Before Conching (ppb)	Dry phase Conching (ppb)	Pasty-Phase Conching (ppb)	Liquid Phase Conching (ppb)
3-methyl-butanal	902	ND	ND	ND
1-propen-2-ol-acetate	51	ND	ND	ND
2,5-dimethyl-pyrazine	1004	ND	ND	ND
2,3-dimethyl-pyrazine	307	ND	ND	ND
2-isopropyl-5-methyhex-2-enal	215	ND	ND	ND
Nonanal	2575	2349	858	669
Trimethyl-pyrazine	1977	11	ND	ND
Acetic acid	2471	6581	9797	5353
Tetramethyl-pyrazine	16599	7431	5901	3525
Benzaldehyde	4774	239	478	124
1,6-octadien-3-ol-3,7-dimethyl acetate	149	27	ND	ND
1,2-dietyhl-trans-cyclobutane	110	ND	ND	ND
3-hydroxy-2-butanone	178	34	ND	81
2-methyl-propanoic acid	200	ND	ND	76
5-methyl-2-furancarboxaldehyde	119	ND	ND	ND
2-decanone	209	ND	ND	ND
5-methyl-2-(1-methylethy)-cyclohexanol	413	ND	ND	ND
4-hydroxy-butanoic acid	89	ND	ND	ND
Benzeneacetaldehyde	949	ND	ND	ND
3-methyl-butanoic acid	907	ND	ND	ND
Benzyl-carboxylic acid	76	ND	ND	ND
2-phenylethylester-acetic acid	982	105	78	67
3-methyl-benzoate-1-butanol	577	ND	ND	ND

Table 4.5 cont'd

Compound	Before Conching (ppb)	Dry Phase Conching (ppb)	Pasty Phase Conching (ppb)	Liquid Phase Conching (ppb)
Hexanoic acid	156	310	56	35
2-methoxy-phenol	203	ND	ND	ND
Benzyl alcohol	193	ND	ND	ND
Hexanoic acid	152	68	ND	124
5-methyl-2-phenyl-2-hexanal	350	ND	ND	ND
Nonanoic acid	231	ND	ND	ND
2-furanmethanol	ND	ND	ND	211
Pentanoic acid	186	ND	ND	66
Isoproponal	ND	ND	ND	896
1-methyl-cyclohexene	302	298	168	141
Decane	ND	ND	310	615
3-methyl-pentane	ND	ND		999
Ethanone	ND	260	676	94
2-methyl-1-prapanol	ND	ND	ND	1786
1,1-diflouoro-dodecane	ND	404	578	1806
2-furanone	ND	235	ND	ND
Octal	ND	ND	ND	ND
1,1-oxybis-2-praponol	ND	204	153	131
1,6-heptadien	ND	188	ND	ND
1,3-dioldisobutyrate-pentan	ND	26	ND	ND
2-propyl-2-benzoyloxy-3,3,3-trifluropropoate	ND	75	ND	ND
Benzeneethanol	ND	353	3300	395
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-one	ND	1185	3031	1648

Table 4.5 cont'd

Compound	Before Conching (ppb)	Dry Phase Conching (ppb)	Pasty Phase Conching (ppb)	Liquid Phase Conching (ppb)
2-decen-1-ol	ND	420	298	32
1-hexadecene	ND	25	34	ND
Hexadecanoic acid	ND	5	53	ND
2-methyl-heptadecane	ND	2706	3600	5748
2-hexanone	ND	50	350	48
2-oxo-ethylester-propanoic acid	ND	139	ND	ND
5,6-dihydro-1H-pyridin-2-one	ND	112	ND	ND
4-hydroxy-3-methoxy-benzoic acid	ND	506	622	ND
1,2-benzenedicarboxylic acid	ND	18	293	38
Methylester-heneicosanoic acid	ND	128	126	99
N-octane	ND	1189	ND	ND
1-methyl-cyclohexene	ND	ND	17	ND
Phenylacetaldehyde	ND	ND	113	ND
1-phenyl-ethanone	ND	ND	104	ND
3-hexen-1-ol-benzoate	ND	ND	92	ND
Octanoic acid	ND	ND	135	ND
5-methyl-2-(1-methylethyl)-cyclohexanol	ND	ND	374	ND
Cyclopentacycloheptene	ND	ND	235	ND
Ethylester-heptanoic acid	ND	ND	4	ND
Decanal	ND	ND	161	ND
2-phenoxy-ethanol	ND	ND	1493	ND
Phenylethy acetate	ND	ND	535	ND
Nonanoic acid	ND	ND	317	ND

Table 5 cont'd

Compound	Before Conching (ppb)	Dry Phase Conching (ppb)	Pasty Phase Conching (ppb)	Liquid Phase Conching (ppb)
Benzeneacetaldehyde	ND	ND	268	149
1-methoxy-4-benzene	ND	ND	67	ND
2-Undecanone	ND	ND	27	ND
1,1-dimethylethylester-butanoic acid	ND	ND	31	ND
2,3-dimethyl-5-propylpyrazine	ND	ND	157	ND
Triacetin	ND	ND	570	ND
Decanoic acid	ND	ND	25	ND
5-methyl-2-phenyl-2-hexanal	ND	ND	1229	ND
2,6-bis-(1,1-dimethylethyl)-phenol	ND	ND	397	ND
Octadecanoic acid	ND	ND	299	ND
Ethylester-hexadecanoic acid	ND	ND	155	ND
3-hexene	ND	ND	ND	683
2-methylene-1,3-diphenyl-propanediol	ND	ND	ND	21
1-methyl-2-phenylethanol	ND	ND	ND	165
Octadecane	ND	ND	ND	80
3,4-dimethylpentanol	ND	ND	ND	69
1-(5-hexenyl-1-methyl)-hyrazine	ND	ND	ND	9
9-Octadecenoic acid	ND	ND	ND	45
3-phenyl-2-propenal	ND	ND	ND	91
Propanoic acid	ND	ND	ND	104
Benzocyclobuten	ND	ND	ND	429
2-cyclobutene	ND	ND	ND	705
4-methyl-hexanal	ND	ND	ND	373
4-tridecene-2-decenal	ND	ND	ND	1629

ND: Not detected

As seen from this Table, the number of acids in the dry phase is 10; it increased to 16 in the pasty phase and decreased again to 11 in the liquid phase. The decrease was mostly observed in both quantity and number of acids to be in the liquid phase.This shows that conching is of vital importance for producing a chocolate with a mild flavor. As pointed out by Minife, (1989) a decrease in the amount of acids, tetramethyl-pyrazine, benzaldehyde and increase in the amount of 2-methyl propanol and 2-methyl-heptadecane are considered to be an indication of quality of conching, which was the case in this study.

3-methyl-butanal, nonanal, benzaldeyde, isopropanal, octanal, phenylacetaldeyde, decanal, hexanal and propanal were the identified aldehydes during the three phases. The quantity of these aldehydes was observed to vary from phase to phase, namely 2588 ppb in dry phase, 4156 ppb in pasty phase and 4458 ppb in liquid phase. Counet et al., (2002) pointed out that the concentration of Strecker aldehydes decreased partially during conching. Schnermann & Schieberle, (1997) stated that the concentration of isopentylpyrazine, tri-or tetramethylpyrazine decreased during conching of dark chocolate. In the present study 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, trimethyl-pyrazine and tetramethylpyrazine were the major detected pyrazines in all three phases.

2-furanone, 1,1-oxybis-2- praponol, 1,6-heptadien, 2-oxo-ethylester-propanoic acid were some of the compounds which were detected only in dry phase but they were not in the detection limit of SPME in the pasty and liquid phases. Phenylacetaldeyde, 1-phenylethanone, 2-phenoxyethanol, and phenylethyl acetate were identified just in pasty phase but did not appear in the liquid phase.

The total amount of volatiles decreased from 37606 to 27848 ppb in dry phase, increased to 38073 ppb in pasty phase and finally decreased to 30197 ppb in liquid phase. It was observed that there was remarkable change in the total amount of alcohols, acids, pyrazines, and hydrocarbons in all phases. Total amount of acids was 7755 ppb in dry phase, 11991 ppb in pasty phase and 6765 ppb in liquid phase. The alcohol concentration was 977 ppb in the dry phase, 5618 ppb in pasty phase and 2958 ppb in the liquid phase. While the concentration of pyrazines decreased continuously (7442 ppb in the dry phase, 5901 ppb in the pasty phase and 3525 ppb

in liquid phase), those of hydrocarbons increased during all phase of conching (3029 ppb in the dry phase, 5009 in the pasty phase and 10777 ppb in liquid phase). Ketone concentration was 579 ppb in the dry phase, increased to 1053 ppb in the pasty phase and decreased again to 142 in the liquid phase.

4.2.1 Odor Identifications

The odor qualitiy of the volatile compounds were identified by GC-MS-olfactometer before and after conching and shown in Table 4.6

Table 4.6 Odor qualities of chocolate volatile compounds before and after conching.

Retention Time (min.)	Compound (before conching)	Odor description
0.79	3-methyl-butanal	Chocolate
0.47	1-propen-2-ol-acetate	Fatty
4.78	2,5-dimethyl-pyrazine	Nutty, green
5.10	2,3-dimethyl-pyrazine	Cooked, nutty
5.20	2-Isopropyl-5-methylhex-2-enal	Fruity
5.61	Nonanal	Floral
5.82	Trimethyl-pyrazine	Cocoa, roasted
6.41	Acetic acid	Sour
6.69	Tetramethyl-pyrazine	Bean like
7.37	Benzaldeyde	Nutty
7.44	1,6-octadien-3-ol,3,7-dimethyl-acetate	Flowery
7.59	1,2-diethyl-trans-cyclobutane	Like laurel
7.70	3-hydroxy-2-butanone	Fruity
7.83	2-methyl-propanoic acid	Rancid
7.97	5-methyl-2-furancarboxaldehyde	Sweet, caramel like
8.11	2-decanone	No smell
8.15	5-methyl-2-(1-methylethy)-cyclohexanol	Like laurel
8.70	4-hydroxy-butanoic acid	Fruity
8.75	Benzeneacetaldehyde	Pungent

Table 4.6 cont'd

Retention Time (min.)	Compound (before conching)	Odor description
8.99	3-methyl-butanoic acid	Cheese
10.26	Benzyl-carboxylic acid	Sweety
10.60	2-phenylethyester-acetic acid	Vinegar
10.82	3-methyl-benzoate-1-butanol	Fruity
10.90	Hexanoic acid	No smell
11.11	2-methoxy-phenol	Woody
11.27	Benzyl alcohol	Almond like
11.46	Phenylethanol	Sweet, honey
11.95	α-ethly-diene-benzeneacetaldehyde	Fruity
12.50	Ethanone	Fruity
12.99	Phenol	Spicy
13.12	4-propyl-2-hydroxycyclopent-2-en-1-one	Caramel
13.38	Decylphenyester-carbonic acid	Sour
13.66	3-phenyl-2-propenal	Potato like
13.80	Hexanoic acid	Fatty
14.10	5-methyl-2-phenyl-2-hexenal	Cocoa
16.12	Nonanoic acid	Rancid

Retention Time (min.)	Compound (after conching)	Odor descriptiom
0.23	3-methylpentane	Odorless
0.47	2-methyl-1-propanol	Sweet
0.77	3-methyl-butanal	Fruity
1.14	Decane	Sharp, bad
1.58	2-methyl-heptadecane	No smell
1.75	3-hexene	Green

Table 4.6 cont'd

Retention Time (min.)	Compound (after conching)	Odor description
2.57	Hexanoic acid	Sour
2.86	1,1-difluoro-dodecane	Camphor like
3.16	1-methyl-cyclohexene	Spicy
3.20	2-propanol	Sweet
3.35	Cyclohexene	Sweet
3.80	Ethanone	Fruity
3.92	1-methyl-2-phenylethanol	Rose
4.21	Tetramethyl-pyrazine	Bean like
4.39	Octadecane	No smell
4.45	Nonanal	Floral
4.66	Benzeneethanol	Citrus
5.58	2-decen-1-ol	No smell
5.79	3,4-dimethylpentanol	Sharp
6.24	2-hexanone	Fatty
6.40	Acetic acid	Sour, vinegar
6.66	Tetramethyl-pyrazine	Bean like
6.80	2-phenylethylester-acetic acid	Vinegar
6.95	9-octanoic acid	Rancid
7.37	Benzaldehyde	Nutty
7.40	2-methyl-heptadecane	No smell
7.48	Isopropanol	No smell
7.69	3-hydroxy-2-butanone	Creamy
7.83	2-methyl-propanoic acid	Fruity
8.07	Propanoic acid	Rancid

Table 4.6 cont'd

Retention Time (min.)	Compound (after conching)	Odor description
8.68	3-methyl-butanoic acid	Banana
10.59	Benzenebutanol	Floral
10.90	Pentanoic acid	Rancid
11.11	2-methoxy-phenol	Smoky
11.37	Phenylethanol	Sweet,honey
12.49	Ethanone	Fruity
13.65	3-phenyl-2-propenol	Potato like
22.28	Octane	Gasoline
22.79	1,2-benzenedicarboxylic acid	Floral
24.20	2-decenal	Fatty

Frauendorfer & Schieberle (2006) reported that acetic acid is an important compound which has a significant effect on chocolate aroma since it may provide undesired flavor, vinegar like odor. Akhtar et al., (2010) assumed that the acetic acid was produced from oxidation of aldehydes and alcohols and released from esters by mechanicaland/or thermal mechanisms during the long conching process.

Ramli et al., (2006) claimed that aldehydes are one of the main groups formed from the roasting process in chocolate manufacture. It is believed that some aldehydes, such as 2-methylbutanal, 2-methylpropanal, benzaldehyde and phenylacetaldehyde are important compounds in chocolate flavor (Counet et al., 2002). Schnermann & Schieberle, (1997) reported that phenylacetaldehyde was an odor-active compound in dark chocolate. Pyrazine is one of the major groups formed in the Maillard reaction during roasting. Schnermann & Schieberle, (1997) stated that, among 525 identified volatiles in roasted cocoa bean, one fifth of them were predominate pyrazine fractions.

In the present work 2,5dimethylpyrazine, 2,3 dimethylpyrazine, trimethyl and, tetramethylpyrazines were observed to have a nutty odor while nonanal, 3-hydroxy-

2-butanone, 4-hydroxy-butanoic acid, 3-methyl-1-butanol, and α-ethyl-benzeneacetaldeyhde, have fruity odor. Odor of acetic acid, 2-methyl-propanoic acid nonanoic acid, pentanoic acid was rancid and sour.

4.2.2 Quantification of total polyphenols, caffeine, and theobromine

Arora et al.,(2000) stated that the flavonoids represent a ubiquitous and abundant group of polyphenol consumed in diet, primarily from fruits and vegetables, derived from plants and act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals and to stabilize membranes by decreasing membrane fluidity. Cocoa and its derived products are rich in flavonoids, characterized as flavan-3-ols or flavanols and include the monomeric forms, (-) epicatechin and (-) catechin and the oligomeric form of the monomeric units (Wollgast & Anklam, 2000). Steinberg et al., (2003) claimed that, most of antioxidant activity in chocolate is due to its polyphenol content.

Benowitz, (1990) stated that the methylxanthines such as caffeine and theobromine are regularly consumed from a variety of foods. Consumers want to know the methylxanthines content of foods because of their widespread consumption. Methylxanthines have been reported to have physiological effect on various body systems, including the central nervous, cardiovascular and respiratory systems (Nehlig et al., 1992; Spiler, 1998).

A calibration curve with caffeine, theobrimine and catechin standards shown in appendix A9, A10 and A11 was prepared and an equation was obtained by using these calibration curves. The quantities of caffeine, theobrimine and total polyphenol were calculated by using these equations. The amounts of caffeine, theobrimine and total polyphenol content were observed to decrease by 10.51, 31.62 and 2.97 % respectively as a result of conching, as shown in Table 4.7. This is known to be due to high temperature and high shearprevailing during conching.

Table 4.7 Variation in the quantity of caffeine, theobrimine, and total polyphenol of dark chocolate during conching.

	Before conching (ppm)	**After conching** (ppm)
Caffeine	59.93±0.05	53.63±0.04
Theobrimine	8.38±0.06	5.73±0.05
Total polyphenol	292±0.01	283.3±0.1

±standard deviation

4.3 Conching Kinetics

Chocolate has a specific flavor depending on genotype and processing steps such as roasting, fermentation and conching which is the last and most important step contributing to the flavor development. Although the major development in flavor takes place in this step the mechanism of its formation is still not too clear.

As known, conching is a batch and time-dependent process. Generally conching time varies within a range of 6 to 12 hours depending on the type of process. This is quite a longtime for an industrial process and therefore in addition to the aroma development, structural changes affecting the chocolate texture are naturally expected.

In the present study, dark chocolate sampling was carried out at 30-minutes intervals to see the variation of both quality and quantity of the volatile compounds within the conching time. A combination of GC/MS/SPME was used for this purpose. Toluene was used as internal standard for the quantitative analysis. The significance of terms in the model was found by analysis of variance (ANOVA) for each response as shown in Table 4.8. The significance was judged by determining their probability levels.

The results showed that the quantity of acids, aldehydes, pyrazines, ketones, hydrocarbons, alcohols, and their sum is polynomially affected by time. The influence of time was found to be highly significant ($p<0.05$).

Table 4.8 Statistical values of responses

Response	F value	P value	R value
Acids	26.62	0.0002	0.9089
Aldehydes	24.95	0.0002	0.9034
Pyrazines	52.81	0.0001	0.9519
Ketones	63.55	0.0001	0.8640
Hydrocarbons	21.39	0.0004	0.8891
Alcohols	26.65	0.0002	0.8551
Esters	246.83	0.0001	0.9611
Sum of volatiles	19.58	0.0005	0.8131

The significance of each polynomial coefficient was statistically determined. Large F values and small p-values are thought to be significant. The related Figures giving response as a function of time are shown in appendix A21. The data given in Table 4.8 are significant at 95% level. Design model equations obtained as a result of fitted data are given in equation (1)-(8):

$Ln(acids) = 9.6 + 0.079*t - 0.91*t^2$ ..... (1)

$Ln(aldehyde) = 8.92 + 2.07*t - 1.03*t^2 - 1.38*t^3$ .....(2)

$Ln(pyrazine) = 9.01 + 1.35*t - 1.37*t^2 - 0.91*t^3$ .....(3)

$Log_{10}(ketone) = 3.33 + 0.9*t$ .....(4)

$Ln(hydrocarbone) = 8.51 + 1.78*t - 1.12*t^2 - 1.17*t^3$ .....(5)

$Log(alcohol) = 4.01 - 4.71E\text{-}003*t - 1.31*t^2$ ..... (6)

$Log(acetate) = 2.74 + 0.49*t$ ..... (7)

$1/(total) = 1.607E\text{-}005 - 6.25E\text{-}006*t - 1.38E\text{-}005*t^2$ .....(8)

Ln: natural log

(in parenthesis) quantities in ppb

As shown Figure 4.3, the quantities of all volatile compounds representing different functional groups increased till 270 minutes from the start of conching. From then on, a significant decrease in their quantities was observed. This increasing-decreasing trend is seen to be quantitatively predominant for the acids whose quantity reached a value higher than 20,000 ppb for a conching time of 270 minutes.

Acids are known to cause sourness in dark chocolate. The conching process is again seen to be effective in further reducing their quantities after the roasting process (Lopez, 1983; Jinap & Dimick, 1991; Jinap et al., 1995). The total amount of acids was observed to fall below 5,000 ppb after 300 minutes of conching time.

Rodriguez et al., (2011) studied the effect of fermentation time and drying temperature on the volatile compounds in cocoa liqueur. They found that total concentration of aldehydes and pyrazines increased with increasing fermentation time. They declared that the total quantity of aldehydes was 11 mg/kg after fermentation. Owusu et al., (2010) studied effect of fermentation, roasting and conching processes on dark chocolate's aroma volatiles. They claimed that fermentation, roasting and conching were the main processes affecting aroma production of dark chocolate. They found that high roasting and conching temperatures, long fermentation and long conching times are responsible from increasing the quantity of volatiles. They did not report any data related with variation of concentration of the mentioned volatiles with time.

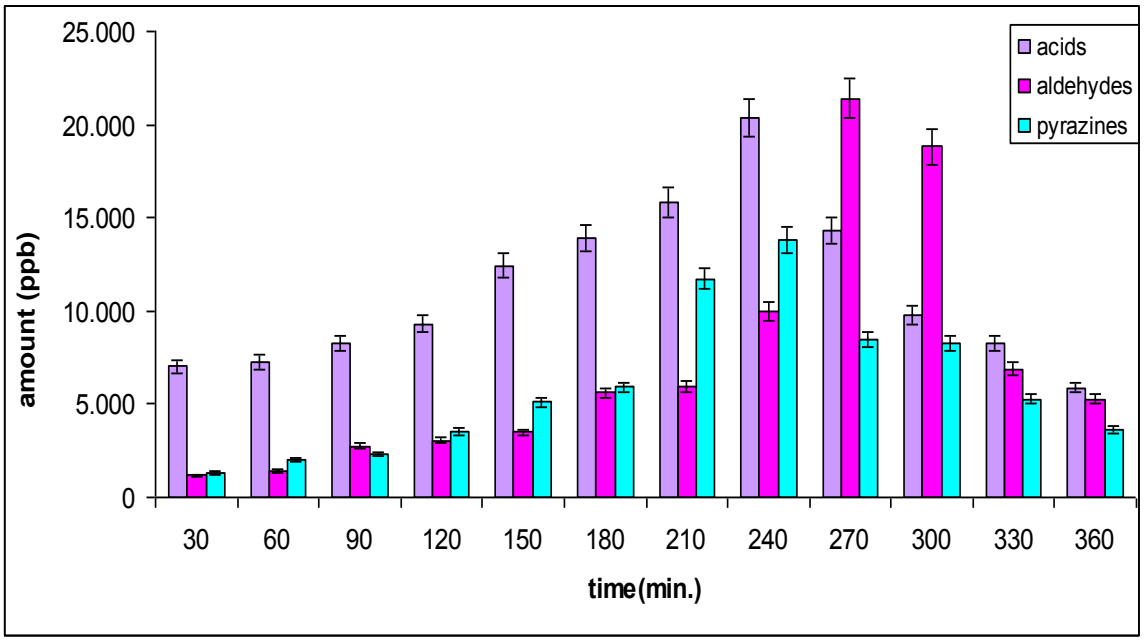


Figure 4.3 Variation in the quantity of acids, aldehydes and pyrazines with time during conching

The amount of pyrazines was about 2,000 ppb at the beginning of conching and it reached a top value of 20,000 ppb after 270 minutes. Owusu et al., (2010) suggested a-6 hour conching at 80°C to be effective in reducing the quantity of pyrazines and aldehydes formed as a result of high roasting temperature, but they did not mention the removal kinetics of the mentioned compounds.

The maximum quantities of ketones, hydrocarbons, and alcohols were observed to correspond to 270, 240, and 210 minutes respectively from the beginning of conching as seen in Figure 4.4. The sum of their quantities was above 18,000 ppb; however after 270 minutes, their concentrations were observed to decrease gradually.

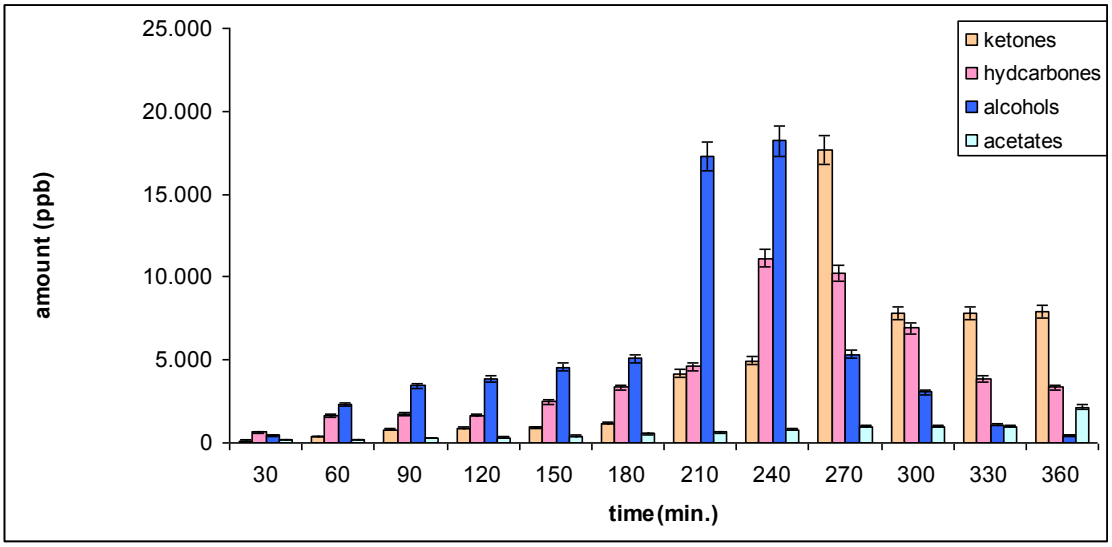


Figure 4.4 Variation in the quantities of ketones, hydrocarbons, alcohols and esters with time during conching

Frauendorfer et al., (2008) claimed that high alcohols are the cause the flowery and candy flavor while esters contribute to a fruity flavor to dark chocolate. (Jinap et al., 1998). On the other hand, sun-drying is reported to decrease the amount of alcohols (Portillo et al.,2009).

In the present work, the amount alcohols, ketones and hydrocarbons were reduced to values below 2,000 8,000, and 4,000 ppb respectively at the end of conching (360 min.). In contrast to ketones; the concentration of hydrocarbons, alcohols, and esters (acetates in this case) increased continually during conching and they reached 4,000 pbb.

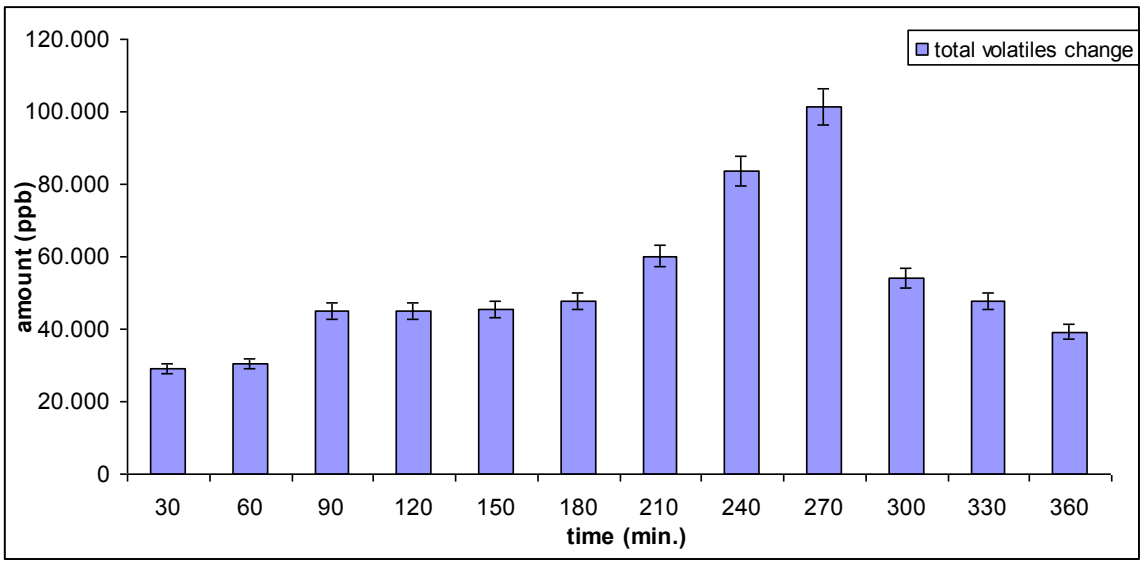


Figure 4.5 Variation in the total quantities of dark chocolate volatiles with time during conching

As seen in Figure 4.5, the total quantity dark chocolate volatiles increased slightly up to 180 minutes of conching. This step was followed by a sharp increase up to 270 minutes at which time total amount of volatiles reached 100,000 ppb. However at the end of conching this value was reduced back to below 50,000 ppb. Dark chocolate had a mellow flavor at the end of conching, because the amount of acids, alcohols and other functional groups, causing unpleasant odor and taste, decreased whereas groups, likepyrazines and esters, causing pleasant odors, increased. Counet et al., (2002) studied the change in the odorant concentration before and after conching. They found that no key odorant was synthesized and while the concentration of some compounds increased, that of some other compounds decreased during conching. Again no indication as the kinetics of odorants was made.

4.3.1 Optimization of Conching Time

General factorial design of Design Expert (ver 7.0.0) was used to determine the optimum time yielding minimum quantities of undesirable acids, and lower alcohols, and maximum quantities of desirable pyrazines, aldehydes and ketones during flavor development of dark chocolate conching. In this study, time was selected in the range as shown in Table 4.9.

Table 4.9 Optimization range of process parameter and responses.

Response (ppb)	Parameter (min.)	Goal	Lower Limit	Upper Limit
Acids	Time	minimize	5,890	20,364
Aldehydes	Time	maximize	1,160	21,411
Pyrazines	Time	maximize	1,323	13,813
Ketones	Time	maximize	142	17,666
Hydrocarbons	Time	minimize	658	11,140
Alcohols	Time	minimize	441	18,217
Esters	Time	maximize	171	2,166
Total volatiles	Time	maximize	29,186	10,1217

According to the Design Expert Program, the optimum time is 354 minutes for dark chocolate conching carried out at 80°C, and 600-1500 rpm. Under these conditions, the quantities of acids, aldehydes, pyrazines, ketones, hydrocarbons, alcohols, esters and total volatiles were determined to be 5890, 6085, 3685, 1573, 3391, 602, 1632, and 43667 ppb respectively. Aroma profile for the conching is given in the Table 4.10

Table 4.10 Dark chocolate's aroma profile at the optimum conching time.

Retention Time (min.)	Compound	Odor Description	Quantity (ppb9
0.09	Acetic acid	Rancid	5,353
1.58	2-methyl-heptadecane	Chocolate	5,747
1.75	3-hexene	Green	682
2.39	Benzaldehyde	Nutty	123
2.57	Hexanoic acid	Sour	124
3.20	2-propanol	Pungent	131
3.35	Cyclohexene	Sweet	141
3.41	2-methylene-1,3-diphenyl-1,3-propanediol	No smell	21

Table 4.10 cont'd

Retention Time (min.)	Compound	Odor Description	Quantity (ppb)
3.56	Benzeneacetaldehyde	Almond	149
3.80	Ethanone	Fruity	94
3.92	1-methyl-2-phenylethanol	Rose	165
4.21	Tetramethyl-pyrazine	Bean like	3,524
4.39	Octadecane	Honey	80
4.45	Nonanal	Coffee	669
4.66	Benzeneethanol	Floral	394
5.10	2,3-dihyro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Caramel	1,648
5.51	Hexanoic acid	Sour	35
5.58	2-decen-1-ol	Fruity	298
5.79	3,4-dimethyl-pentanol	No smell	69
5.86	1,5-hexenyl-1-methyl-hyrazine	Meaty	10
5.91	2-methyl-heptadecane	Chocolate	54
6.01	Nonanal	Coffee	33
6.24	2-hexanone	No smell	48
6.80	2-phenylethylester-acetic acid	Rancid	67
6.95	9-octadecenoic acid	Sour	44
8.07	Propanoic acid	Rancid	104
22.79	1,2-benzenedicarboxylic acid	Sweet	38
23.45	4-methyl-hexanal	Chocolate	373
23.85	Heneicosanoic acid-methyl ester	Rancid	126
24.20	2-decenal	citrus	1,629

4.4 Development of an Aromatic Dark Chocolate with Cinnamon, Aniseed and Ginger as Additives

4.4.1 Determination of Quantity of Spices Added

The range percentage of spices added to dark chocolate was determined firstly by a sensory panel then the data obtained were evaluated through the Response Surface Methodologyto decide on the final percentages. A range of 1 to 5% was then specified for the mentioned spices. The influence of spices on the hardness, viscosity, moisture, sensory quality, and total color change was investigated. Hardness, viscosity, moisture and color of dark chocolate were used as target parameters to determine the optimum spice concentration, keeping the traditional structure of dark chocolate.The data related with experimental design for the optimization and the relevant figures are given in appendices A22, A23, A24, A25, A26, A27 and A28. The optimum spice concentrations determined as such are given in Table 4.11

Table 4.11 Optimum percentages of spice additives

Parameters	Aniseed Goal	Cinnamon Goal	Ginger Goal	Lemon peel powder Goal
Viscosity	Target	Target	Target	Target
Texture	Target	Target	Target	Target
Total color change	Minimize	Minimize	Minimize	Minimize
Moisture	Target	Target	Target	Target
Sensory	Target	Target	Target	Target
Optimum percentage	1.6	4.5	2.5	2

*****target: accepted quality parameters of dark chocolate;

*goal: desired values of experimental design

Thus the optimum spice concentrations were determined to be 1.6 for aniseed, 5 for cinnamon, 2.5 for ginger and 2.0 for lemon peel powder, all in percent.

4.4.2 Experimental Results for the Development of Spice-Enriched Aromatic Chocolates

Flavor is the basic factor determining consumer preference in the global world. In chocolate industry, many nuts such as peanut, pistachio nut and almond are being used to contribute to increase the consumer acceptance. Spices are also used as flavoring agents in many foods almost everywhere. As it was pointed out in the Introduction section, the present study aims producing a functional chocolate enriched with selected spice powders. Thus, with the contribution of these spices, a "synergetic effect" regarding chocolate flavor is expected to be created.

GC/MS/SPME, GC/MS/O and sensory analysis were used to assess the flavor of spice-chocolate samples and that of dark chocolate both qualitatively and quantitatively. Toluene was used as internal standard for the quantitative determination of the aroma compounds. Dilution factor for determination of the odor activity values of the aroma compounds was determined by the following procedure: The sample was reduced to the half of the previous quantity until no odor was perceived. Odor activity value of each compound was calculated using this dilution factor. A comparison giving the quantities and odor activity values of the volatiles of dark chocolate and the corresponding spice chocolates is shown in Tables 4.12, and 4.13.

Table 4.12 Volatile compounds and their odor qualities in cocoa mass and dark chocolate

Compound	**Cocoa Mass (ppb)-OAV***	**Odor Description**	**Dark Chocolate (ppb)-OAV***	**Odor Description**
3-methyl-butanal	902-1.74	Chocolate	ND	
1-propen-2-ol-acetate	51-0.09	Fatty	ND	
2,5-dimethyl-pyrazine	1004-1.94	Nutty	ND	
2,3-dimethyl-pyrazine	307-0.59	Cooked	ND	
2-Isopropyl-5-methyhex-2-enal	215-0.42	Unknown	ND	

Table 4.12 cont'd

Compound	Cocoa Mass (ppb)-OAV*	Odor Description	Dark Chocolate (ppb)-OAV*	Odor Description
Nonanal	25754.99	Unknown	ND	ND
Trimethyl-pyrazine	1977-3.83	Cocoa	ND	ND
Acetic acid	2471-4.49	sour	5353	
Tetramethyl-pyrazine	16599-32.16	Bean like	3525	
Benzaldehyde	4774-9.25	Nutty	124	
1,6-octadien-3-ol-3,7-dimethyl-acetate	149-0.28	Flowery	ND	
1,2-diethyl-trans-cyclobutane	110-0.21	Like laurel	ND	
3-hydroxy-2-butanone	178-0.34	Fruity	ND	
2-methyl-propanoic acid	200-0.38	Rancid	104	
5-methyl-2-furancarboxyaldeyhde	119-0.23	Sweet, Caramel	ND	
2-decanone	209-0.41	No smell	ND	
5-methyl-(1-methylethyl)-cyclohexanol	413-0.8	Like laurel	ND	
4-hydroxy-butanoic acid	89-0.17	Fruity	ND	
Benzeneacetaldehyde	949-1.84	Pungent	ND	
3-methyl-butanoic acid	907-1.75	Cheese	ND	
Benzyl-carboxylic acid	76-0.14	Sweety	ND	
2-phenylethylester-acetic acid	982-1.90	Vinegar	ND	

Table 4.12 cont'd

Compound	Cocoa Mass (ppb)-OAV*	Odor Description	Dark Chocolate (ppb)-OAV*	Odor Description
1-butanol-3-methyl-benzoate	577-1.11	Fruity	ND	
Hexanoic acid	156-0.3	No smell	123	
5-methyl-2-phenyl-2-hexanal	350-0.68	Cocoa	ND	
Nonanoic acid	231-0.44	Rancid	ND	
Pentanoic acid	186-0.36	Rancid	ND	
2-methyl-heptadecane	ND		5747-11.13	Chocolate
3-hexene	ND		683-1.32	Green
2-propanol	ND		131-0.25	Pungent
Cyclohexene	ND		141-0.27	Sweet
Benzeneacetaldeyde	ND		149-0.28	Almond
1-methyl-2-phenylethanol	ND		165-0.32	Rose
Octadecane	ND		80-0.15	Honey
2-decen-1-ol	ND		298-0.57	Fruity
Nonanol	ND		34-0.06	Cucumber
2-hexanone	ND		49-0.09	No smell
2-phenylethylester-acetic acid	ND		68-0.13	Rancid
2-Octadecenoic acid	ND		45-0.08	Oily
2-cyclobutene	ND		705-1.36	No smell
4-methyl-hexenal	ND		373-0.72	Chocolate
Methylester-heneicosonaic acid	ND		126-0.24	Rancid
2-decenal	ND		1629-3.15	citrus

OAV*: odor activity value
ND: not detected

As seen in Tables 4.12, and 4.13, a total of 13 compounds were identified for the aniseed chocolate after conching. 6 of them (3 aldehydes, 1 alcohol, 1 ketone and 1 acid) came from the original dark chocolate while the remaining 7 compounds, such as estragole, tricyclo (Undec-9-ene, 2,6,6,9 tetramethyl), benzaldehyde-4-methoxy, originated from aniseed with high odor activity values. This means that the related spice gives aniseed flavor to dark chocolate. Especially, 4-methoxy-(1-propenyl) benzene or anethole and estragole are the main compounds with strong aniseed odor. The concentration and odor activity values of these compounds were determined to be 17,164 ppb-32.26 and 6,351 ppb-12.31 respectively. As expected, 3-methyl-butanal and tetramethyl-pyrazine, whose concentrations and odor activities were determined as 4,458 ppb-3.53 and 19,777 ppb-32.16 respectively, were observed to have the characteristic chocolate odor. In spite of the fact that the concentration of the spice-chocolate aroma is comparable with that of dark chocolate, the spice aroma did not suppress the characteristic dark chocolate aroma as shown in Tables 4.13 and 4.12. In other words, both aniseed and original dark chocolate flavors are perceived comparatively at the same time.

Table 4.13 Volatile compounds and their odor qualities in aniseed chocolate

Compound	**Quantity (ppb)**	**OAV***	**Odor Description**
(2-aziridinylethyl) amine	2293	4.44	Unknown
Acetic acid	842	1.63	Sour, vinegar
Tetramethyl-pyrazine	4458	8.64	Bean like
5-ethyldecane	714	1.38	Fruity
Estragole	17164	33.26	Aniseed
Tricyclo (Undec-9-ene-2,6,6,9-tetramethyl)	4162	8.06	Lilac
1-methoxy-4-(1-propenyl)-benzene	6351	12.31	Aniseed
Butanamide-guadine-carbonate	8316	16.11	Unknown
Phenylethyl-alcohol	965	1.87	Sweet, honey
1-methoxy-4-(1-propeny)	17268	34.22	Aniseed
Ethanone	302	0.58	Fruity

Table 4.13 cont'd

Compound	Quantity (ppb)	OAV*	Odor Description
4-methoxy-benzaldehyde	1871	3.62	Almond like
3-phenyl-2-propenal	7908	15.32	Potato like
2H-1-benzopyran-2-one	1317	2.55	Sweet

OAV*:odor activity value

25 compounds for ginger-chocolate and 21 compounds for cinnamon-chocolate were identified. For ginger chocolate, 3 of these compounds were aldehydes (5,637 ppb), 2 were acids (209 ppb), 2 were alcohols (2,458 ppb) and 2 were ketones (1,535 ppb); for cinnamon chocolate, 7 were aldehydes (3,3847 ppb), 3 were acids (1,367 ppb), 1 was alcohol (1,405 ppb), 2 were ketones (1,099 ppb) and 1 was phenol (199 ppb). As shown in Tables 4.14 and 4.15 the reduction in the quantity and number of these volatiles during conching is not related to the presence of spices. In other words, the spice volatiles did not affect the reduction in the number of the mentioned volatile compounds after conching.

Table 4.14 Volatile compounds and their odor qualities in ginger chocolate

Compound	Quantity (ppb)	OAV*	Odor Description
Acetic acid	356	0.69	Sour,vinegar
Benzaldehyde	965	1.87	Nutty
2-Octanone	806	1.56	Green
1,2,4,5-tetrazin-3-amine	369	0.72	No smell
Benzyl-4-nitrophenylcarbonate	1063	2.06	Unknown
7,11-dimethyl-1,6,10-dodecatriene	1419	2.75	Apple
5-methyl-1-phenyl-1-hexanone	-	-	Peppermint
3-methyl-butanoic acid	108	0.20	Cheese
3,7,11-trimethyl-1,3,6,10-dodecatetraene	318	0.62	Green
1-methanol-3-cyclohexene	623	1.21	Lilac
Benzyl alcohol	-	-	Almond like

Table 4.14 cont'd

Compound	Quantity (ppb)	OAV*	Odor Description
Alpha-cedrene	89	1.79	Woody
1H-benzocycloheptene	4228	8.19	Unknown
Alpha-farnasene	6855	13.28	Green,woody
4-(4-hydroxy-3-methoxyphenyl)-2-butanone	18404	35.66	Ginger
2-phenylethylester-acetic acid	871	1.68	Sour, vinegar
1-methoxy-4-(1-propenyl)-benzene	26301	50.97	Spicy
2-methoxy-phenol	681	1.31	Woody
Phenylethyl alcohol	1835	3.55	Sweet, honey
(1-ethyl-2-propenyl)-benzene	84	0.16	Sweet
Ethanone	729	1.41	Fruity
3,7,11-trimethyl1,3,6,10-dodecatetraene	298	0.57	Green
3-methoxy-benzeldahyde	1375	2.66	Almond like
3-phenyl-2-propenal	3297	6.39	Potato like
Butanoic acid	101	0.19	Butter
1-octenyl-benzene	716	1.38	No smell

Of the total volatiles, 15 compounds originated from ginger, and 11 compounds from cinnamon, which give a characteristic spicy flavor to dark chocolate. As known, the main compounds, giving their characteristic odors to ginger and cinnamon, are 4-(4-hydroxy-3-methoxyphenl)-2-botanone (18404 ppb-35.66) and cinnamaldeyde (18677-36.5) respectively. Ginger chocolate was observed to be the most aromatic among the whole spice chocolates studied, as this was concluded from the relatively higher odor activity values of compounds making up the ginger volatiles.

Table 4.15 Volatile compounds and their odor qualities in cinnamon chocolate

Compound	Quantity (ppb)	OAV*	Odor Description
6-ethyl-2-methyl-decane	81	0.15	Unknown
Acetic acid	1116	2.16	Sour, vinegar
Copaene	1007	1.95	Woody
Benzaldehyde	511	0.99	Nutty
Bicyclo (6-methyl -3,1,1,-heptane)	49	0.09	Unknown
8-methyl-6,8-nonadien-2-one	26	0.05	No smell
2-octanone	542	1.05	Green
Bicyclo(2-methyl-3,1-hexan-2-ol)	-	-	Floral, fruity
2,3,3-trimethyl-pentane	91	0.17	Smoky
4-methylene-cyclohexene	321	0.62	Citrus
Copaene	713	1.38	Woody
Benzenepropanal	950	1.84	No smell
5-methyl-pentanal	219	0.42	Green
Ethanone	557	1.08	Fruity
3-methyl-1-butanol-benzoate	748	1.44	Floral, fruity
Phenylethyalcohol	750	1.45	Sweet, honey
2-methoxy-phenol	200	0.38	Smoky
3-phenyl-2-propenal	3072	5.95	Potato like
Cinnamaldehyde	18677	35.55	cinnamon
Propyl-propanedioic acid	26	0.05	Apple
4-(1-phenylethyl)-phenol	167	0.32	No smell
2H-1-benzopyran-2-one	2157	4.18	Green

OAV*:odor activity value

The olfactometric analysis of mentioned samples is given in Table 4.12, 4.13, 4.14 and 4.15. The results show that the odor of spice-chocolates is formed by the contribution of the original cocoa and spice odorants as expected. While some of the odorants have characteristic odors, some of them are odorless. For instance 1,2,4,5-tetrazin-3-amine and 1-octenyl-benzene were observed to be odorless although their concentrations were in the range of 369-716 ppb. Again as expected, olfactometric analysis showed that various compounds having similar molecular structures may have different odors and vice versa. For example both being alcohols, phenylethanol, has a sweet top note while1-butanol has a fruity odor. Thirteen different odors for aniseed, twenty eight for ginger and twenty five for cinnamon chocolates were sniffed by the olfactometer. As a conclusion, the main compounds determining the spice-chocolate odors are 1-methoxy-4-(1-propenyl)-benzene (6,351 ppb) for aniseed chocolate, 4-(4-hydroxy-3-methoxyphenl)-2-botanone(18,404 ppb) for ginger chocolate and cinnamaldehyde (28,677 ppb) for cinnamon chocolate.

The acceptability test assessed by the sensory panel is given in Figure 4.6 and Table 4.16.

Table 4.16 One-sample T test for acceptability of the sensory panel with a test value=7 for dark spice-chocolates

Chocolate type	t	df	Sig. (2-tailed)	Mean difference	Lower	Upper
Ginger chocolate	1.732	11	0.111	0.50000	-0.135	1.135
Aniseed chocolate	0.638	11	0.536	0.25000	-0.612	1.112
Cinnamon chocolate	1.000	11	0.339	0.25000	-0.300	0.800

Trained panelists carried out sensory evaluation of the spice chocolate samples. The sensory data thus obtained, were compared to instrumental GC/MS/O data to search for a relationship. Chocolate with spices were evaluated in terms of the overall acceptability such as texture, odor and taste. According to the results obtained by the panelists, the chocolate samples were sensorily acceptable. Among these, ginger chocolate was concluded to be more aromatic and more acceptable.

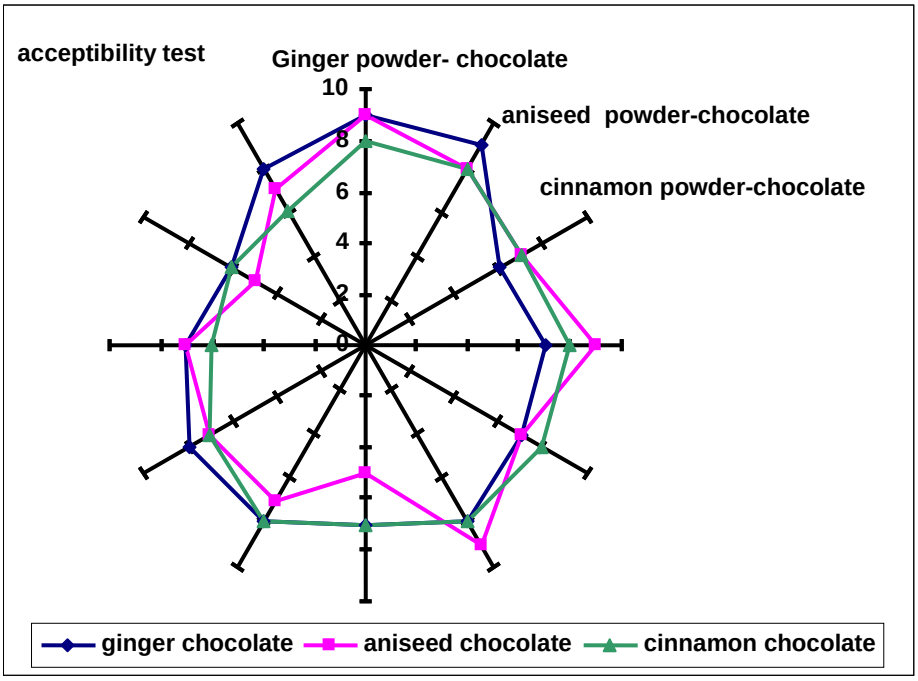


Figure 4.6 Acceptability of aniseed, ginger, and cinnamon chocolates as assessed by the panel

4.5 Development of Lemon and Pistachio Nut Chocolate with Lemon Peel Powder and Pistachio Nut Paste.

Qualitative and quantitative identification of flavor and odor of dark chocolate with and without additives before and after conching was carried out in GC/MS and GC/MS/O. SPME was used for extraction of flavor of dark chocolate. Toluene was used as internal standard for quantitative determination of aroma compounds. Dilution factor for odor activity value with SPME was determined by the following procedure: quantity of sample was reduced the half of its previous value until odor of sample was not perceived. Odor activity value of compounds was qualified using the dilution factor. Change in the dark chocolate volatiles with and without additives is shown in Tables 4.17 and 4.18.

Table 4.17 Change in the aroma profile of lemon chocolate after conching

Retention Time (min.)	Compound	Odor Description	Quantity (ppb)	OAV*
3.19	D-Limonene	Lemon	29136	56
3.73	1-methyl-1,4-cyclohexadiene	Lemon	6109	11.8
4.07	1-methyl-3-(1-methylethyl)-benzene	Flowery	1043	2.0
6.43	Acetic acid	Vinegar	2242	4.3
6.68	Tetramethyl-pyrazine	Bean like	3341	6.5
7.39	Benzaldehyde	Nutty	1056	2.0
7.45	4-methylene- bicyclo (3.1.0)-hexene	Citrus	266	0.5
7.97	Trans-alpha-bergamotene	Lemon	3725	7.2
8.18	7,11-dimethyl-1,6,10-dodecatriene	Fruity	776	1.51
8.70	1,24,5-tetrazin-3-amine	Unknown	269	0.5
8.76	Benzeneacetaldehyde	Cocoa	403	0.8
8.81	2,6-dimethyl-2,6-octadiene	Rose	3161	6.1
9.01	3-methyl-butanoic acid	Cheese	327	0.63
9.15	2,6-dimethyl-1,3,5,7-octatetraene	Floral	2371	4.59
9.26	1-methanol -3-cyclohexene	Lilac	2345	4.71
9.58	Cyclohexanol	Unknown	27979	54
9.62	1H-benzocycloheptene	No smell	17019	32.9
9.72	3,7-dimethyl-2,6-octadienal	Lemon	9547	18.5
9.90	1R-Alpha-pinene	Green	25563	49.5
10.11	Benzene-1-(1,5-dimethyl-4-hexenyl)	Unknown	2239	4.33
10.60	6,6-dimethyl-bicyclo (3.1.1.)-heptane	Herbal	2739	5.3
10.84	2-phenyllethylester-acetic acid	Vinegar	530	1.0
11.45	1,2-dibenzyloxybenzene	Unknown	255	0.4
11.68	Phenylethyl alcohol	Sweet	1128	2.18

Table 4.17 cont'd

Retention Time (min.)	Compound	Odor Description	Quantity (ppb)	OAV*
11.97	(1-ethyl-2-propenyl)-benzene	Sweet	78	0.2
12.51	Ethanone	Fruity	571	1.0
12.74	Biphenyl	Floral	1867	3.6
13.39	1H-pyrrole-2-carboxaldehyde	No smell	159	0.3
13.69	3-phenyl-2-propenal	Potato like	131	0.25
13.80	2-(aminoxy)-propanoic acid	Rancid	61	0.1
14.05	1,1-dichloro-2-propanone	Unknown	10	0.01
14.10	1-octenyl-benzene	No smell	319	0.6
24.26	2H-1-benzopyran-2-one	Sweet	356	0.7

Table 4.18 Change in aroma profile of pistachio nut chocolate after conching

Retention time (min.)	Compound	Odor description	Quantity (ppb)	*OAV
4.34	Acetic acid, anhydride	Sour	22	0.04
5.82	Trimethyl-pyrazine	Cocoa, roasted	62	0.1
6.45	Acetic acid	Sour	235	0.5
6.67	Tetramethyl-pyrazine	Bean like	3600	6.9
7.38	Benzaldehyde	Nutty	709	1.3
7.44	1-phenyl- 1,2 propanedione	Butter	24	0.05
7.58	(S)-3,4-dimethylpentanol	Unknown	107	0.2
7.70	Acetic acid anhydride	Sour	19	0.03
7.84	2-oxo-ethyl ester-propanoic acid,	Apple	27	0.05
8.11	2-Octanone	Green	132	0.3
8.59	2-methyl-1-propanol (isobutanol)	Sweet	103	0.2
8.69	4-hydroxy-butanoic acid,	Butter	73	0.1
8.76	2,7-dibenzyloxyfluorene-9-one	Unknown	230	0.5
8.87	1-phenyl -1-butanone,	Flowery	29	0.06
9.00	Acetic acid	Sour, vinegar	104	0.2
9.56	2,2,4-trimethyl-3-phenyl-hex-5-en-3-ol	No smell	65	0.1
9.89	1,2-benzenedicarbonitrile	Pungent	118	0.2
10.26	3-benzyloxy-2-fluoro-benzaldehyde,	Sweet	109	0.2
10.59	2-phenylethyl ester-acetic acid,	Fruity	553	1.1
10.75	1-(2-methyl-1-cyclopenten)-ethanone	Woody	235	0.4
10.81	1-butanol- 3-methyl-benzoate	Fruity	577	1.1
10.91	Butanoic acid	Sour, butter	62	0.1
11.11	2-methoxy-Phenol	Woody	295	0.6
11.27	1,2-ethanediol, 1,2-diphenyl-(R'R)	Unknown	78	0.2
11.35	Phenylethyl alcohol	Sweet, honey	1037	2.1
11.55	3-phenyl -2-propenal	Potato like	916	1.8
12.50	Ethanone	Fruity	399	0.7

Table 4.18 continued

Retention time (min.)	Compound	Odor description	Amount (ppb)	*OAV
16.12	3-methoxy-3-methyl 2-butanone	Pisctachio nut	15897	30.8
24.26	2H-1-Benzopyran-2-one	Sweet	4631	8.9

As seen in Tables 4.17 and 4.18, 35 compounds for lemon chocolate and 32 compounds for pistachio nut chocolate were identified after conching. For lemon chocolate, of the total 35 compounds, 3 aldehydes, 4 acids, 3 alcohols and a ketone were identified to originate from dark chocolate while the remaining 24 compounds, which were observed to have high odor activity values, came from lemon peel powder. This means that they impart their characteristic lemon flavor to dark chocolate. The total quantities of lemon peel powder volatiles was observed to be (1,456 ppb) for aldehydes, (285 ppb) for acids, (1,128 ppb) for alcohols, (521 ppb) for ketones and (3,341 ppb) for pyrazines while the those for the pistachio paste chocolate were: (3,297 ppb) for aldehydes,(686 ppb)for acids,(1,249 ppb) for alcohols (368 ppb) for ketones and (3,662) ppb for pyrazines. The data shows that there is a reduction in the amount and number of the mentioned compounds. It is concluded that this reduction is not related to the spices added but it is linked to the conching. Because drop in the number and quantity of these substance was almost the same for all chocolates with additives.

Olfactometric analysis of these chocolates is given in Tables 4.17 and 4.18. As seen, lemon chocolate is the most aromatic chocolate because the OAV's of its compounds is higher than 1. Although 15 compounds were identified for pistachio nut chocolate, since their OAV's was below 1, pistachio nut chocolate was deduced not to be an aromatic chocolate.

Results show that the characteristic dark chocolate odor with additives is mixture of various odorants. Some of them have their specific odors while others are odorless. For instance 1,2,4,5-tetrazin-3-amine and 1-octenyl-benzene are odorless although concentration of these compound are 369 and 716 ppb. They do not contribute to the odor of chocolate with additives. Generally, pyrazines have a nutty odor whereas

acids have a vinegar-likeodor. It was concluded that the basic compound which determines the odor of chocolate with additives is d-limonene (58,282 ppb) for lemon chocolate.

4.6 Comparison Aroma Profiles of Cinnamon, Aniseed, Lemon-Peel Chocolate to Chocolate with Citral, Cinnemaldehyde, and Aniseed Essential Oil

Qualitative and quantitative identification of dark chocolate flavor and odor with and without essential oils was carried out in GC-MS and GC-MS-O. SPME was used for the analysis of dark chocolate flavor. Toluene was used as internal standard for quantitative determination of aroma compounds. Dilution factor for the odor activity value with SPME was determined by the method described in section 3.18. The comparison in the variation of dark chocolate volatiles with those of natural additives and essential oil is shown in Tables 4.19, 4.20 and 4.21.

Table 4.19 Comparison of aroma profiles of cinnamon chocolate and dark chocolate with cinnamaldeyde

Cinnamon powder-chocolate			
Compounds	**Quantity (ppb)**	**Odor activity value**	**Odor description**
6-ethyl-2-methyl-decane	80	0.15	Unknown
Acetic acid	1116	2.16	Sour, vinegar
Copaene	1007	1.95	Woody
Benzaldehyde	511	0.99	Nutty
Bicyclo (3.1.1.heptane,6-methyl)	48	0.09	Unknown
8-methyl -6,8-nonadien-2-one	25	0.05	No smell
2-Octanone	220	0.42	Green
Bicyclo (3.1. hexan-2-ol, 2-methyl)	542	1.05	Floral, fruity
2,3,3-trimethyl-pentane	90	0.17	Smoky
4-methylene-cyclohexene	321	0.62	Citrus
Benezenepropanal	950	1.84	No smell
5-methyl-pentanal	219	0.42	Green

Table 4.19 cont'd

Ethanone	557	1.08	Fruity
1-butanol-3-methyl- benzoate	748	1.44	Floral, fruity
Phenylethylalcohol	750	1.45	Sweet, honey
2-methoxy-phenol	199	0.38	Smoky
3-phenyl-2-propenal	3072	5.95	Potato like
Cinnamaldehyde	28677	55.5	Cinnamon
4- (1-phenylethyl)-phenol	166	0.32	No smell
Propyl-propanedioic acid	31	0.06	Apple
2H-1- Benzopyran-2-one	21577	4.18	Green

Chocolate with Cinnnamaldehyde

Compounds	**Quantity (ppb)**	**Odor activity value**	**Odor description**
Acetic acid	1156	2.24	Sour, vinegar
Bezaldehyde	120	0.23	Nutty
2-methyl-pentanal	11266	21.83	Sweet, fruity
1,2,4,5-tetrazin-3-amine	221	0.43	Unknown
Benzyl-4-nitrophenyl carbonate	350	0.68	Floral
5-methyl-1-phenyl-1-hexanone	222	0.43	Spicy
3-methyl -butanoic acid	47	0.09	Cheese
3,7,-trimethyl-1,3,6,10-Dodecatetraene	407	0.79	Apple
Dodecatetraene	186	0.36	Green
Benzenebutanal	1278	2.47	Fruity, floral
1-methoxy-4-(1-propenyl)-benzene	450	0.87	Sweet
1-butanol- 3-methyl benzoate	376	0.73	Pungent,fruity

Table 4.19 cont'd

Chocolate with Cinnnamaldehyde			
Compounds	**Quantity (ppb)**	**Odor activity value**	**Odor description**
3-hydroxy-butanal	339	0.65	Cocoa
3-phenyl-2-propenal	970	1.88	Potato like
Phenylethyl alcohol	616	1.19	Sweet, honey
Ethanone	295	0.57	Fruity
Cinnamaldehyde	32650	63	Cinnamon

Table 4.20 Comparison of aroma profiles of aniseed powder-chocolate and dark chocolate with aniseed essential oil

Aniseed powder-chocolate		**Odor**	
Compound	**Quantity (ppb)**	**Activity Value**	**Odor Description**
(2-Aziridinylethyl) amine	2293	4.44	Unkown
Acetic acid	842	1.63	Sour, vinegar
Tetramethyl-pyrazine	4458	8.64	Bean like
5-ethyldecane	714	1.38	Fruity
Estragole	17164	33.26	Aniseed
Tricyclo (2,6,6, tetramethyl -undec-9-ene)	4162	8.06	Lilac
1-methoxy-4-(1-propenyl)-benzene	6351	12.31	Aniseed
Phenylethyl alcohol	3386	6.56	Sweet, honey
Ethanone	965	1.87	Fruity
4-methoxy-benzealdehyde	302	0.58	Almond-like
3-phenyl-2-propenal	1871	3.62	Potato like
2H-1-Benzopyran-2-one	1317	2.55	Sweet
Chocolate with Aniseed Essential Oil		**Odor**	
Compound	**Quantity (ppb)**	**Activity Value**	**Odor Description**
1-propen-2-ol-acetate	1241	2.41	Fatty
4-methyl-3-penten-2-one	9543	18.49	Potato, green
4-hydroxy-4-methyl-2-pentanone	730	1.41	Cocoa
Acetic acid	1081	2.09	Sour, vinegar
Estragole	15966	30.44	Aniseeed
Bicyclo- heptanes-2-cyclopropylidene	1089	2.11	Unkown
1-methoxy-4-(1-propenyl)-benzene	2941	5.69	Aniseed
4-methoxy-benzaldehyde	3652	7.07	Floral
3-phenyl-2-propenal	8833	17.11	Potato

Table 4.21 Comparison of aroma profile of lemon chocolate and dark chocolate with citral

Lemon Peel Powder-Chocolate Compound	**Quantity (ppb)**	**Odor Activity Value**	**Odor Description**
D-Limonene	58272	112	Lemon
1-methyl -1,4-cyclohexadiene	6109	11.84	Lemon
1-methyl-3-(1-methylethyl)-benzene	1043	2.02	Flowery
Acetic acid	2242	4.34	Sour, vinegar
Tetramethyl-pyrazine	3341	6.47	Bean like
Benzaldehyde	1056	2.04	Nutty
Bicyclo (3.1.0) –hexane- 4-methylene	266	0.52	Citrus
Trans-alpha-bergamotene	3725	7.22	Lemon
7,11-dimethyl -1,6,10-dodecarriene	766	1.51	Fruity
1,2,4,5-tetrazin-3-amine	269	0.52	Unknown
Benzeneacetaldehyde	403	0.78	Cocoa
2,6-dimethyl-2,6-Octadiene	3161	6.12	Rose
3-methyl-butanoic acid	327	0.60	Cheese
2,6-dimethyl-1,3,5,7-Octatetraene	2371	4.49	Floral
1-methanol 3-cyclohexene	2435	4.71	Lilac
Cyclohexanol	55957	108	Unknown
1H-benzocycloheptene	17019	32.98	No smell
3,7-dimethyl -2,6-Octadienal	9547	18.50	Lemon
1R-Alpha-pinene	51127	99	Green
Benzene-1-(1,5-dimethyl-4-hexenely)	2239	4.33	Unknown
Bicyclo (3.1.1.) heptanes, 6,6-dimethyl	2739	5.30	Herbal
2-phenylethyl ester-acetic acid	530	1.02	Vinegar
Beta-myrcene	1730	3.35	Fruity
1,2-dibenzyloxybenzene	255	0.49	Unknown
Phenylethy alcohol	1128	2.18	Sweet, honey
Benzene-(1-ethyl-2-propenyl)	78	0.15	Sweet
Ethanone	521	1.01	Fruity

Table 4.21 cont'd

Lemon Peel Powder-Chocolate Compound	**Quantity (ppb)**	**Odor Activity Value**	**Odor Description**
1H-pyrole-2-carboxyaldhyde	159	0.31	No smell
3-phenyl-2-propenal	131	0.25	Potato like
2-(aminoxy)-propanoic acid	61	0.11	Rancid
1-octenyl-benzene	319	0.62	No smell
1,1-dichloro-2-propanone	-	-	Unkown
2H-1-Benzopyran2-one	356	0.69	Sweet
Chocolate with Citral Compound	**Quantity (ppb)**	**Odor Activity Value**	**Odor Description**
2- (bis(2-cyanoethy)amino-acetamide,	-	-	Unknown
3,3,6,6,-tetramethyl- tricylo(2,4)-hexene	893	1.73	Green
1,2,4,5-tetrazin-3-amine	91	0.17	Unkown
Trans-3-caren-2-ol	28114	54	Lemon
3,7-dimethyl- 2,6-Octadienal	85925	166	Lemon
1-(1,5-dimethyl-4-hexenyl)-benzene	363	0.7	Unkown
Benzene-butanol	446	0.86	Flowery
1-methoxy-4-(1-propenyl)-benzene	363	0.7	Sweet
2H-pyran-2-methanol	67	0.13	Fruity

34, 24 and 16 compounds were identified for lemon-peel, cinnamon and aniseed chocolate respectively whereas 14, 16, and 9 compounds were identified for dark chocolate with citral, cinnamaldeyde and aniseed essential oils. 21, 12 and 12 of them were odor active for lemon, cinnamon and aniseed powder while 3, 5 and 8 of them were odor active for dark chocolate with citral, cinnamaldeyde and aniseed essential oils respectively.

Data obtained from GC-MS showed that chocolate with natural spices was more aromatic than chocolate with citral, cinnamaldeyde and aniseed essential oil. Both chocolates gave a spicy aroma. However chocolates with natural spices were more acceptable and contained more odor active compounds than that of with citral, cinnamaldeyde and aniseed essential oil as this was demonstrated by the sensory panel. D-Limonene was mostly responsible from the lemon aroma for chocolate with lemon peel, whereas 2,6-Octadienal-3,7-dimethyl give the same aroma for the chocolate with citral. Lemon odor was perceived in both chocolates but their tastes were different. According to the aroma analysis, lemon peel powder contains more than 40 different volatiles which were considered to give chocolate the pleasant taste and odor. Also these compounds are thought to interact with the cocoa mass during conching and some new compounds such as 6,6-dimethylbicyclo(3.1.1) heptane formed as a result of this interaction. Vieira et al.,(2008) studied the impact of limonene on the physical properties of reduced fat chocolate. They reported that addition of limonene decreased hardness and viscosity of chocolate and facilitated the production and improving the eating quality of reduced fat chocolate.

No change was observed in the hardness, color, and viscosity of chocolate after the addition of citral, cinnamaldehyde and aniseed essential oil. Because they were less than 0.1 % of the total chocolate mass. The chocolate obtained with this way was considered to be a new product in terms of its characteristic flavor. As stated in Table 4.22, the panelists' response was not bad for citral, cinnemaldehyde, and aniseed chocolates but not as acceptable as chocolates with natural powders. Mironescu et al.,(2008) studied chocolate with the addition of chili pepper as a new product. They found that variation in the chili concentration did not affect the perception of the

chilli after a certain level. Chu et al.,(2003) optimized the formulation of chocolate peanut spread. Results showed that optimum formulation for chocolate peanut spread were 29-65% peanut, 9-41% chocolate mass and 17-36% sugar. Schumacher et al.,(2009) investigated effect of addition of quinoa on chemical and sensory properties of dark chocolate. They declared that protein and vitamin E content of dark chocolate increased with increasing percentage of quiona while quantity of polyphenols decreased

Table 4.22 One-sample T test for flavor and texture profiles with a test value=7

	Softness		Hardness		Effort		Thickness		Coarseness		Additive Smell		Chocolate Smell		Additive Taste		Chocolate Taste		Sweetness		Bitterness	
	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p
Citral chocolate	3.188	0.009	2.691	0.021	2.000	0.071	2.590	0.025	2.152	0.054	1.609	0.136	4.733	0.001	3.071	0.011	3.463	0.050	3.767	0.003	3.079	0.010
Lemon chocolate	-0.804	0.438	3.458	0.005	0.561	0.586	3.458	0.05	2.548	0.027	4.062	0.002	2.803	0.017	3.000	0.012	3.546	0.05	1.593	0.139	2.803	0.017
Cinnamal deyde chocolate	1.593	0.139	1.603	0.137	1.239	0.241	1.076	0.305	1.773	0.104	-0.944	0.365	-0.185	0.857	-1.682	0.1210	-0.178	0.862	0.167	0.870	0.504	0.504
Cinnamon chocolate	-1.935	0.079	-0.699	0.499	-2.399	0.035	-1.836	0.094	-2.057	0.064	0.000	1.000	2.345	0.039	1.609	0.136	-0.248	0.809	0.432	0.674	0.417	0.417
Aniseed chocolate	1.173	0.266	1.149	0.275	1.301	0.220	1.000	0.339	0.897	0.389	4.311	0.001	1.449	0.175	2.419	0.034	2.345	0.039	1.735	0.111	0.015	0.015
Aniseed (essential oil) chocolate	3.023	0.012	2.548	0.027	3.527	0.005	4.750	0.001	1.332	0.210	1.865	0.089	1.735	0.111	2.028	0.067	3.527	0.005	1.301	0.220	0.053	0.053

One sample T test was used in order to evaluate sensory test results. Results of statistical analysis of panel test are shown in Table 4.22. 7-(Good) was chosen as test value and 11 attributes were assessed according to this value. “t”, “-“ and “p” in the Table 4.22 represent difference, direction of attributes from this value and confidence interval respectively. As seen from this Table, there is no difference statistically for bitterness, chocolate taste, additive taste, hardness and softness of dark chocolate with lemon peel powder, aniseed powder and aniseed essential oil. However, there is a significant difference for bitterness, chocolate taste, additive taste, and hardness of chocolate with cinnamaldeyde, citral and aniseed essential oil.

As seen from Table 4.23, both citral and lemon chocolates are statistically satisfactory. The acceptability test showed that aniseed powder and cinnamon powder chocolates are statistically satisfactory whereas the chocolates obtained by the corresponding essential oils are not statistically satisfactory.

Table 4.23 One-sample T test for acceptability panel test with a test value=7 for dark chocolate with additives

Chocolate type	t	df	Significant. (2-tailed)	Mean difference	Lower	Upper
Lemon peel chocolate	2.345	11	0.039	0.66667	0.041	1.292
Lemon (citral) chocolate	2.569	11	0.026	1.000	0.140	1.86
Aniseed powder chocolate	0.638	11	0.536	0.25000	-0.612	1.112
Aniseed (essential oil) chocolate	-0.167	11	0.870	-0.083	-1.180	1.020
Cinnamon-powder chocolate	1.000	11	0.339	0.25000	-0.300	0.800

4.7 Influence of Addition of Ginger, Aniseed, Cinnamon, Lemon Peel Powder and Pistachio Nut Paste on the Texture, Viscosity, Melting Point and Particle Size of Dark Chocolate.

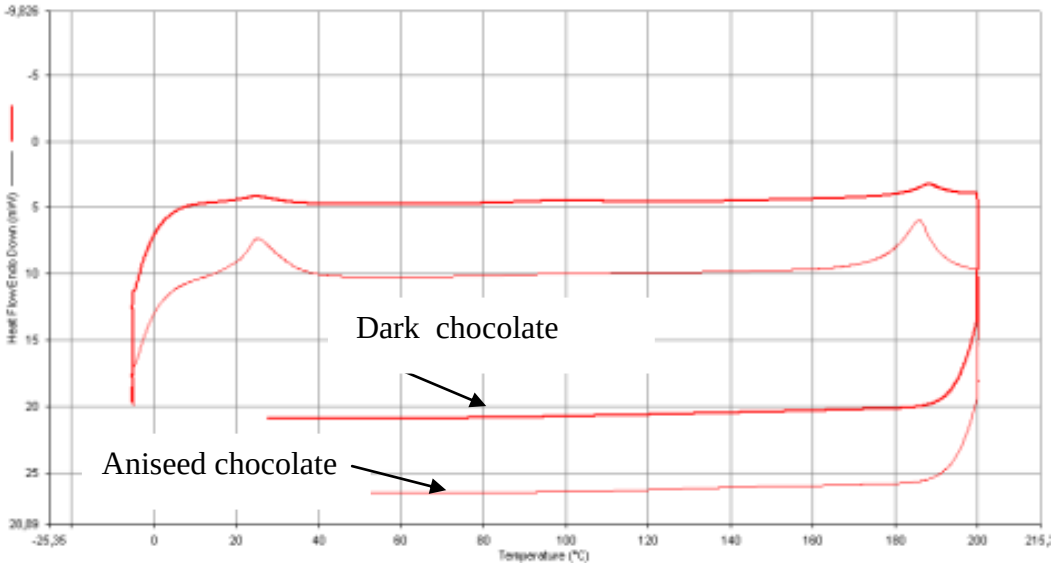
Chocolate was formulated with same amount of additives in order to compare the effect of additives on the viscosity, texture, melting point and particle size. The formulations of dark chocolates with and without additives are shown in Table 4.24.

Table 4.24 Formulations of dark chocolate without and with additives. All figures are in % (w/w).

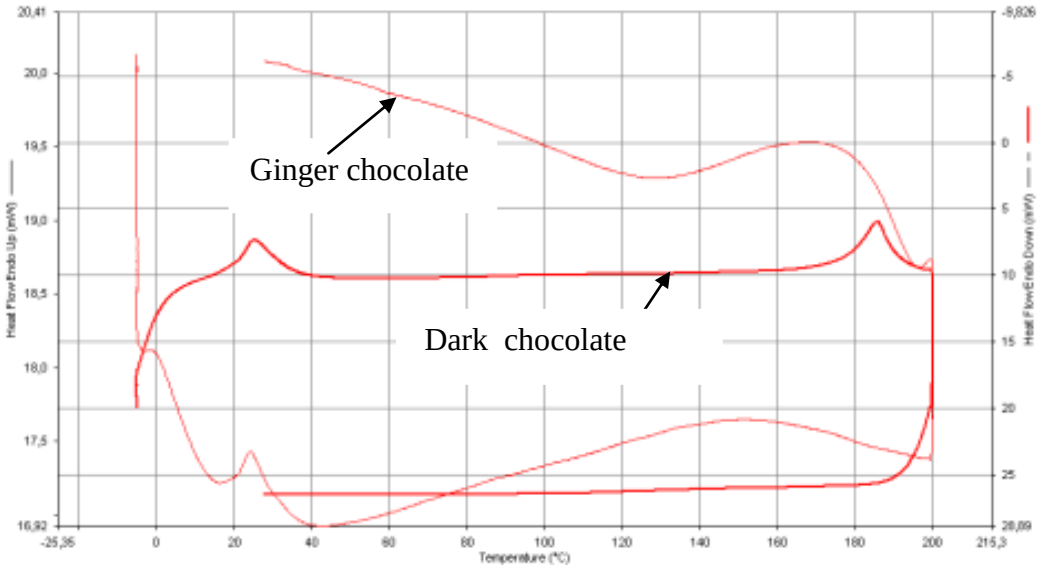
Ingredients	Dark chocolate	Aniseed Chocolate	Ginger chocolate	Cinnamon Chocolate	Lemon peel chocolate
Cocoa mass	45.07	43.07	43.07	43.07	43.07
Sugar	39.14	39.14	39.14	39.14	39.14
Added-cocoa butter	15.45	15.45	15.45	15.45	15.45
Lecithin	0.34	0.34	0.34	0.34	0.34
Additive	-	2	2	2	2

4.7.1 Effect of Additives on Melting Point of Dark Chocolate

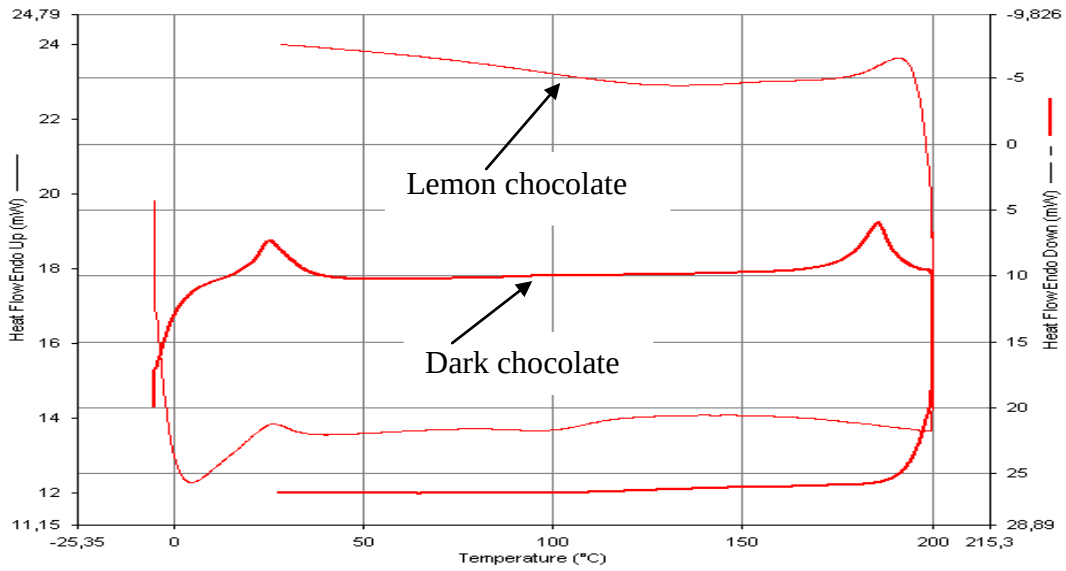
The thermal behavior of chocolate with additives was studied in DSC in order to see their effect on the melting point. Figure 4.7 shows a typical DSC melting curve for dark chocolate with and without additives. As seen from the figures, the fat melting profile corresponding to each additive is different. As it is known, the peak temperature for melting is the average of melting point of chocolate and the onset of melt indicates the time when the fat just starts to melt.



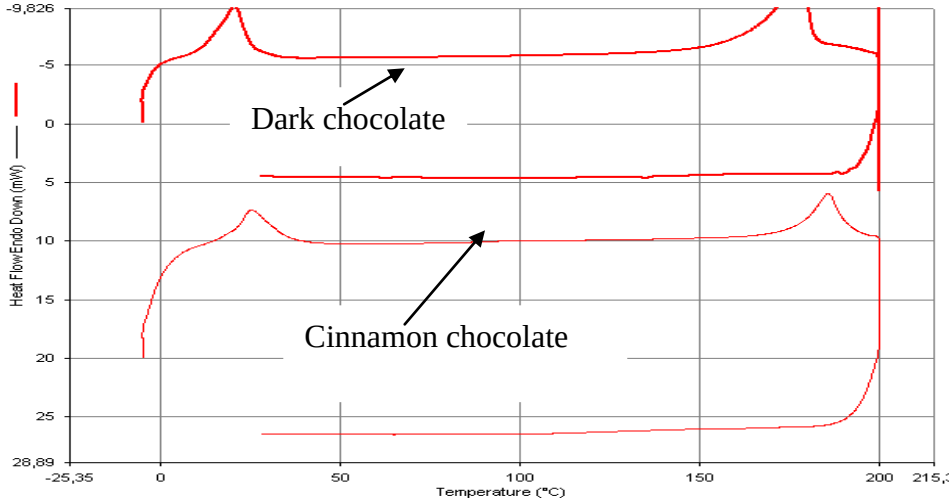
(a) aniseed-dark chocolate



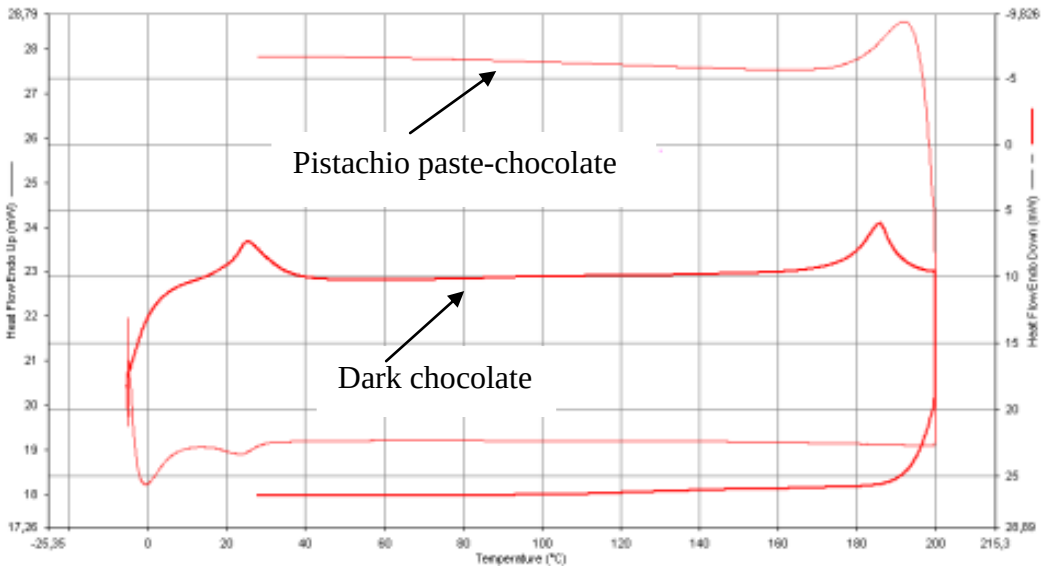
(b) ginger-dark chocolate



(c) lemon–dark chocolate



(d) cinnamon-dark chocolate



(e) pistachio nut-dark chocolate

Figure 4.7 DSC thermograms showing the fat melting profiles of dark chocolate with and without additives for each case.

Melting properties of chocolate are important because of their contribution to the taste and sensation in the mouth. Marangoni & McGauley (2003) stated that the structure of fat in a food product is an important property that strongly influences its perceived mechanical and melting properties. The melting point was observed to be 25.26 °C for the control sample (dark chocolate without additive) while it was 24.71, 25.81 and 24.57 °C for aniseed, lemon and ginger chocolate respectively as shown in Table 4.25. These results show that aniseed decreases, while ginger and lemon peel increases the melting point of the dark chocolate within the proportion they added. Especially ginger was observed to be more effective in increasing the melting point in comparison with lemon peel and aniseed powders probably due to its higher fiber content.

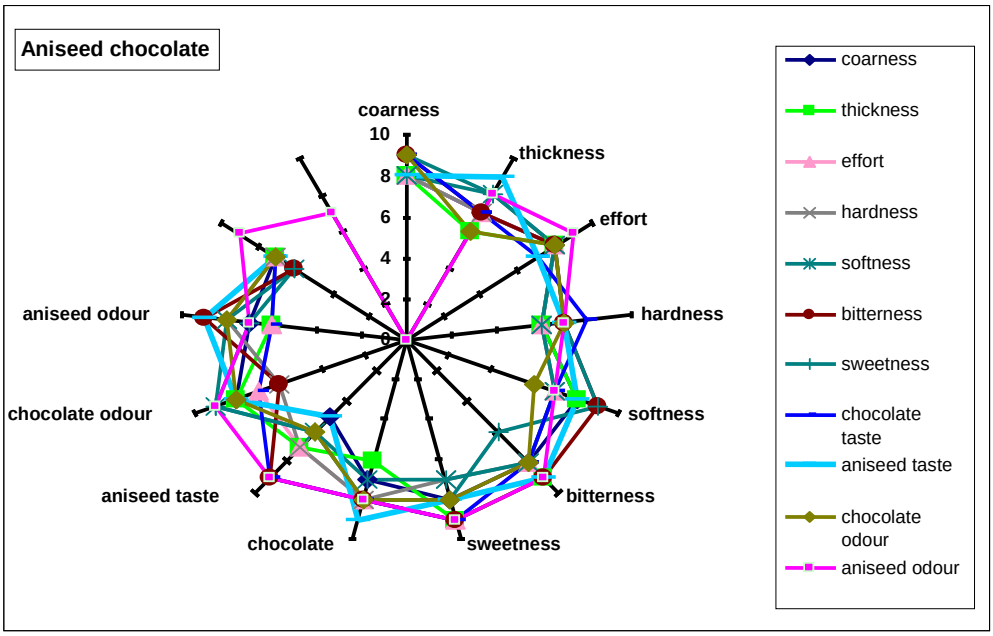
Table 4.25 Melting properties of dark chocolate with and without additives.

Chocolate type	**T_0 (°C)**	**T_{end} (°C)**	**T_{peak} (°C)**	**ΔH_{melt}(J/g)**
Dark chocolate	19.29±0.07	34.13±0.08	25.26±0.05	20.66±0.04
Aniseed powder chocolate	19.91±0.08	33.06±0.09	25.49±0.1	19.74±0.05
Lemon peel chocolate	19.81±0.2	33.75±0.5	24.57±0.06	19.68±0.07
Ginger powder chocolate	20.59±0.3	31.13±0.6	25.81±0.05	19.79±0.04
Pistachio paste chocolate	19.14±0.01	27.52±0.03	23.92±0.08	19.05±0.2

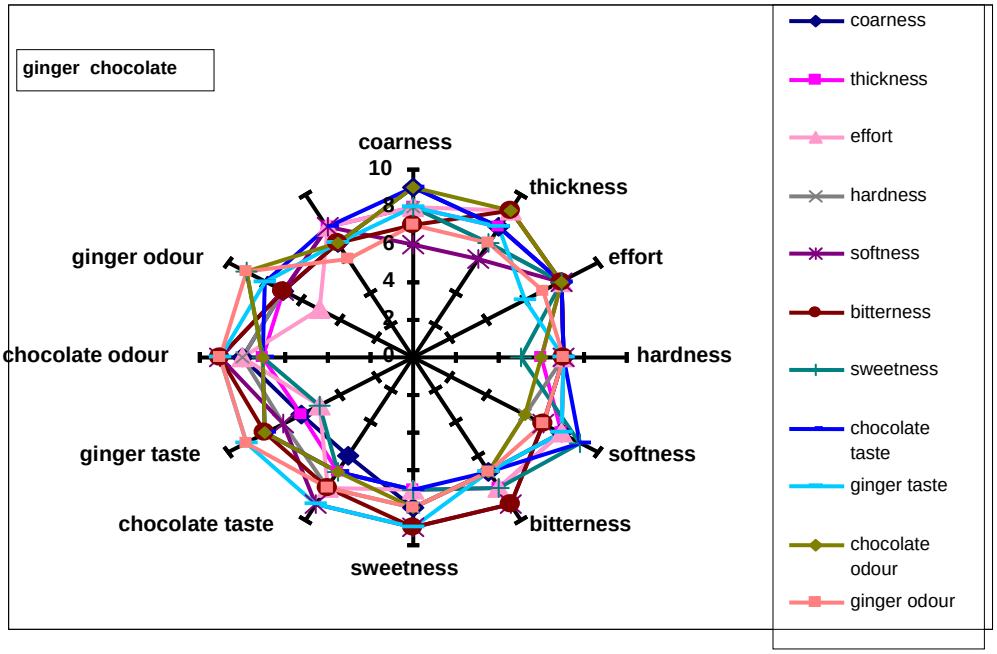
4.7.2 Effect of Additives on Texture of Dark Chocolate

Texture measurement is important for processed foods in order to obtain an end product which has desired quality and to control the process steps of food product. In this study, sensory texture evaluation is combined with instrumental measurement. Sensory attributes (texture, taste and smell) of dark chocolate with additives was assessed by 12 trained panelists. Hardness, which is the most common method to measure food texture properties, was applied in the instrumental analysis of sample at room temperature. Hardness determines the physical rigidity of chocolate and relates directly to sensory properties during consumption.

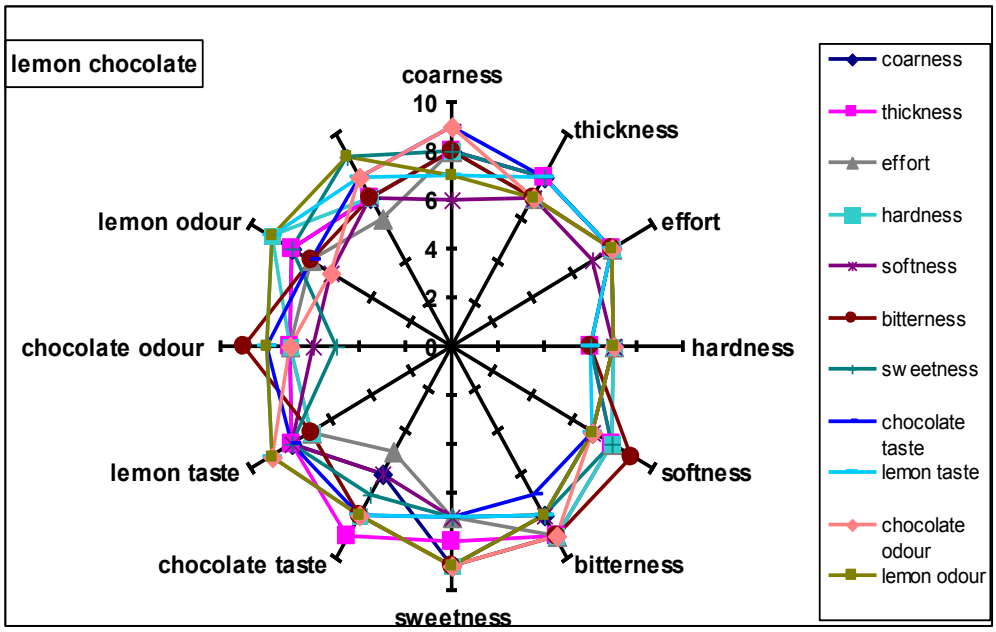
Sensory assessment of texture and flavor profiles of aniseed, ginger, lemon-peel and cinnamon chocolates is given in Figures 4.8-a-b-c-d-e respectively. In these figures, sweetness, effort, coarseness and thickness refer successively to the taste associated with sucrose, work required to melt, perception of particles against the roof of the mouth, perception of the viscosity of the melted chocolate sample in the mouth. A nine point hedonic scale was used where 1= super bad and 9= super good .


Aniseed chocolate
coarness
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
aniseed taste
chocolate odour
aniseed odour
10
8
6
4
2
0
coarness
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
aniseed taste
chocolate odour
aniseed odour

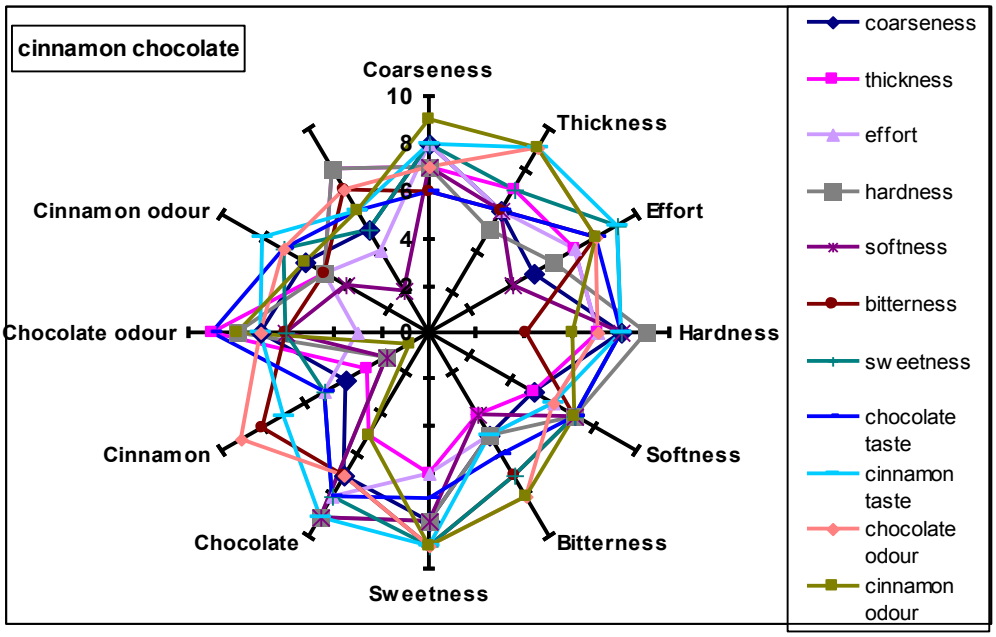
(a)


ginger chocolate
coarness
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
ginger taste
chocolate odour
ginger odour
10
8
6
4
2
0
coarness
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
ginger taste
chocolate odour
ginger odour

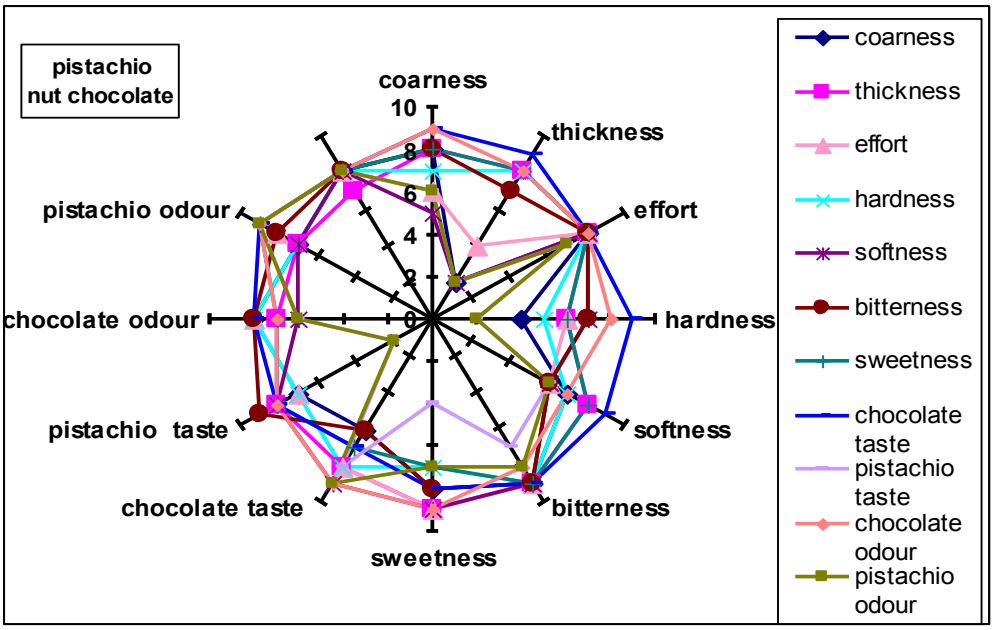
(b)


lemon chocolate
coarness
10
8
6
4
2
0
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
lemon taste
chocolate odour
lemon odour
coarness
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
lemon taste
chocolate odour
lemon odour

(c)


cinnamon chocolate
Coarseness
10
8
6
4
2
0
Thickness
Effort
Hardness
Softness
Bitterness
Sweetness
Chocolate
Cinnamon
Chocolate odour
Cinnamon odour
coarseness
thickness
effort
hardness
softness
bitterness
sweetness
chocolate taste
cinnamon taste
chocolate odour
cinnamon odour

(d)



(e)

Figure 4.8-a,b,c, d, and e, flavor and texture profiles of aniseed, ginger, lemon-peel, cinnamon and pistachio paste chocolate as assessed by the panel.

One sample T test was used in order to evaluate sensory test panel. Results of statistical analysis of panel test are shown in Table 4.26. As seen in Table 4.26, there is no difference statistically for bitterness, chocolate taste, additive taste, hardness and softness of ginger, lemon-peel and aniseed chocolates. However, there is a significant difference for bitterness, chocolate taste, additive taste, hardness and odor softness of cinnamon chocolate. Sweetness, chocolate odor and additive odor, coarseness and effort for ginger, lemon and aniseed chocolates were higher than 7 while these attributes were lower than 7 for cinnamon and pistachio nut chocolates. Panel test analysis shows that lemon, ginger and aniseed chocolate is satisfactory in terms of texture and flavor but cinnamon and pistachio nut chocolates are not satisfactory.

Table 4.26 One-sample T test for flavor and texture profiles panel test with a test value=7

	Softness		Hardness		Effort		Thickness		Coarseness		Additive Smell		Chocolate Smell		Additive Taste		Chocolate Taste		Sweetness		Bitterness	
	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p
Ginger chocolate	2.000	0.071	2.171	0.053	1.164	0.269	1.483	0.166	1.603	0.137	2.028	0.067	1.865	0.89	3.188	0.09	3.447	0.05	0.672	0.515	3.527	0.005
Lemon chocolate	-0.804	0.438	3.458	0.005	0.561	0.586	3.458	0.05	2.548	0.027	4.062	0.002	2.803	0.017	3.000	0.012	3.546	0.05	1.593	0.139	2.803	0.017
Cinnamon chocolate	-1.935	0.079	-0.699	0.499	-2.399	0.035	-1.836	0.094	-2.057	0.064	0.000	1.000	2.345	0.039	1.609	0.136	-0.248	0.809	0.432	0.674	-0.842	0.417
Aniseed chocolate	1.173	0.266	1.149	0.275	1.301	0.220	1.000	0.339	0.897	0.389	4.311	0.001	1.449	0.175	2.419	0.034	2.345	0.039	1.735	0.111	2.872	0.015
Pistachio Nut chocolate	0.000	1.000	1.449	0.175	0.583	0.571	3.000	0.012	-0.290	0.777	-1.318	0.214	5.631	0.0001	-1.961	0.076	7.340	0.0001	3.000	0.012	2.345	0.039

Dark chocolate samples with and without additives were analyzed in terms of hardness, which is defined as kg force needed for a needle to penetrate the chocolate by 1 mm. As seen in Table 4.27, chocolate samples with additives are harder than dark chocolates without additives. Especially, ginger and lemon peel powders are more effective on hardness.

Table 4.27 Hardness (kg force) measurement results of chocolate without and with additives

Chocolate type	Hardness (kg force)
Dark chocolate	1.529±0.10
Lemon peel chocolate	2.836±0.40
Cinnamon chocolate	2.118±0.70
Aniseed chocolate	2.552±0.20
Ginger chocolate	2.547±0.09
Pistachio paste chocolate	2.425±0.3

Sensory analysis of cinnamon chocolate texture was consistent with the results obtained instrumentally but those of ginger chocolate were not.

Table 4.28 One-sample T test for acceptability panel test with a test value=7 for dark chocolate with additives

Chocolate type	t	df	Significance (2-tailed)	Mean difference	Lower	Upper
Lemon chocolate	2.345	11	0.039	0.66667	0.041	1.292
Ginger chocolate	1.732	11	0.111	0.50000	-0.135	1.135
Aniseed chocolate	0.638	11	0.536	0.25000	-0.612	1.112
Pistachio nu chocolate	-1.968	11	0.075	-0.8333	-1.765	0.098
Cinnamon chocolate	1.000	11	0.339	0.25000	-0.300	0.800

Acceptability tests for cinnamon, pistachio nut, aniseed, ginger, and lemon chocolates assessed by the panel are shown in Figure 4.9 and Table 4.28. According to the panel test results, lemon, cinnamon, ginger, and aniseed chocolates were acceptable in terms of taste and texture but those of pistachio nut chocolate were not.

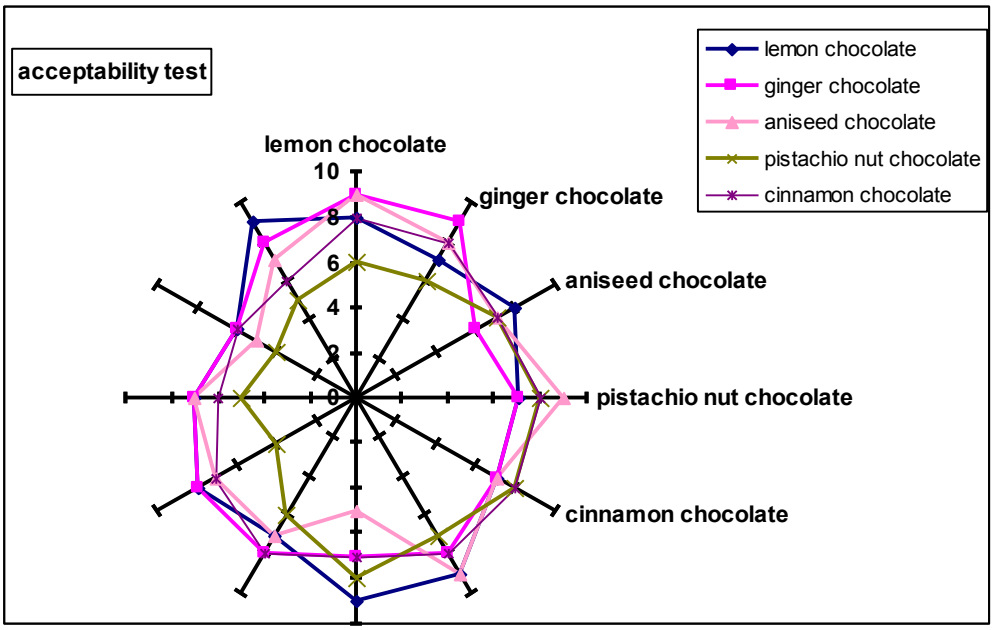
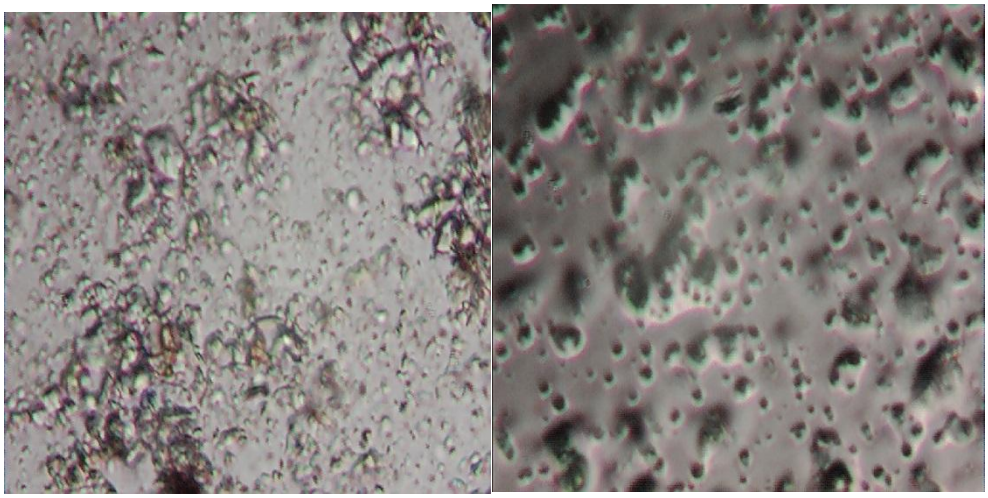


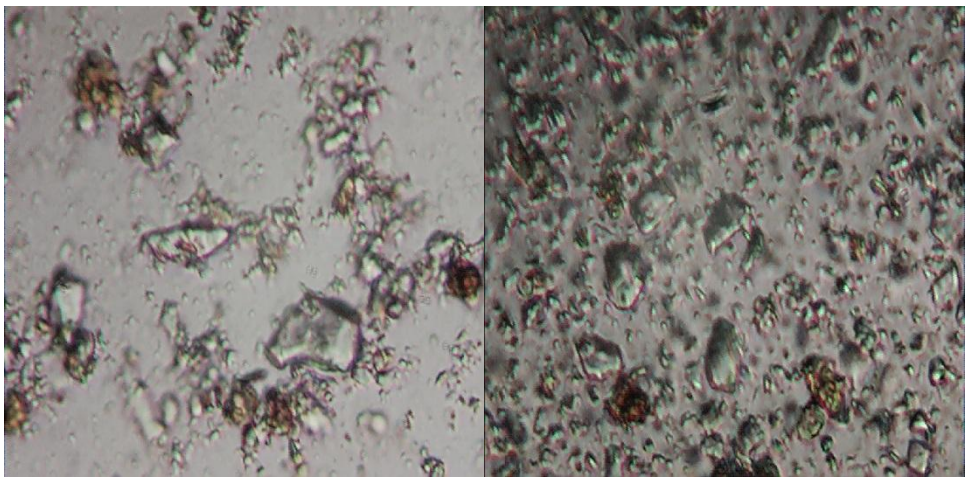
Figure 4.9 Acceptability of aniseed, ginger, lemon, cinnamon and pistachio nut chocolates as assessed by the panel

4.7.3 Effect of Additives on Particle Size Distribution of Dark Chocolate

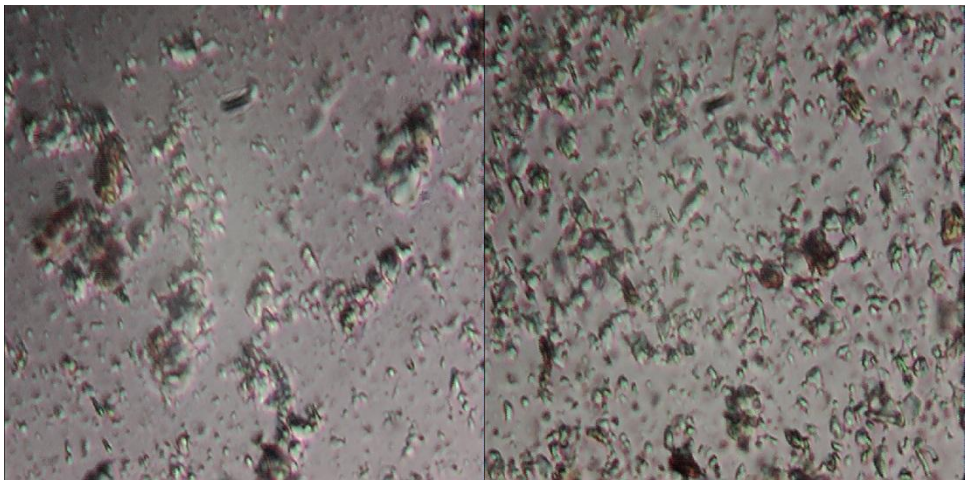
Particle size of chocolate with and without additives was determined by using polarized light microscopy. Four regions were determined under the microscopy and the largest particle in this region was identified. Dimension of this particle was measured by using software of microscopy. Images of particle size distribution of dark chocolate with and without additives are shown in Figure 4.10.



Dark chocolate Aniseed powder -chocolate



Cinnamon powder- chocolate Lemon peel powder-chocolate



Ginger powder- chocolate Pistachio nut paste-chocolate

Figure 4.10 Digital images showing particle size distribution of dark chocolate with and without additives

Particle size distribution is reported to have an impact on the rheological, textural and sensorial properties of chocolate. Large particles cause a sense like grittiness in the mouth. Maximum acceptable particle size of dark chocolate is 35µm. Dark chocolate is unacceptable above this value due to high viscosity and poor texture (Beckett, 2000). Particle size distribution of these regions was given in Table 4.29 in which there are no particles above 35µm for aniseed, lemon, pistachio nut paste and ginger chocolates when dark chocolate was taken as reference. However, hardness and viscosity of these chocolates were higher than those of dark chocolate. This may be due to the fibrous structure of these additives. As known, fiber absorbs the cocoa butter and causes an increase in the viscosity and hardness of mentioned chocolates. Cinnamon chocolate contains particles above 35µm; therefore the coarseness of

cinnamon chocolate is below 7. Instrumental and sensory analysis, on the other hand, was consistent for cinnamon chocolate.

Table 4.29 Particle size distribution of dark chocolate with and without additives

Chocolate type	Region1 (μm)	Region 2(μm)	Region 3(μm)	Region 4(μm)
Dark chocolate	21.56	28.44	16.62	25.83
	17.59	24.55	11.29	21.49
	20.83	13.35	19.61	18.67
	16.68	14.91	17.20	22.71
Aniseed powder chocolate	23.03	30.77	26.17	16.99
	33.92	29.60	24.89	22.77
	26.54	30.42	28.85	32.27
	27.02	24.78	21.78	23.92
Lemon powder chocolate	22.76	23.19	11.71	15.26
	19.81	16.49	20.08	16.18
	19.35	19.93	23.10	13.50
	21.09	15.37	18.02	20.80
Ginger powder chocolate	24.23	21.76	21.15	24.87
	25.97	20.79	16.05	24.18
	26.19	19.36	29.47	23.57
	22.67	21.57	22.87	25.05
Cinnamon powder chocolate	29.21	31.38	37.10	35.35
	20.72	23.47	38.97	34.32
	21.74	14.86	40.52	27.69
	16.35	20.30	28.42	17.86
Pistachio nut paste chocolate	31.07	17.61	34.07	30.14
	25.62	21.35	20.42	20.75
	25.21	35.63	21.66	25.48
	22.80	33.02	11.41	18.95

4.7.4 Effect of Additives on Viscosity of Dark Chocolate

Chocolate rheology is important in terms of flavor perception and sensory qualities. The rheology of chocolate affects the exact weight control during enrobing, shell making and molding process. Vavreck, (2004) claimed that some parameters such as conching conditions, particle size distribution, fat content, emulsifiers, and tempering influence rheological properties of chocolate.

Chocolate exhibits a non-Newtonian flow behavior due to the presence of high concentration of suspended solids. Chocolate is characterized by the presence of a yield value, however, it has been found not to obey to the Bingham model. Steiner, (1958) proposed an adaptation of the model given by Casson for printing ink to

measure its viscosity and yield value. The Casson equation and Casson parameters were accepted by International Office of Cocoa, Chocolate and Sugar Confectionary. Three main parameters used in the rheological assessment of chocolate are yield stress, plastic viscosity and thixotrophy. Yield stress is the amount of energy required to start flow. Plastic viscosity is the amount of energy required to keep the chocolate flowing. Thixotrophy, on the other hand, is the time-dependent behavior of the apparent viscosity. Yield stress is critical in relation to surface coating. The plastic viscosity relates to ease of pumping, filling and coating properties. (Afoakwa et al., 2008). Rheological measurements of dark chocolate (control) and samples with additives were carried out at 40°C in duplicates. The mean values were reported with standard deviation. The Casson equation and new recommended method by Servais et al., (2004) were used to model the rheological measurements as shown in Table 4.30. The new method involves recording: (1) the shear stress at a shear rate of 5 s^{-1} to represent the yield stress of the sample, (2) the viscosity at a shear rate of 30s^{-1} to represent the high shear viscosity. The relationships between the Cason plastic viscosity, Casson yield value, yield stress, and apparent viscositywere evaluated considering the recommended method which aims to find a new practical way for determining relevant and reliable parameters reflecting the yield stress and apparent viscosity.

Table 4.30 Rheological parameters of dark chocolate without and with additives

Chocolate type	**Casson viscosity (Pa.s)**	**Casson yield stress (Pa)**	**Apparent viscosity (Pa.s)**	**Yield stress (Pa)**
Dark chocolate	1.568±0.01	7.412±0.09	3.053±0.07	23.01±0.06
Cinnamon powder chocolate	1.901±0.07	7.588±0.08	11.20±0.05	32.27±0.06
Ginger powder chocolate	1.850±0.07	2.686±0.04	8.802±0.03	25.06±0.30
Aniseed powder chocolate	1.968±0.03	2.409±0.05	10.25±0.10	30.02±0.70
Lemon peel powder chocolate	2.017±0.20	2.178±0.30	7.355±0.40	21.20±0.09
Pistachio nut paste chocolate	2.300±0.25	2.376±0.38	7.350±0.45	21.70±0.19

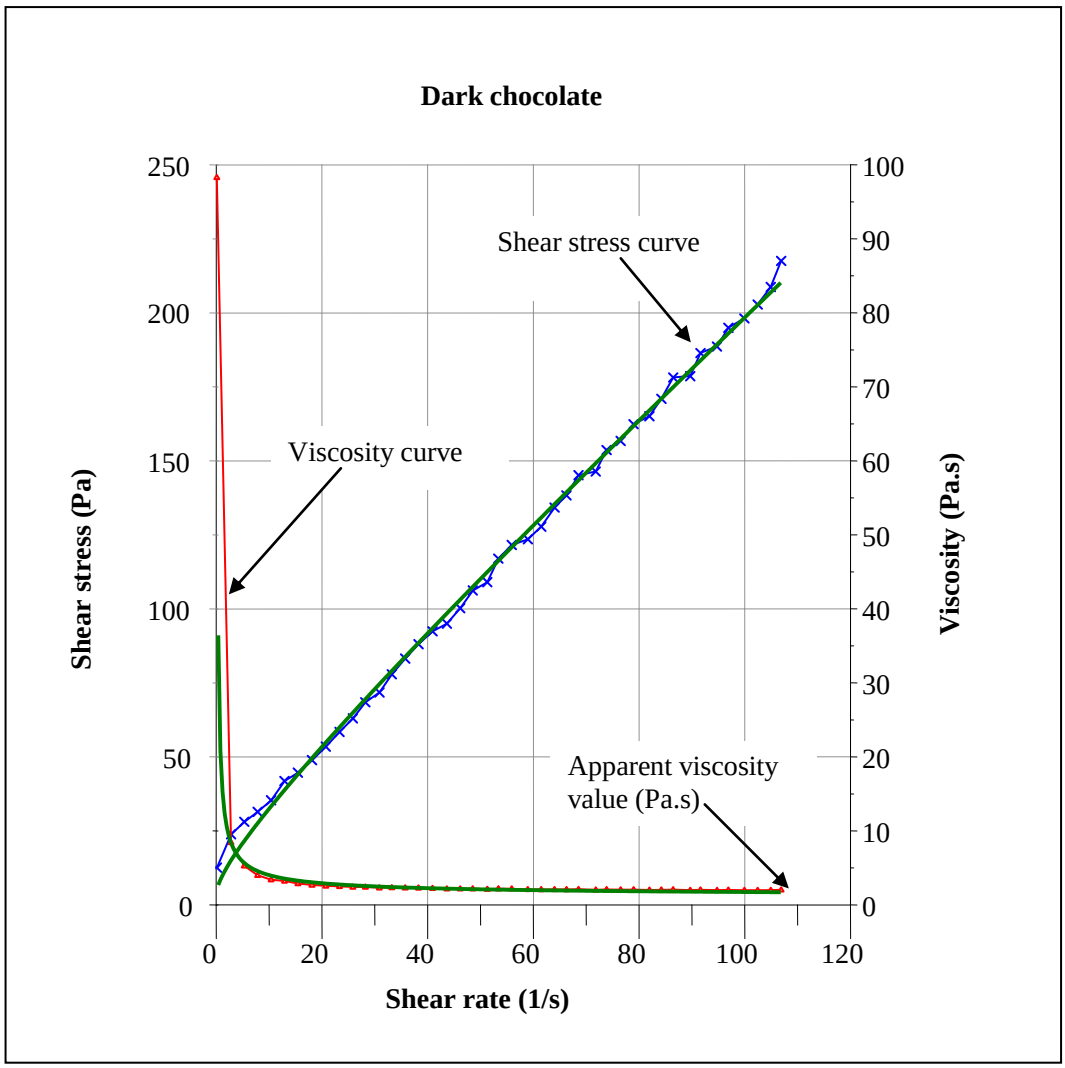


Figure 4.11 A typical rheology plot illustrating measurement of apparent viscosity and yield stress for dark chocolate.

Flow parameters for chocolate with additives were determined by using the procedure recommended by the OICC (Office International du Cacao et du Chocolat,1973) for determining Casson parameters. The Casson equation was used to relate shear stress and shear rate. Regression analysis was performed to calculate the Casson parameters from the flow curve. The influence of additives on the chocolate rheology is clearly seen when Figure 4.11 is compared with Figure 4.12. Figures related with other types of chocolates are given in appendices A29, A30, A31 and A32.

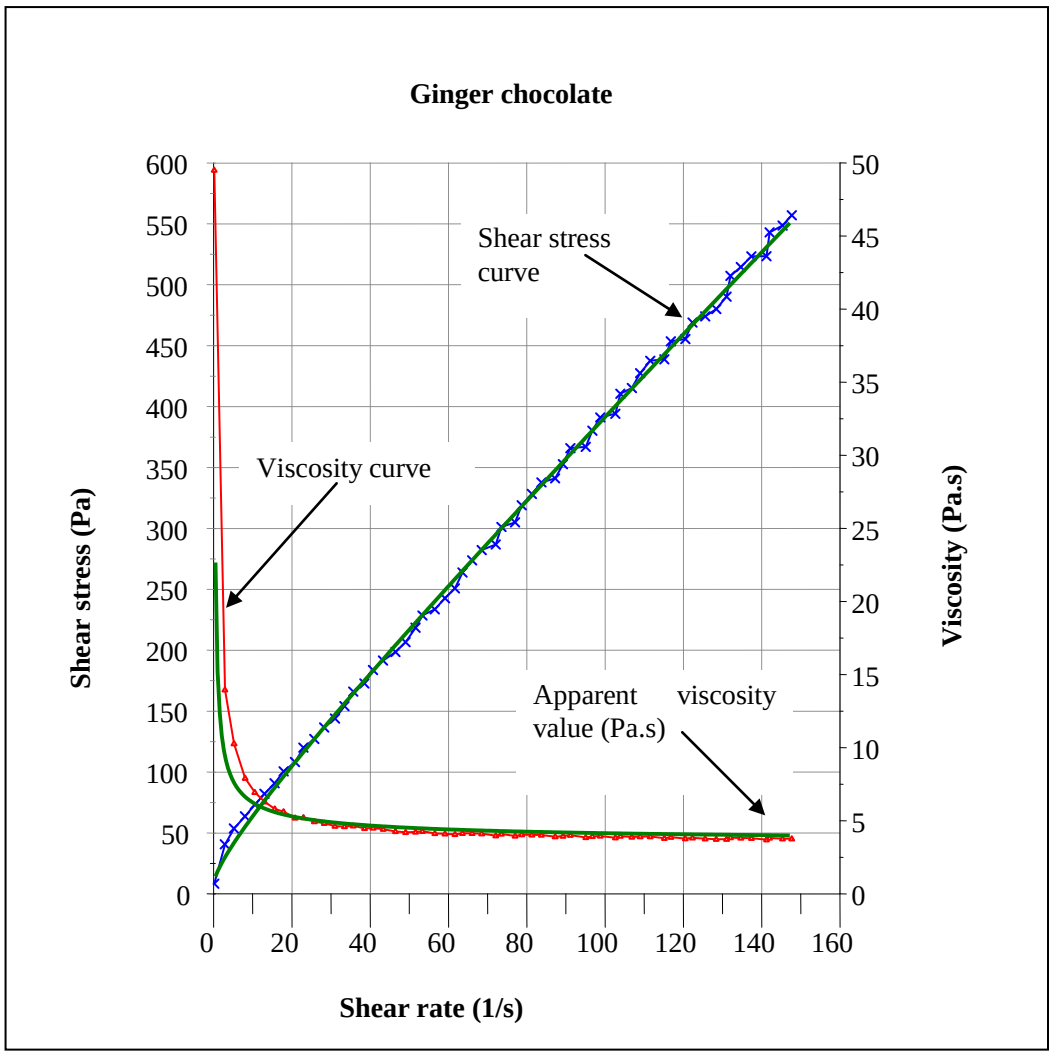


Figure 4.12 A typical rheology plot illustrating measurement of apparent viscosity and yield stress for ginger chocolate

As seen in Figures 4.11, 4.12 and Table 4.30, there is no significant difference between the plastic viscosity of dark chocolate and chocolate with additives. It is a fact that particle size of additives under 30 microns is an important parameter in terms of being close to viscosity of dark chocolate and chocolate with additives. The yield stress of cinnamon, pistachio nut and ginger chocolates are higher than those of dark chocolate. As stated previously, this is attributed to the fibrous structure of these substances. Especially ginger, lemon and cinnamon absorb the cocoa oil and increase the viscosity of chocolate.

Priscilla Efraim et al., (2011) studied the effect of three types of phytosterols (encapsulated pine phytosterol powder, oil-based soy phytosteroli powder soy

phytosterol) addition on the rheological properties of chocolate. They observed that application of encapsulated pine phytosterol powder influence of chocolate rheology. A different study related with chocolate rheology is performed by Afoakwa et al., (2007). They studied effect of particle size on the viscosity of chocolate and they found that particle size (D90 (90% finer than this size) affect the rheological properties of chocolate.

4.7.5 Influence of Additives on Color of Dark Chocolate

Color is an important attribute to the food industry. Consumers frequently look at a product and make a judgment decision largely based on overall appearance including color. A suitable color model is necessary to fully describe the visual appearance of a food material. Hunter L*, a*, b* scale are commonly used for determination of color difference in food industry. This system is dependent on the measurement of *L, a* and*b*. The *L* value represents lightness and change from 0 (black) to 100 (white). The value *a* changes from –*a* (greenness) to +*a*(redness) while *b* values is from –*b* (blueness) to +*b* (yellowness). Δ*E* is an equally weighed combination of the coordinate (*L*, a*, b**) differences. It represents the magnitude of difference in color but does not indicate direction of color. As seen in Figure 4.13, pistachio nut caused a relatively higher change in the lightness of the chocolate in comparison with other additives. This parameter is the most informative to show the color change. As shown in Figure 4.13, the effects of the additives on the yellowness of all the samples are negligible.

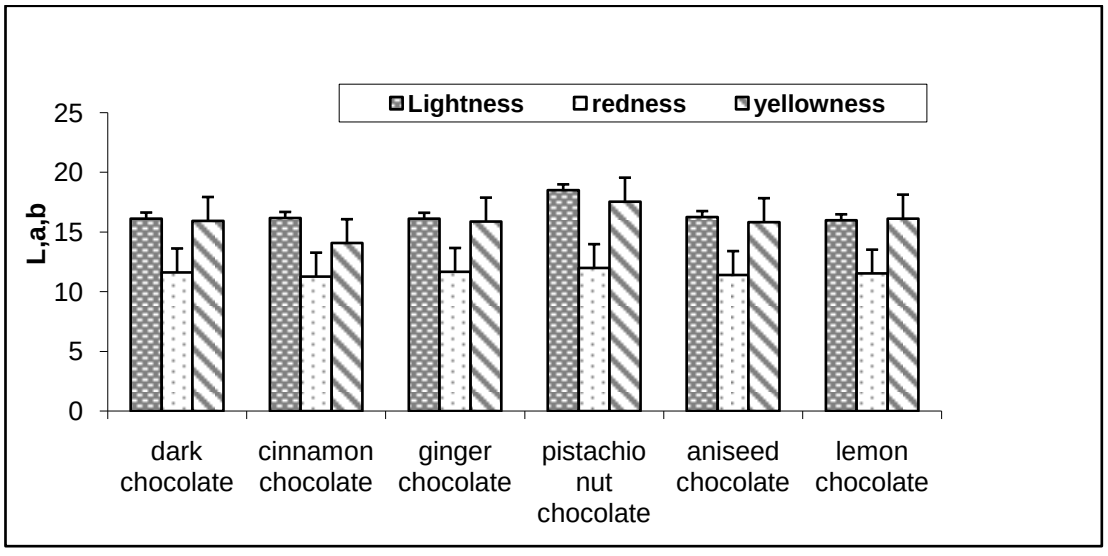


Figure 4.13 *L*, *a*, and, *b* value of chocolate with additives.

As seen from Figure 4.14, color of ginger, aniseed and lemon chocolates are close that of dark chocolate. Total color change in these chocolate is small. As mentioned before, the reason of small change in the total color is the small percentage of these additives.

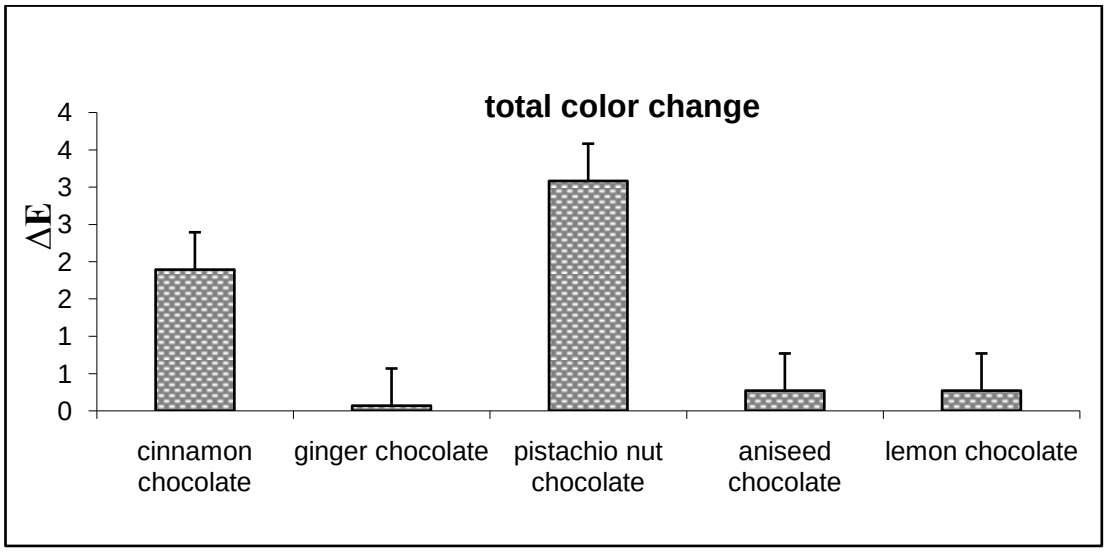


Figure 4.14 Total color change of chocolates with additives

4.7.6 Influence of Additives on the Total Polyphenol Content of Dark Chocolate

Arora et al.,(2000), stated that the flavonoids represent a ubiquitous and abundant group of polyphenol consumed in diet, primarily from fruits and vegetables, derived from plant and act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals and their ability to stabilize membranes by decreasing membrane fluidity. Cocoa and its derived products are rich in flavonoids, characterized as flavan-3-ols or flavanols and include the monomoric forms, (-) epicatechin and (-) catechin and the oligomeric form of the monomeric units (Wollgast & Anklam, 2000). Steinberg et al., (2003) declared that most of antioxidant activity in the chocolate comes from polyphenol content. All fractions of cocoa bean polyphenols have been identified to have antioxidant property.

As seen in Figure 4.15, some type of additives increased total polyphenol. There is a significant increase in the total polyphenol content of chocolate containing spices like cinnamon, ginger and aniseed despite their low percentages.

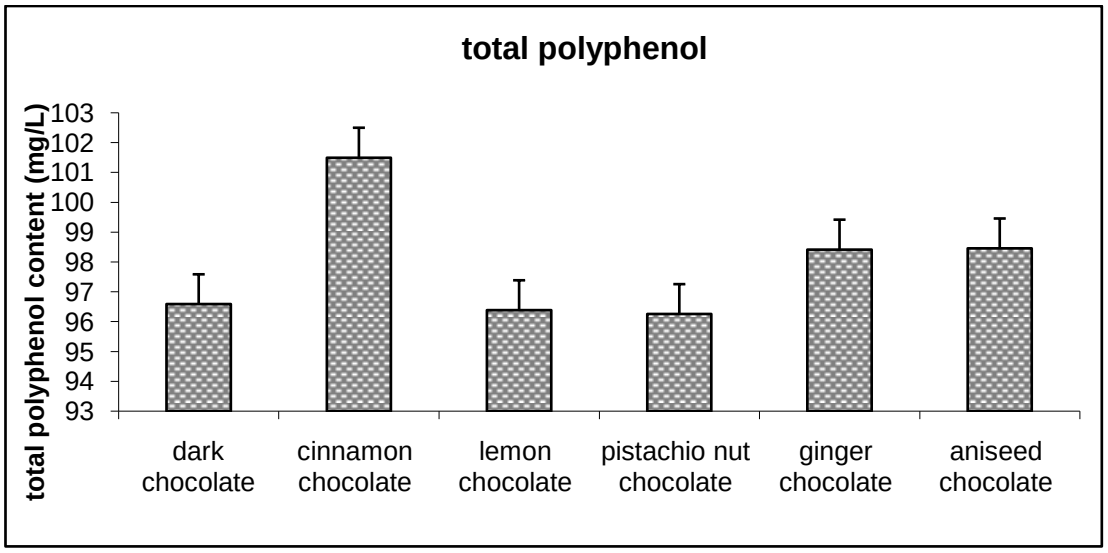


Figure 4.15 Total polyphenol change of chocolate with additives

It was observed that lemon and pistachio nut do not have any influence on the total polyphenol content of chocolate. Karen et al., (2008) studied the strength of the relationship between polyphenol-rich nonfat cocoa solid content (NFCS) and polyphenol content. They found that in the dark chocolate NFCS is linearly related to total polyphenols and also extra component such as milk, or butter might influence polyphenol content.

CONCLUSIONS

1. For dark chocolate without additives, the total volatile concentration in the headspace was observed to increase in pasty phase and decrease in the liquid phases of conching. Odor qualities of samples were determined to be fruity, green, sweet, caramel and chocolate in pasty and liquid phases. The total polyphenol, caffeine and theobromine contentswere observed to decrease in the course of conching.
2. The volatile concentration in the headsspace of the sample was observed to increase up to 300 minutes during conching. It decreased after that time.
3. Spicy chocolates with cinnamon, aniseed and ginger powders were developed. As a result of both instrumental and sensory tests, they were shown to be acceptable with respect to their textures and aroma profiles.
4. Chocolate with lemon peel powder was, similarly, found to have acceptable texture and aroma whereas the sample prepared with pistachio nut paste was not.
5. Results showed that samples prepared with pure citral, pure cinnamldehyde, and aniseed essential oil got less scores compared to those prepared with natural spice powders and lemon peel powder.
6. Ginger powder and lemon peel powder were determined to be the most effectives in increasing the melting point and the hardness of dark chocolate respectively. Particle size distribution of dark chocolate was observed not to be affected by the addition of additive powders.
7. Cinnamon powderwas found to be the most effective additive on the total polyphenol content of dark chocolate.

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APPENDICES

A1. Experimental design of parameters optimization of SPME extraction of sample in the design expert

Run	Factors			Responses			
	Temperature (°C)	**Time (min.)**	**Fiber type**	**Acids**	**Alcohol**	**Other**	**Total**
1	55	37.5	D	4	5	5	14
2	55	37.5	C	5	4	2	11
3	55	37.5	C	4	5	11	20
4	55	37.5	A	9	5	6	20
5	55	37.5	C	4	4	4	12
6	34	37.5	D	5	4	1	10
7	76	37.5	C	6	2	17	25
8	55	37.5	A	8	4	8	20
9	70	60.0	C	9	8	24	41
10	55	37.5	C	4	4	2	10
11	40	60.0	A	4	4	3	11
12	40	60.0	C	5	1	3	9
13	40	15.0	A	2	2	2	6
14	55	6.0	C	4	1	5	10
15	55	37.5	D	5	5	4	14
16	34	37.5	C	5	4	9	18
17	55	37.5	D	3	4	2	9
18	55	37.5	B	3	3	5	11
19	55	37.5	B	6	3	9	18
20	34	37.5	A	5	4	3	11
21	40	60.0	B	4	1	7	12
22	70	60.0	A	5	4	14	23
23	55	6.0	B	5	1	3	9
24	55	37.5	A	9	5	6	20
25	55	70.0	C	5	3	4	12
26	40	60.0	D	5	3	2	10
27	55	37.5	C	4	4	2	10

28	55	37.5	B	3	3	5	11
29	55	70.0	B	4	3	9	16
30	70	60.0	B	5	3	11	19
31	70	15.0	D	5	3	7	15
32	55	37.5	B	3	3	5	11
33	55	6.0	D	4	3	3	10
34	55	6.0	A	3	2	5	10
35	70	15.0	B	3	4	9	16
36	76	37.5	A	8	2	6	14
37	55	70.0	A	4	3	8	15
38	70	60.0	C	9	8	24	41
39	70	15.0	A	2	5	7	14
40	55	37.5	D	4	5	5	14
41	76	37.5	D	7	5	9	21
42	40	15.0	B	4	0	5	9
43	34	37.5	B	5	1	4	10
44	55	37.5	D	4	5	5	14
45	70	15.0	C	5	6	9	20
46	55	70.0	D	7	3	5	15
47	55	37.5	A	9	5	6	20
48	55	37.5	B	3	3	5	11
49	55	37.5	A	9	5	6	20
50	40	15.0	C	3	2	4	9
51	76	37.5	B	10	5	9	24
52	40	15.0	D	3	3	2	8

A2. Actual and predicted value of acids

Run	Actual value	Predicted value	Residual	Leverage
1	1.39	1.53	-0.15	0.084
2	1.61	1.57	0.042	0.072
3	1.39	1.57	-0.18	0.072
4	2.20	1.65	0.55	0.077
5	1.39	1.57	-0.18	0.072
6	1.61	1.33	0.28	0.138
7	1.79	1.77	0.018	0.128
8	2.08	1.65	0.43	0.077
9	2.20	1.89	0.31	0.126
10	1.39	1.57	-0.18	0.072
11	1.39	1.67	-028	0.139
12	1.61	1.59	0.019	0.134
13	0.69	1.33	-0.64	0.140
14	1.39	1.33	0.058	1.40
15	1.61	1.53	0.078	0.084
16	1.61	1.36	0.25	0.140
17	1.10	1.53	-0.43	0.084
18	1.10	1.43	-0.33	0.077
19	1.79	1.43	0.36	0.077
20	1.61	1.44	0.17	0.139
21	1.39	1.45	-0.065	1.140
22	1.61	1.97	-0.36	1.40
23	1.61	1.19	0.42	0.138
24	2.20	1.65	0.55	0.077
25	1.61	1.81	-0.20	0.130
26	1.61	1.55	0.055	0.146
27	1.39	1.57	-0.18	0.072
28	1.10	1.43	-0.33	0.077
29	1.39	1.67	-0.28	0.139

30	1.61	1.75	-0.14	0.140
31	1.61	1.51	0.10	0.147
32	1.10	1.43	-0.33	0.077
33	1.39	1.29	0.093	0.138
34	1.10	1.41	-031	0.139
35	1.10	1.41	-031	0.140
36	2.08	1.85	0.23	0.139
37	1.39	1.89	-0.51	0.142
38	2.20	1.89	0.31	0.126
39	0.69	1.62	-0.93	1.40
40	1.39	1.53	-0.15	0.084
41	1.95	1.74	0.21	0.153
42	1.39	1.11	0.28	0.140
43	1.61	1.22	0.39	0.139
44	1.39	1.53	-0.15	0.084
45	1.61	1.54	0.065	0.135
46	1.95	1.78	0.17	0.156
47	2.20	1.65	0.55	0.077
48	1.10	1.43	-0.33	0.077
49	2.20	1.65	0.55	0.077
50	1.10	1.25	-0.15	0.144
51	2.30	1.64	0.67	0.139
52	1.10	1.21	-0.12	0.137

A3. Actual and predicted value of alcohol

Run	Actual value	Predicted value	Residual	Leverage
1	2.24	2.19	0.041	0.113
2	1.00	0.91	0.087	0.396
3	2.00	2.11	-0.11	0.106
4	2.24	2.15	0.087	0.108
5	2.00	2.11	-0.11	0.106
6	2.00	1.90	0.10	0.411
7	1.00	1.35	-0.35	0.391
8	2.00	2.15	-0.15	0.108
9	2.00	1.44	0.56	0.393
10	2.24	2.11	0.13	0.106
11	2.00	1.94	0.006	0.403
12	2.83	2.26	0.57	0.291
13	1.41	1.66	-0.25	0.408
14	1.73	1.89	-0.16	0.362
15	2.24	2.19	0.041	0.113
16	1.41	2.37	-0.95	0.347
17	2.00	2.19	-0.19	0.113
18	1.73	1.70	0.03	0.108
19	1.41	1.20	0.22	0.407
20	2.00	1.94	0.058	0.398
21	1.00	1.05	-0.048	0.405
22	2.00	1.87	0.13	0.405
23	1.73	1.53	0.20	0.401
24	2.24	2.15	0.087	0.108
25	2.00	2.11	-0.11	0.106
26	1.73	1.84	-0.11	0.400
27	2.00	2.11	-0.11	0.106
28	1.73	1.70	0.03	0.108

29	1.73	1.70	0.03	0.108
30	1.73	2.11	-037	0.407
31	1.73	1.84	-0.11	0.400
32	1.73	1.70	0.03	0.108
33	1.73	1.68	0.051	
34	1.41	1.53	-0.11	0.396
35	2.00	1.73	0.27	0.406
36	1.41	1.94	-0.53	0.398
37	1.73	1.79	-0.062	0.413
38	2.83	2.26	0.57	0.291
39	2.24	1.73	0.50	0.406
40	2.24	2.19	0.041	0.113
41	2.24	2.08	0.16	0.515
42	1.00	0.53	0.47	0.409
43	1.00	0.71	0.29	0.398
44	2.24	2.19	0.041	0.113
45	1.00	1.67	-0.67	0.405
46	1.73	1.73	0.00	0.529
47	2.24	2.15	0.087	0.108
48	1.73	1.70	0.03	0.108
49	2.24	2.15	0.087	0.108
50	2.45	1.93	0.52	0.408
51	2.24	2.28	-0.048	0.398
52	1.73	1.72	0.01	0.417

A4. Actual and predicted value of others

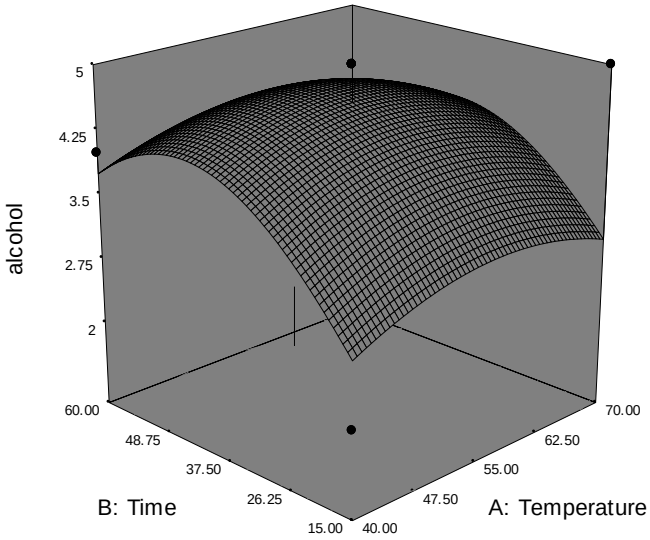
Run	Actual value	Predicted value	Residual	Leverage
1	2.24	2.04	0.20	0.084
2	1.41	2.61	-1.20	0.072
3	3.32	2.61	0.71	0.072
4	2.45	2.41	0.035	0.077
5	2.00	2.61	-0.61	0.072
6	1.00	1.16	-0.16	0.138
7	4.12	3.48	0.64	0.128
8	2.83	2.41	0.41	0.077
9	4.90	3.50	1.40	0.126
10	1.41	2.61	-1.20	0.072
11	1.73	2.05	-0.32	0.139
12	1.73	2.25	-0.52	0.134
13	1.41	1.53	-0.11	0.140
14	2.24	2.24	0.00	0.140
15	2.00	2.04	-0.037	0.084
16	3.00	1.74	1.26	0.140
17	1.41	2.04	-0.62	0.084
18	2.24	2.53	-0.29	0.077
19	3.00	2.53	0.47	0.077
20	1.73	1.54	0.19	0.139
21	2.65	2.17	0.48	0.140
22	3.74	3.30	0.44	0.140
23	1.73	2.16	-0.43	0.138
24	2.45	2.41	0.035	0.077
25	2.00	2.99	-0.99	0.130
26	1.41	1.68	-0.26	0.146
27	1.41	2.61	-1.20	0.072

28	2.24	2.53	-0.29	0.077
29	3.00	2.90	0.10	0.139
30	3.32	3.42	-0.099	0.140
31	2.65	2.40	0.25	0.147
32	2.24	2.53	-0.29	0.077
33	1.73	1.67	0.063	0.138
34	2.24	2.05	0.19	0.139
35	3.00	2.89	0.11	0.140
36	2.45	3.29	-0.84	0.139
37	2.83	2.79	0.034	0.142
38	4.90	3.50	1.40	0.126
39	2.65	2.78	-0.13	0.140
40	2.24	2.04	0.20	0.084
41	3.00	2.91	0.09	0.153
42	2.24	1.64	0.59	0.140
43	2.00	1.66	0.34	0.139
44	2.24	2.04	0.20	0.084
45	3.00	2.97	0.028	0.135
46	2.24	2.42	-0.18	0.156
47	2.45	2.41	0.035	0.077
48	2.24	2.53	-0.29	0.077
49	2.45	2.41	0.035	0.077
50	2.00	1.72	0.28	0.144
51	3.00	3.40	-0.40	0.139
52	1.41	1.15	0.26	0.137

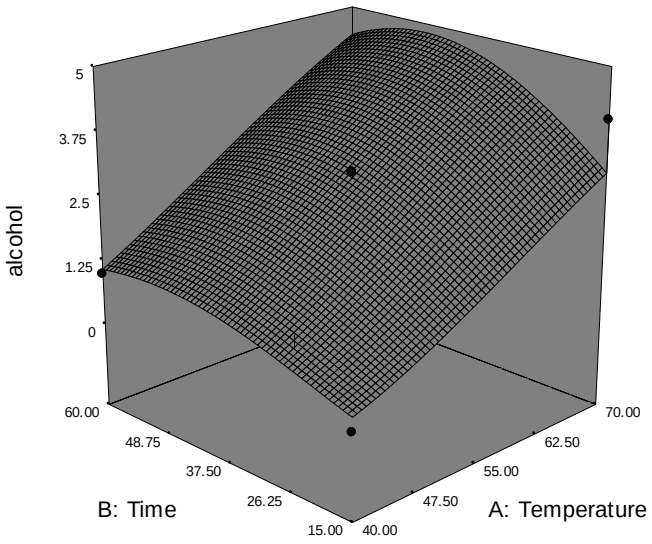
A5. Actual and predicted value of total

Run	Actual value	Predicted value	Residual	Leverage
1	2.64	2.56	0.083	0.084
2	2.40	2.69	-0.30	0.072
3	3.00	2.69	0.30	0.072
4	3.00	2.69	0.30	0.077
5	2.48	2.69	-0.21	0.072
6	2.30	2.12	0.18	0.138
7	3.22	3.13	0.093	0.128
8	3.00	2.69	0.30	0.077
9	3.71	3.17	0.54	0.126
10	2.30	2.69	-0.39	0.072
11	2.40	2.55	-0.15	0.139
12	2.20	2.55	-0.36	0.134
13	1.79	2.21	-0.042	0.140
14	2.30	2.46	-0.15	0.140
15	2.64	2.56	0.083	0.084
16	2.89	2.26	0.63	0.140
17	2.20	2.56	-0.36	0.084
18	2.40	2.56	-0.16	0.077
19	2.89	2.56	0.34	0.077
20	2.40	2.26	0.14	0.139
21	2.48	2.42	0.069	0.140
22	3.14	3.17	-0.034	0.140
23	2.20	2.32	-0.12	0.138
24	3.00	2.69	0.30	0.077
25	2.48	2.94	-0.45	0.130
26	2.30	2.42	-0.11	0.146

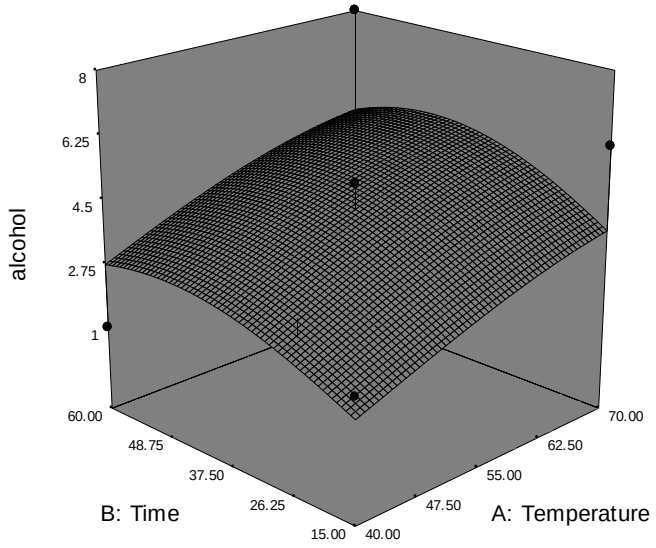
27	2.30	2.69	-0.39	0.072
28	2.40	2.56	-0.16	0.140
29	2.77	2.80	-0.023	0.139
30	2.83	3.03	-0.20	0.140
31	2.71	2.69	0.013	0.147
32	2.40	2.56	-0.16	0.077
33	2.30	2.32	-0.015	0.138
34	2.30	2.45	-0.15	0.139
35	2.77	2.69	0.078	0.140
36	2.64	3.12	-0.48	0.139
37	2.71	2.94	-0.23	0.142
38	3.71	3.17	0.54	0.126
39	2.64	2.83	-0.19	0.140
40	2.64	2.56	0.083	0.084
41	3.04	2.99	0.056	0.153
42	2.20	2.08	0.12	0.140
43	2.30	2.12	0.18	0.139
44	2.64	2.56	0.083	0.084
45	3.00	2.83	0.16	0.135
46	2.71	2.80	-0.094	0.156
47	3.00	2.69	0.30	0.077
48	2.40	2.56	-0.16	0.077
49	3.00	2.69	0.30	0.077
50	2.20	2.21	-0.017	0.144
51	3.18	2.99	0.19	0.139
52	2.08	2.08	0.00	0.137



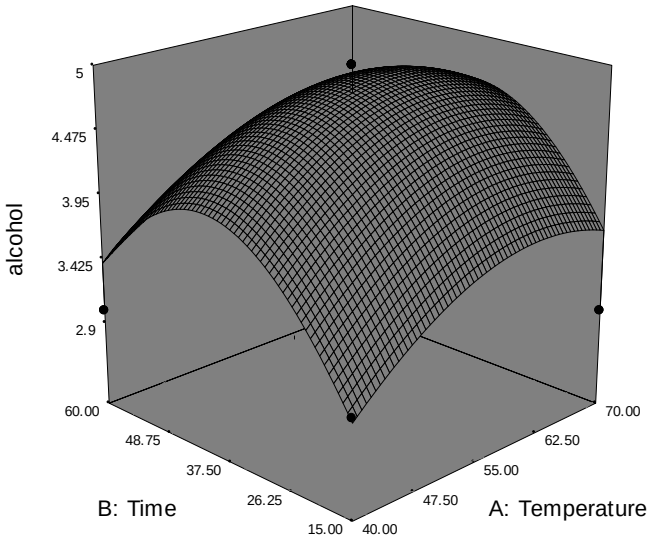
Fiber A



Fiber B

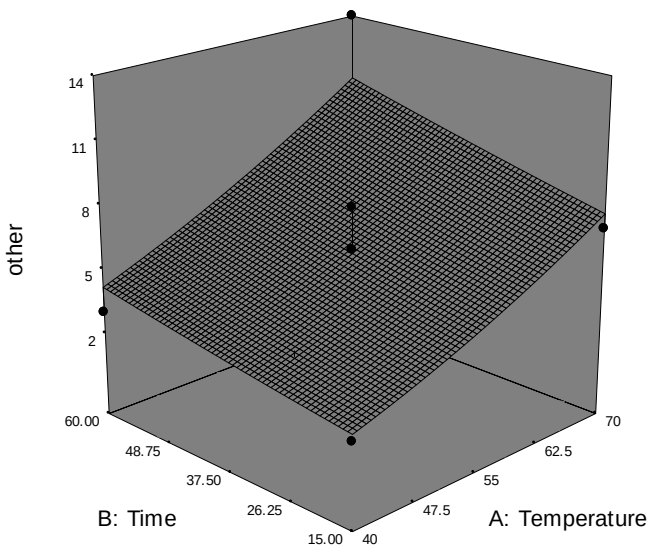


Fiber C

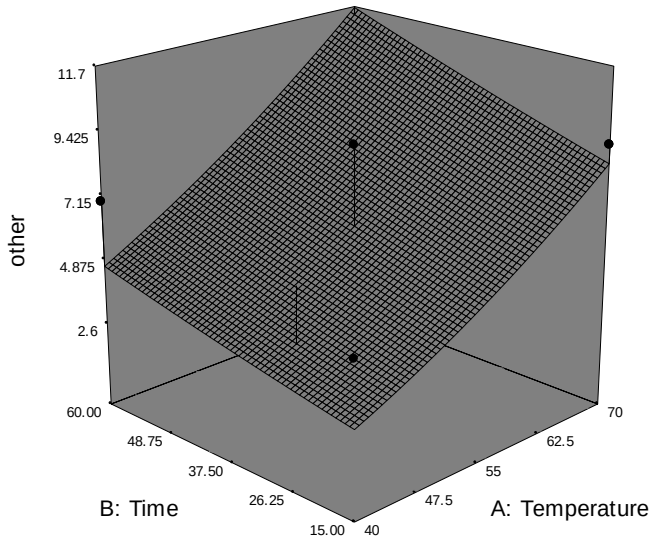


Fiber D

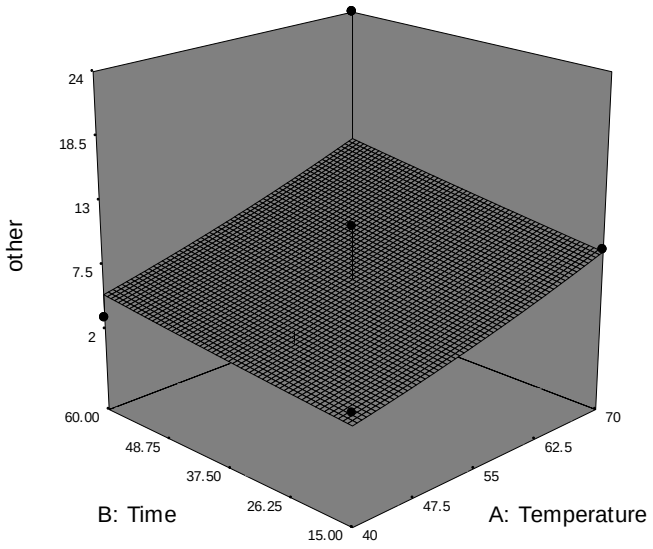
A6. Variation in the number of alcohol as a function of time and temperature and fiber type.



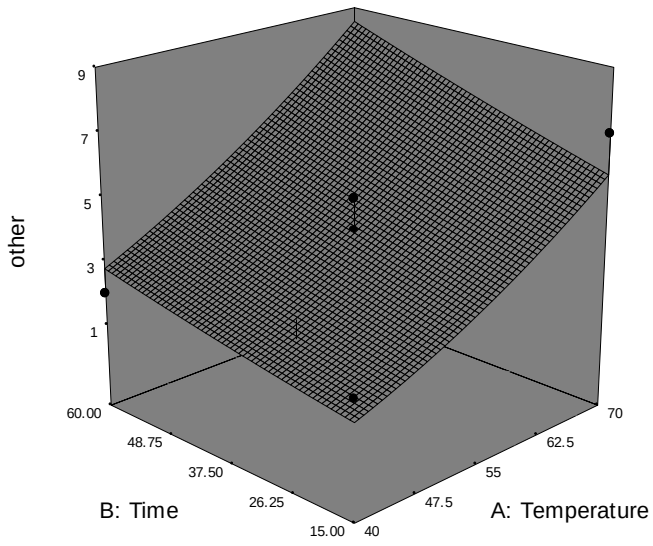
Fiber A



Fiber B

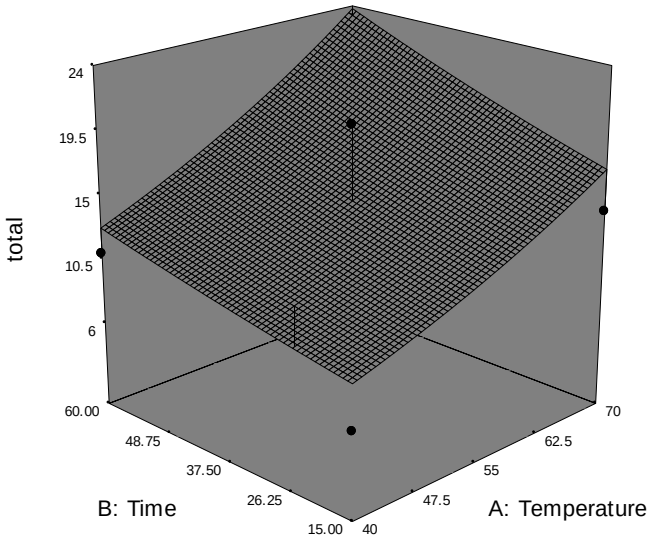


Fiber C

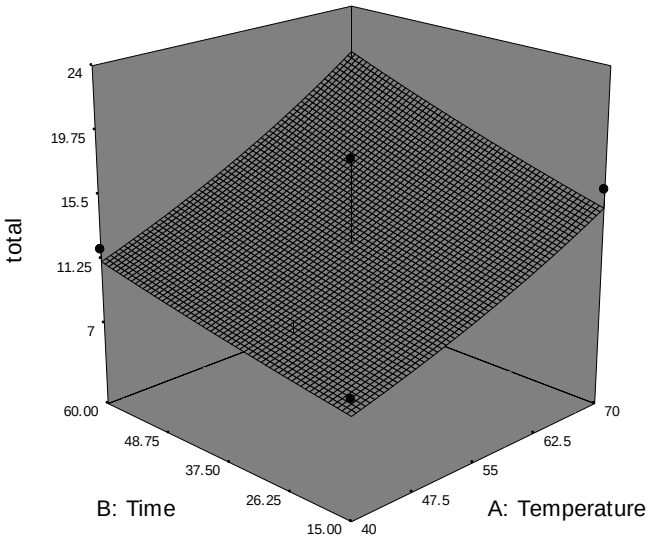


Fiber D

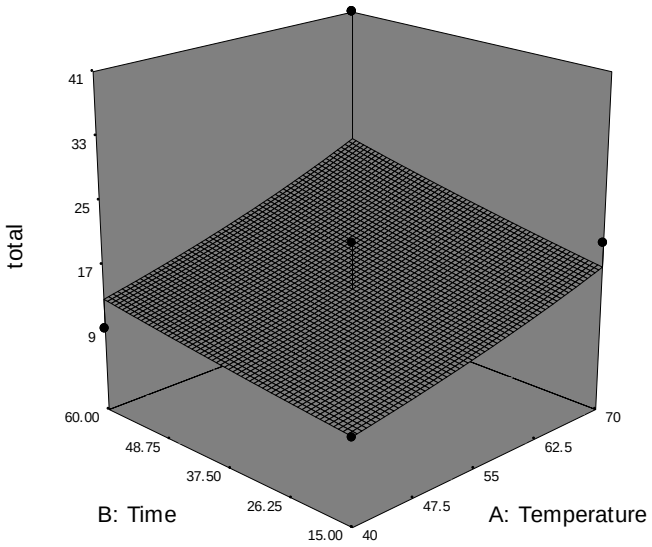
A7. Variation in the number of other as a function of time and temperature and fiber type.



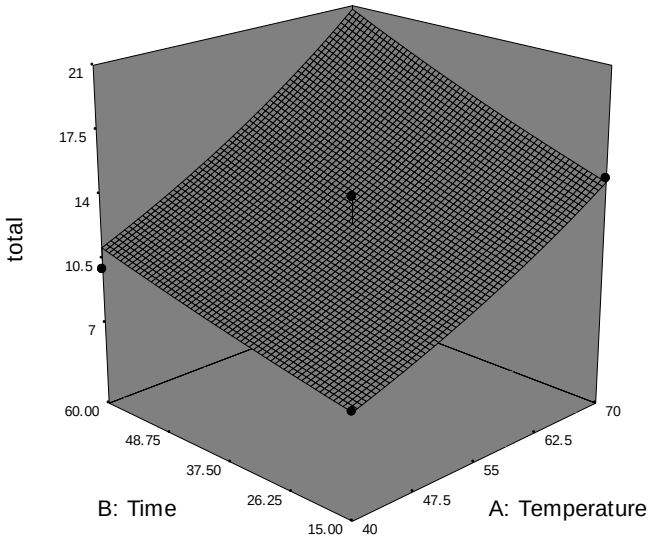
Fiber A



Fiber B

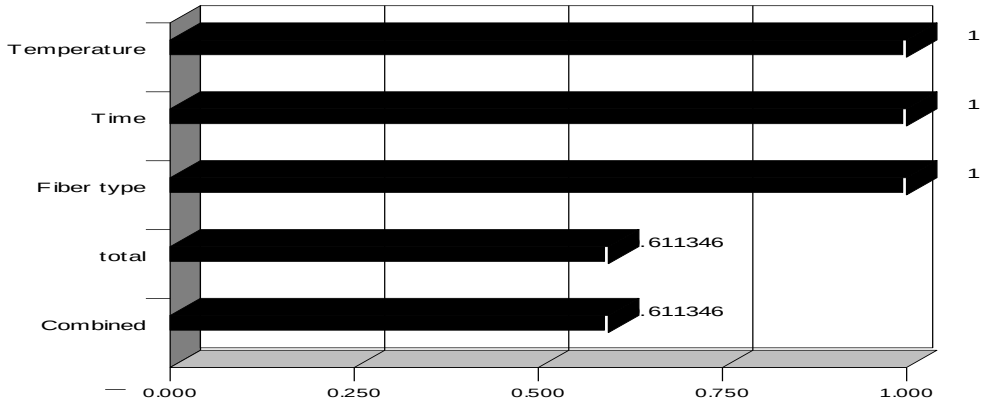


Fiber C

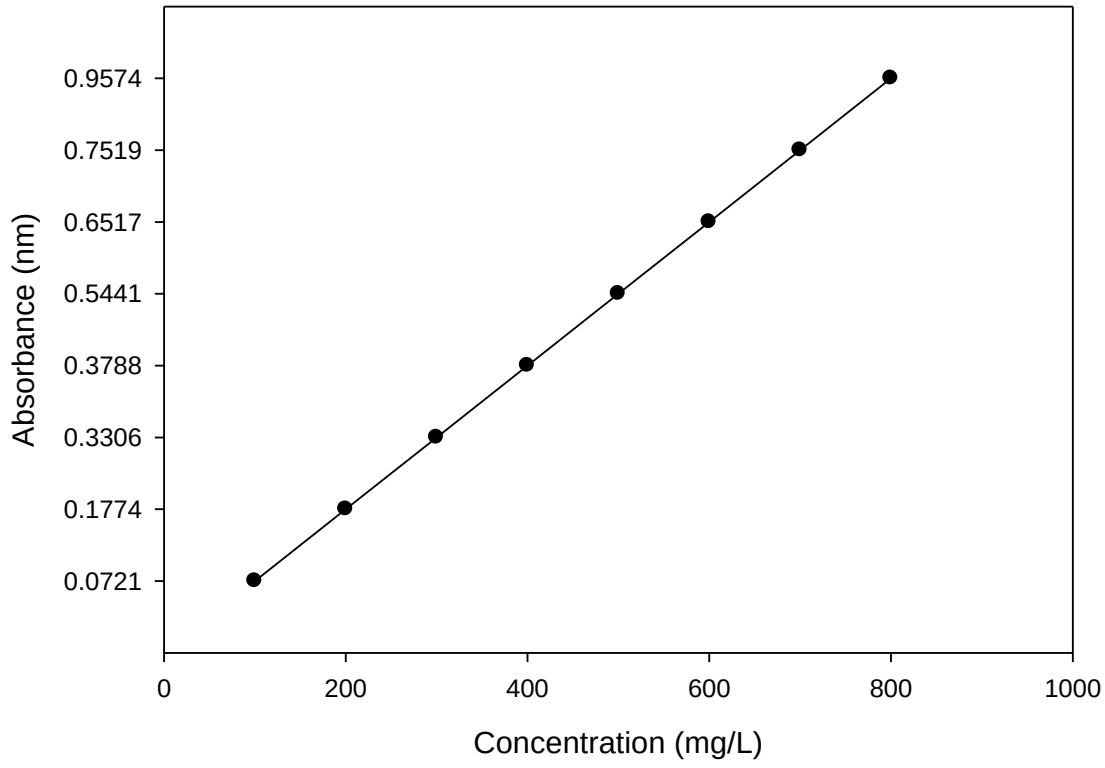


Fiber D

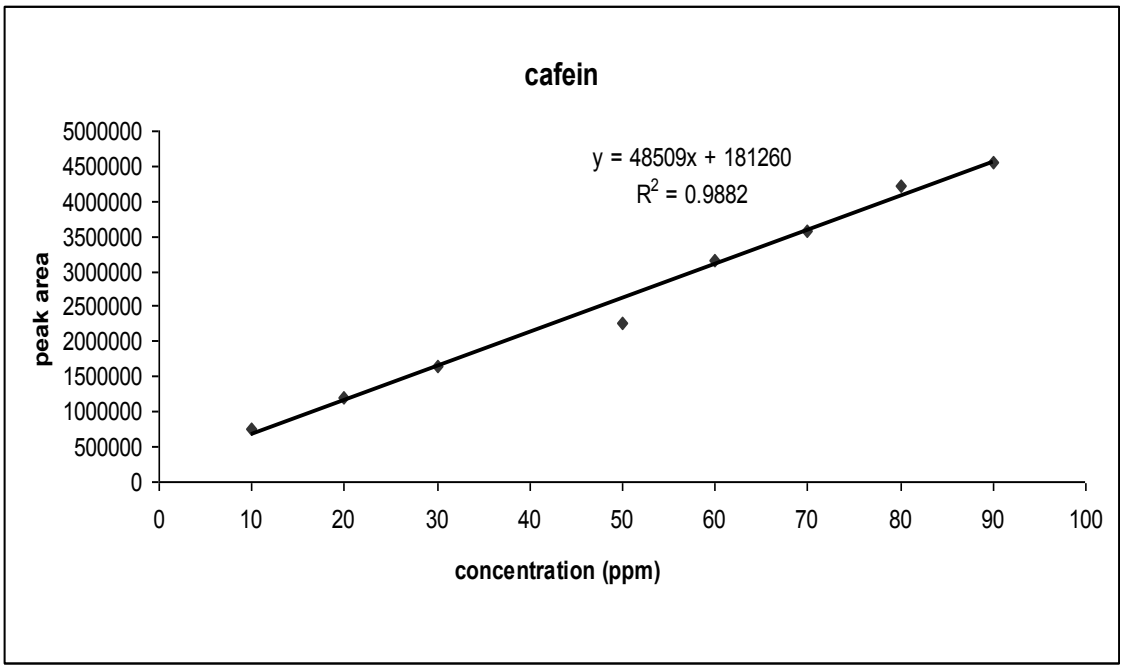
A8. Variation in the number of total as a function of time and temperature and fiber type.



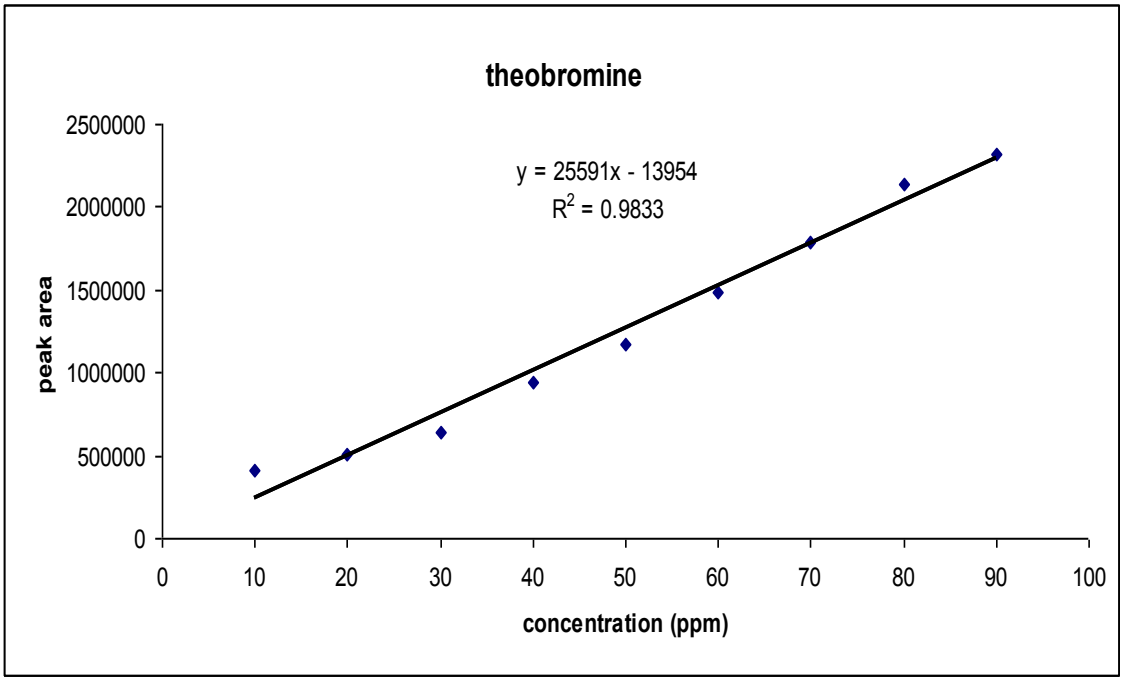
A9. Desirability figure of optimization of SPME extraction parameters



A10. Calibration curve of catectin standard for quantative analysis of total polyphenol



A11. Calibration curve of cafein standard for quantative analysis of cafein



A12. Calibration curve of theobromine standard for quantative analysis of theobromine

A13. Experimental design for optimization of conching time.

Run	Factor	Responses							
	Ttime (min.)	Acids (ppb)	Aldehydes (ppb)	Pyrazines (ppb)	Ketones (ppb)	Hydrocarbones (ppb)	Alcohols (ppb)	Acetates (ppb)	Total (ppb)
1	270	14297	21411	8495	17666	10245	5338	969	101217
2	150	12449	3486	5116	960	2462	4582	414	45273
3	60	7251	1409	2012	403	1609	2333	191	30400
4	300	9765	18802	8295	7843	6908	3048	973	54020
5	360	5890	5263	3612	7881	3350	450	2166	39186
6	330	8268	6882	5263	7800	3898	1078	997	47667
7	240	20364	10009	13813	4962	11140	18217	810	83497
8	210	15814	5970	11726	4197	4602	17247	629	60072
9	120	9311	3066	3533	915	1677	3840	323	45151
10	180	13883	5597	5901	1200	3350	5068	552	47663
11	30	7056	1160	1323	142	658	441	171	29186
12	90	8260	2756	2295	820	1708	3441	301	45048

A14. Actual and predicted value of acids for optimization of conching time

Run	**Actual value**	**Predicted value**	**Residual**	**Leverage**
1	9.57	9.60	-0.032	0.273
2	9.43	9.40	0.030	0.239
3	8.89	8.91	-0.021	0.279
4	9.19	9.41	-0.22	0.255
5	8.68	8.57	0.11	0.76
6	9.02	9.08	-0.055	0.279
7	9.92	9.67	0.25	0.239
8	9.67	9.64	0.024	0.197
9	9.14	9.23	-0.09	0.273
10	9.54	9.55	-7.532E-003	0.197
11	8.86	8.81	0.052	0.76
12	9.02	9.06	-0.038	0.255

A15. Actual and predicted value of aldehydes for optimization of conching time

Run	Actual value	Predicted value	Residual	Leverage
1	9.97	9.52	0.45	0.273
2	8.16	8.31	-0.15	0.239
3	7.25	7.29	-0.04	0.279
4	9.84	9.47	0.38	0.255
5	8.57	8.58	-9.44E-003	0.762
6	8.84	9.17	-0.33	0.279
7	9.21	9.38	-0.17	0.239
8	8.69	9.10	-0.41	0.197
9	8.03	7.89	0.13	0.273
10	8.63	8.72	-0.095	0.197
11	7.06	7.20	-0.14	0.763
12	7.92	7.54	0.38	0.255

A16. Actual and predicted value of pyrazines for optimization of conching time

Run	Actual value	Predicted value	Residual	Leverage
1	9.05	9.26	-0.210	0.273
2	8.54	8.56	-0.021	0.239
3	7.61	7.49	0.120	0.279
4	9.02	9.08	-0.056	0.255
5	8.19	8.08	0.12	0.761
6	8.57	8.70	-0.13	0.279
7	9.53	9.26	0.270	0.239
8	9.37	9.12	0.250	0.197
9	8.17	8.20	-0.032	0.273
10	8.68	8.88	-0.200	0.197
11	7.19	7.20	-0.017	0.768
12	7.74	7.83	-0.096	0.255

A17. Actual and predicted value of ketones for optimization of conching time

Run	Actual value	Predicted value	Residual	Leverage
1	4.25	3.74	0.510	0.127
2	2.98	3.08	-0.100	0.099
3	2.61	2.59	0.012	0.225
4	3.89	3.90	-6.176E-003	0.169
5	3.90	4.23	-0.330	0.295
6	3.89	4.06	-0.170	0.225
7	3.70	3.57	0.120	0.099
8	3.62	3.41	0.210	0.085
9	2.96	2.92	0.041	0.127
10	3.08	3.25	-0.170	0.085
11	2.15	2.43	-0.280	0.295
12	2.91	2.76	0.160	0.169

A18. Actual and predicted value of hydrocarbones for optimization of conching time

Run	Actual value	Predicted value	Residual	Leverage
1	9.23	8.98	0.250	0.273
2	7.81	7.97	-0.160	0.239
3	7.38	6.95	0.430	0.279
4	8.84	8.89	-0.049	0.255
5	8.12	8.00	0.120	0.765
6	8.27	8.58	-0.31	0.279
7	9.32	8.89	0.430	0.239
8	8.43	8.67	-0.230	0.197
9	7.42	7.59	-0.160	0.273
10	8.12	8.34	-0.230	0.197
11	6.49	6.79	-0.300	0.766
12	7.44	7.23	0.210	0.255

A19. Actual and predicted value of alcohols for optimization of conching time

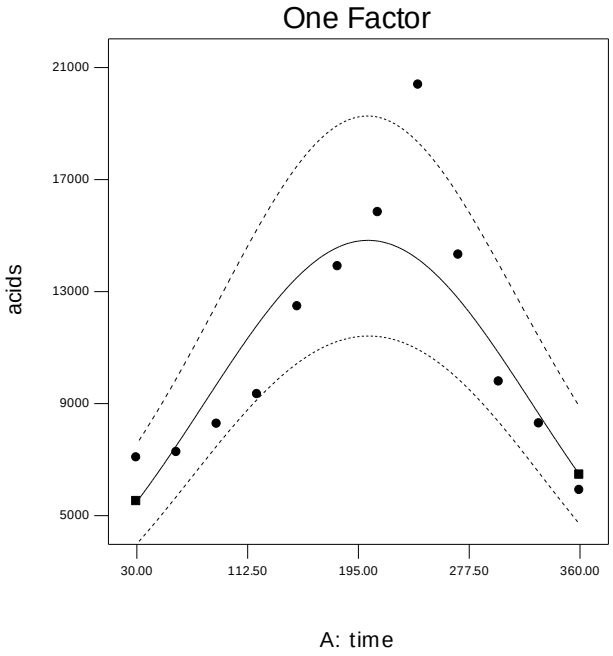
Run	Actual value	Predicted value	Residual	Leverage
1	3.73	3.73	-7.274E-003	0.151
2	3.66	3.91	-0.250	0.169
3	3.37	3.13	0.230	0.277
4	3.48	3.47	0.010	0.169
5	2.65	2.69	-0.038	0.554
6	3.03	3.13	-0.093	0.277
7	4.26	3.91	0.350	0.169
8	4.24	4.00	0.240	0.187
9	3.58	3.74	-0.150	0.151
10	3.70	4.00	-0.290	0.187
11	2.64	2.70	-0.057	0.550
12	3.54	3.48	0.057	0.169

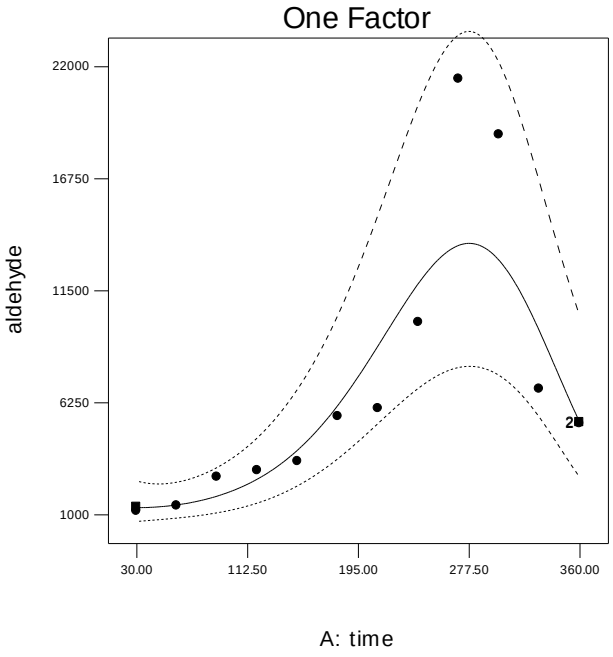
A20. Actual and predicted value of acetates for optimization of conching time

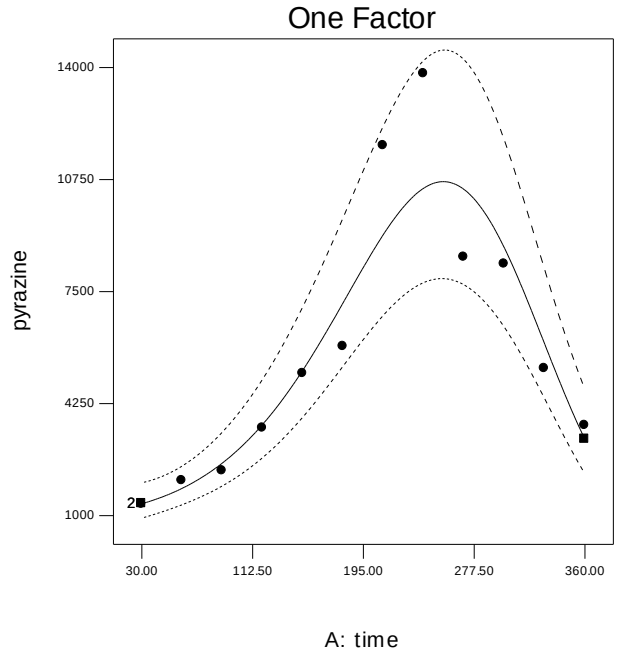
Run	Actual value	Predicted value	Residual	Leverage
1	2.99	2.96	0.024	0.127
2	2.62	2.61	0.011	0.099
3	2.28	2.34	-0.058	0.225
4	2.99	3.05	-0.063	0.169
5	3.34	3.23	0.110	0.295
6	3.00	3.14	-0.140	0.225
7	2.91	2.87	0.035	0.099
8	2.80	2.78	0.014	0.085
9	2.51	2.52	-7.864E-003	0.127
10	2.74	2.70	0.047	0.085
11	2.23	2.25	-0.017	0.295
12	2.48	2.43	0.051	0.169

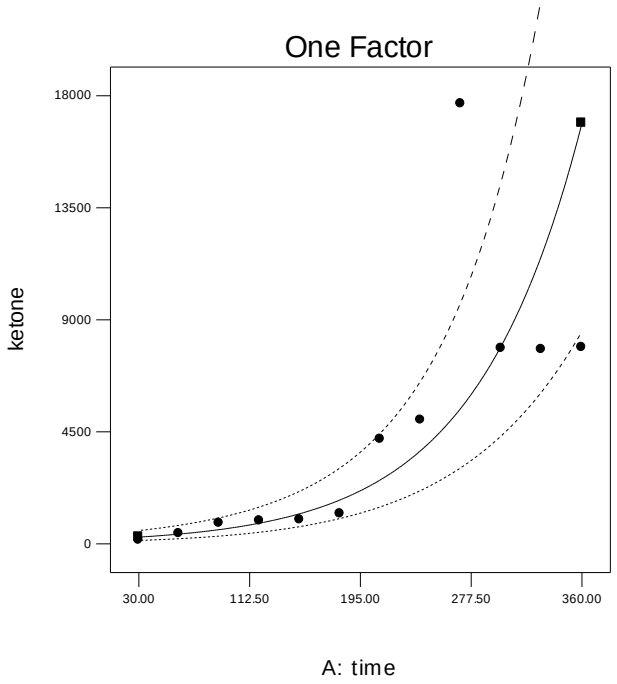
A21. Actual and predicted value of total for optimization of conching time

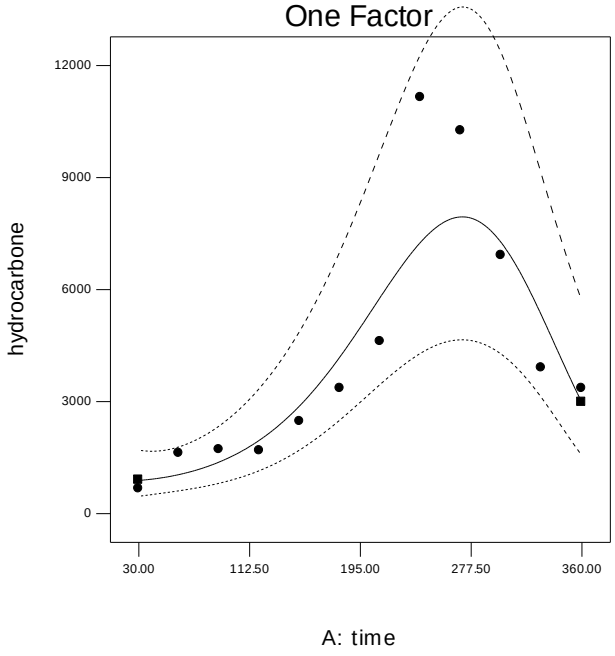
Run	Actual value	Predicted value	Residual	Leverage
1	9.880E-006	1.436E-005	-4.482E-006	0.273
2	2.209E-005	2.011E-005	1.979E-006	0.239
3	3.289E-005	3.063E-005	2.263E-006	0.279
4	1.851E-005	1.624E-005	2.276E-006	0.255
5	2.552E-005	2.588E-005	-3.632E-007	0.765
6	2.098E-005	1.998E-005	9.944E-007	0.279
7	1.198E-005	1.409E-005	-2.110E-006	0.239
8	1.665E-005	1.514E-005	1.511E-006	0.197
9	2.215E-005	2.348E-005	-1.336E-006	0.273
10	2.098E-005	1.724E-005	3.745E-006	0.197
11	3.426E-005	3.386E-005	4.071E-007	0.761
12	2.220E-005	2.708E-005	-4.884E-006	0.255

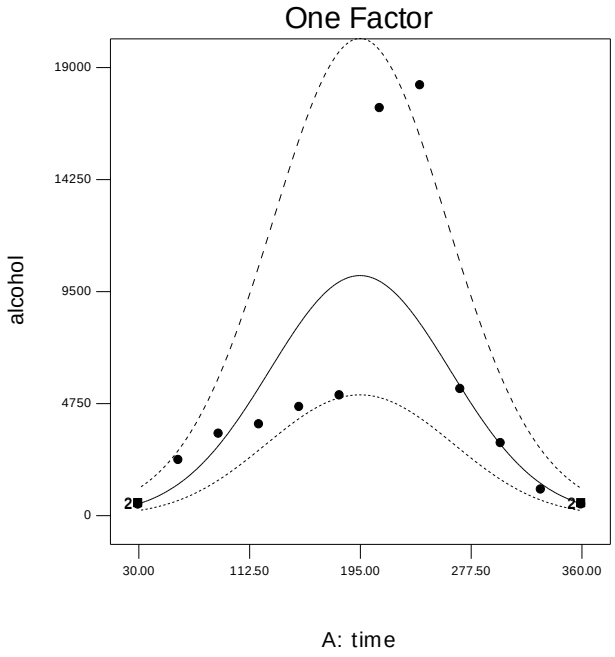

One Factor
acids
21000
17000
13000
9000
5000
30.00
112.50
195.00
277.50
360.00
A: time

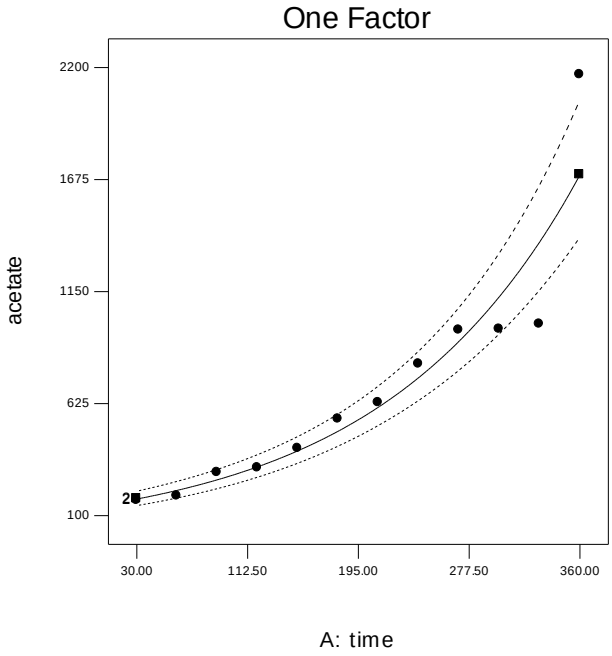

One Factor
aldehyde
22000
16750
11500
6250
1000
30.00
112.50
195.00
277.50
360.00
A: time

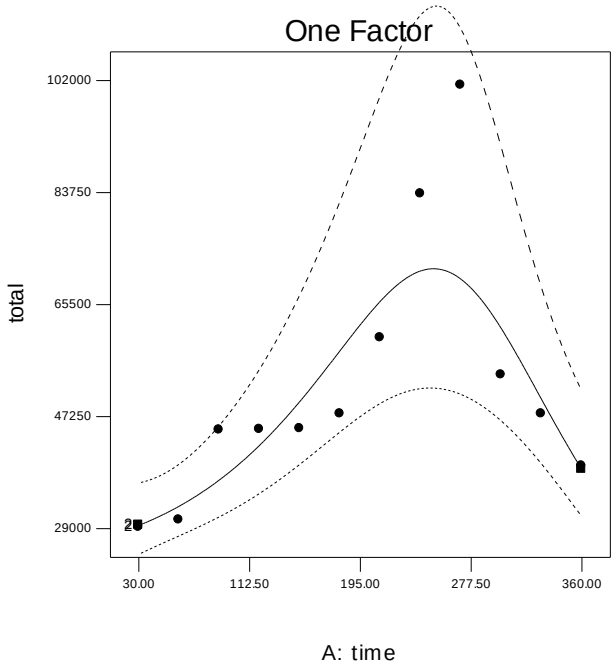

One Factor
pyrazine
14000
10750
7500
4250
1000
2
30.00
112.50
195.00
277.50
360.00
A: time


One Factor
ketone
18000
13500
9000
4500
0
30.00
112.50
195.00
277.50
360.00
A: time


One Factor
hydrocarbone
12000
9000
6000
3000
0
30.00
112.50
195.00
277.50
360.00
A: time


One Factor
alcohol
19000
14250
9500
4750
0
2
2
30.00
112.50
195.00
277.50
360.00
A: time





A22. Change of amount of acids, aldehyde, pyrazine, ketone, hydrocarbone, alcohol, acetate and total during conching.

A23. Experimental design in design expert for optimization of lemon peel powder

Run	Factor	Responses				
	Lemon peel Powder (%)	Viscosity (pa.s)	Hardness (kg force)	Moisture(%)	Total color Change	Sensory analyses
1	1.0	1.205	1.280	0.739	0.363	5
2	3.0	2.044	3.221	0.964	0.505	4
3	4.5	2.186	5.792	1.062	3.070	5
4	4.0	2.078	5.303	1.031	2.960	5
5	1.5	1.999	2.542	0.763	0.480	5
6	5.0	2.518	6.066	1.084	3.310	6
7	1.0	1.966	2.460	0.735	0.360	6
8	1.0	1.967	2.435	0.732	0.359	6
9	3.5	2.074	3.857	0.983	2.140	5
10	3.0	2.036	3.220	0.960	0.501	6
11	2.0	1.308	1.529	0.865	0.490	8
12	5.0	2.514	6.046	1.082	3.280	5
13	5.0	2.512	6.036	1.075	3.270	5

A24. Experimental design in design expert for optimization of ginger

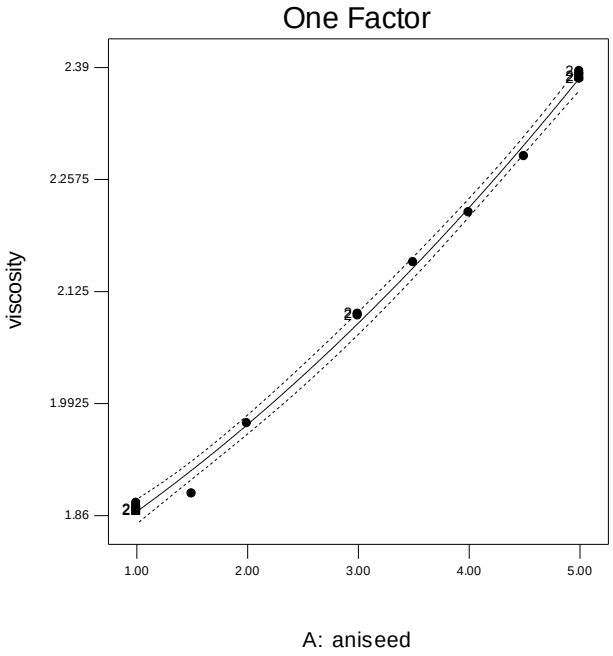
Run	Factor	Responses				
	Ginger (%)	Viscosity (pa.s)	Hardness (kg force)	Moisture(%)	Total color Change	Sensory analyses
1	1.0	1.636	1.611	0.723	0.33	4
2	2.0	1.207	1.504	0.910	0.62	7
3	1.0	1.620	1.610	0.720	0.29	4
4	1.0	1.615	1.605	0.715	0.25	4
5	1.5	1.836	1.789	0.836	0.53	7
6	4.5	2.369	3.306	1.290	3.22	6
7	3.5	2.268	2.887	0.945	3.07	4
8	5.0	2.434	3.521	1.360	3.31	4
9	3.0	2.107	2.597	0.943	0.69	3
10	3.0	2.101	2.590	0.940	0.65	5
11	5.0	2.425	3.514	1.320	3.17	4
12	5.0	2.410	3.507	1.340	3.21	3
13	4.0	2.330	2.967	1.220	3.17	3

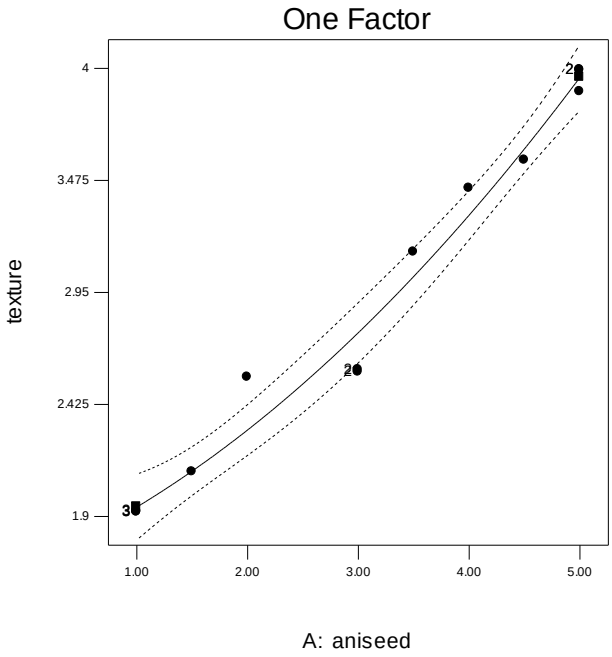
A25. Experimental design in design expert for optimization of cinnamon

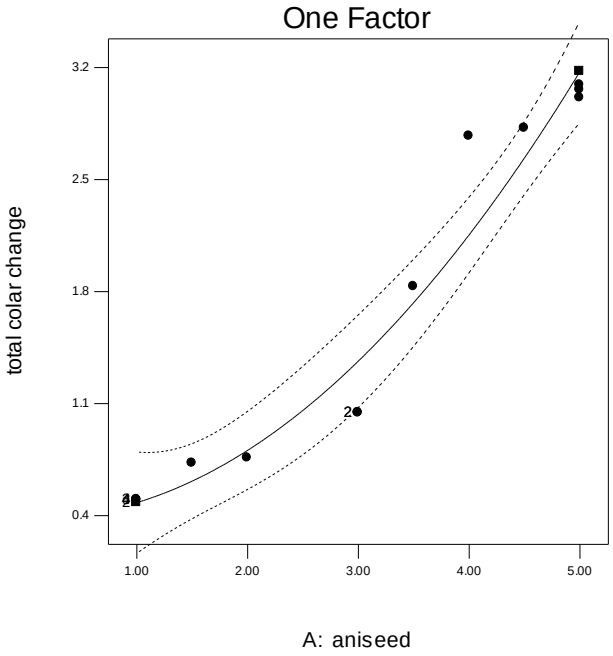
Run	Factor	Responses				
	Cinnamon (%)	Viscosity (pa.s)	Hardness (kg force)	Moisture(%)	Total color Change	Sensory analyses
1	5.0	2.246	3.833	1.021	3.91	3
2	4.0	1.205	2.670	0.972	3.08	9
3	5.0	2.220	3.830	1.019	3.89	9
4	2.5	1.911	2.201	0.790	2.92	3
5	1.0	1.778	1.695	0.648	0.33	4
6	1.5	1.812	2.113	0.702	0.71	4
7	4.5	2.136	2.896	1.000	3.48	5
8	2.0	1.901	2.118	0.767	2.33	5
9	3.0	1.976	2.247	0.907	2.98	4
10	1.0	1.774	1.692	0.645	0.29	4
11	5.0	2.236	3.825	1.015	3.86	4
12	3.0	1.972	2.236	0.901	2.96	3
13	1.0	1.775	1.690	0.636	0.30	4

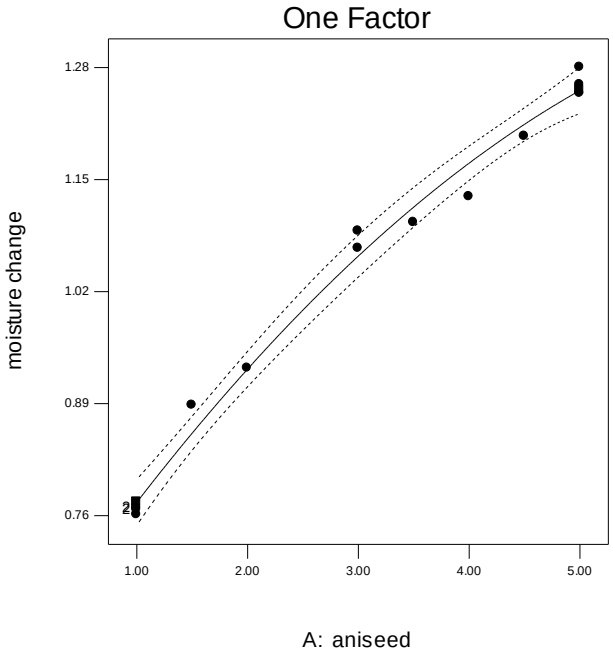
A26. Experimental design in design expert for optimization of aniseed

Run	Factor	Responses				
	Aniseed (%)	Viscosity (pa.s)	Hardness (kg force)	Moisture(%)	Total color Change	Sensory analyses
1	1.0	1.870	1.923	0.769	0.496	5
2	4.5	2.284	3.569	1.200	2.820	4
3	1.0	1.866	1.921	0.767	0.497	6
4	3.5	2.159	3.138	1.100	1.830	4
5	5.0	2.385	3.993	1.280	3.060	5
6	1.0	1.874	1.920	0.761	0.499	5
7	5.0	2.376	3.990	1.260	3.010	7
8	2.0	1.968	2.552	0.931	0.759	7
9	4.0	2.218	3.437	1.130	2.770	4
10	5.0	2.382	3.890	1.250	3.090	4
11	1.5	1.885	1.433	0.888	0.727	4
12	3.0	2.098	2.587	1.090	1.041	5
13	3.0	2.096	2.576	1.07	1.043	4

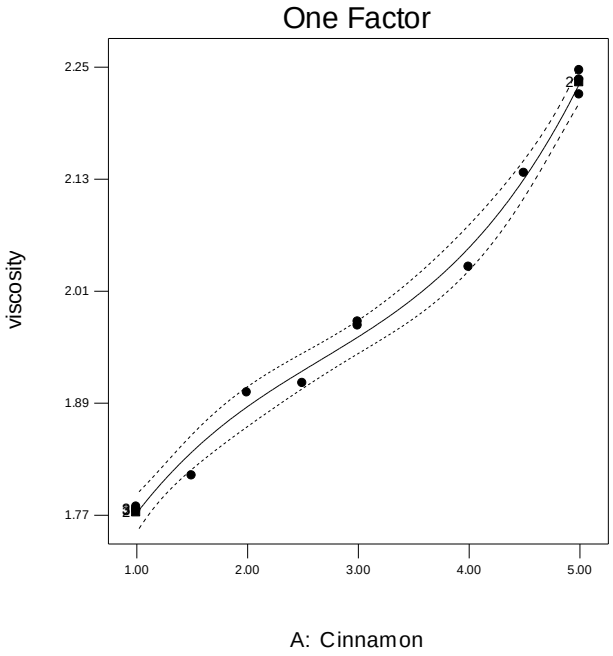

One Factor
viscosity
2.39
2.2575
2.125
1.9925
1.86
1.00
2.00
3.00
4.00
5.00
A: aniseed

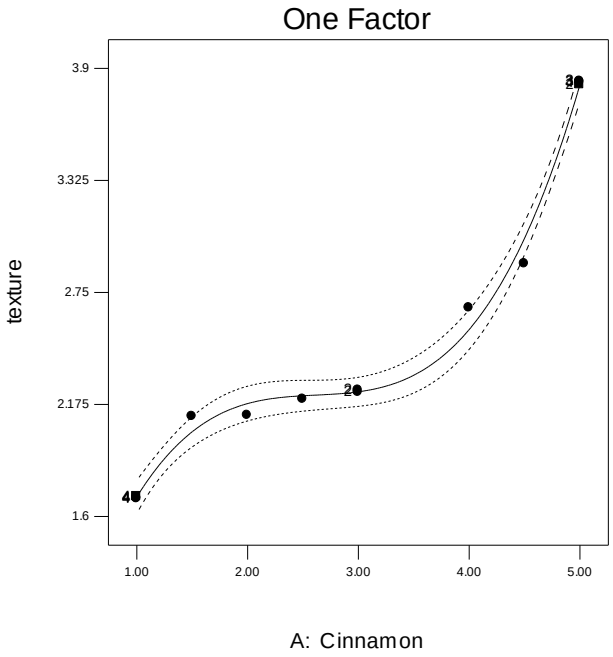

One Factor
texture
4
3.475
2.95
2.425
1.9
1.00
2.00
3.00
4.00
5.00
A: aniseed

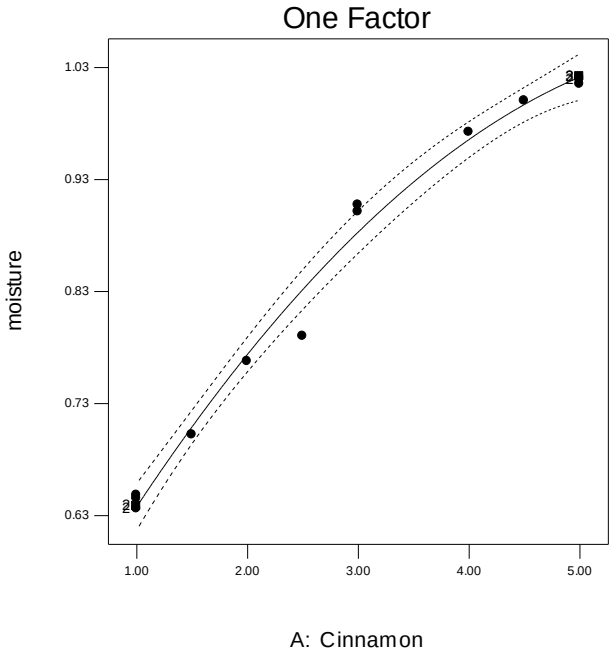


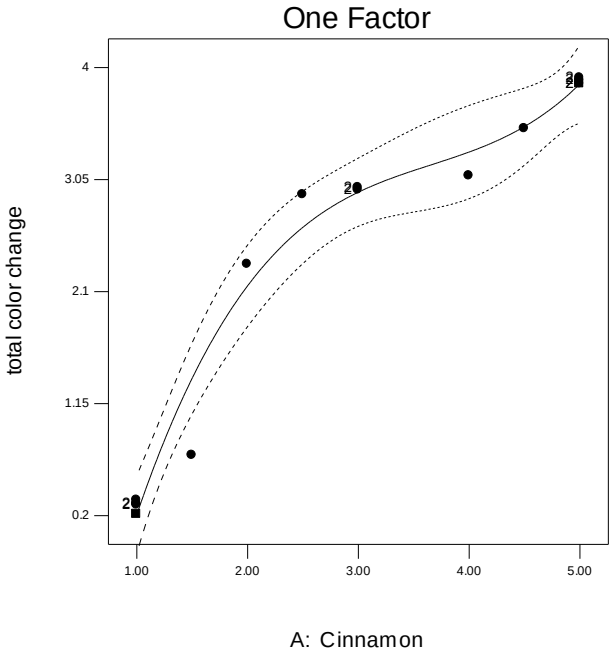


A27. Change in the viscosity, texture, total color and moisture of aniseed chocolate

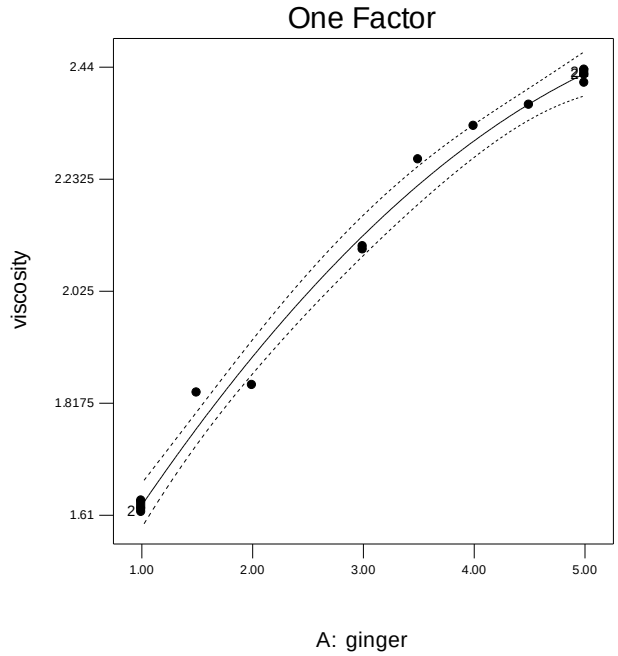

One Factor
viscosity
2.25
2.13
2.01
1.89
1.77
1.00
2.00
3.00
4.00
5.00
A: Cinnamon

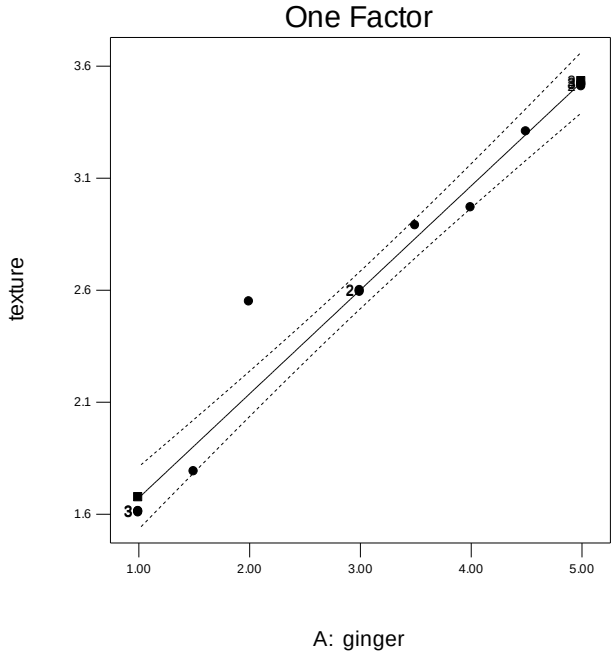

One Factor
texture
3.9
3.325
2.75
2.175
1.6
1.00
2.00
3.00
4.00
5.00
A: Cinnamon

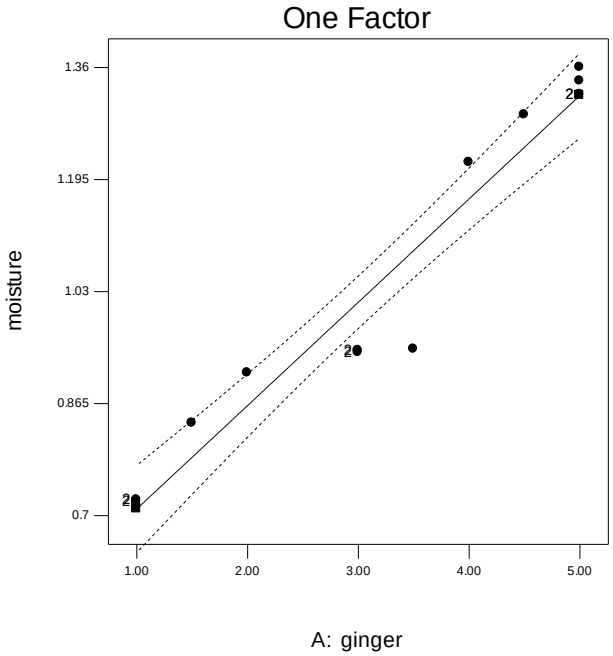


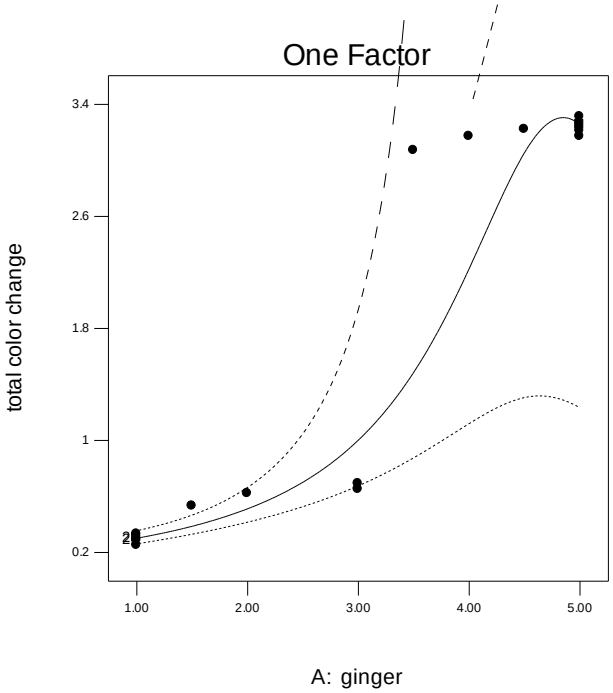


A28. Change in the viscosity, texture, total color and moisture of cinnamon chocolate

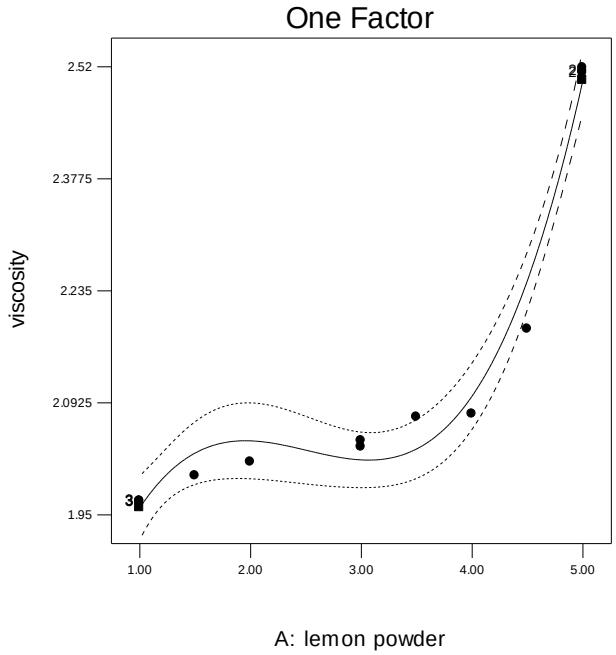

One Factor
viscosity
2.44
2.2325
2.025
1.8175
1.61
1.00
2.00
3.00
4.00
5.00
A: ginger

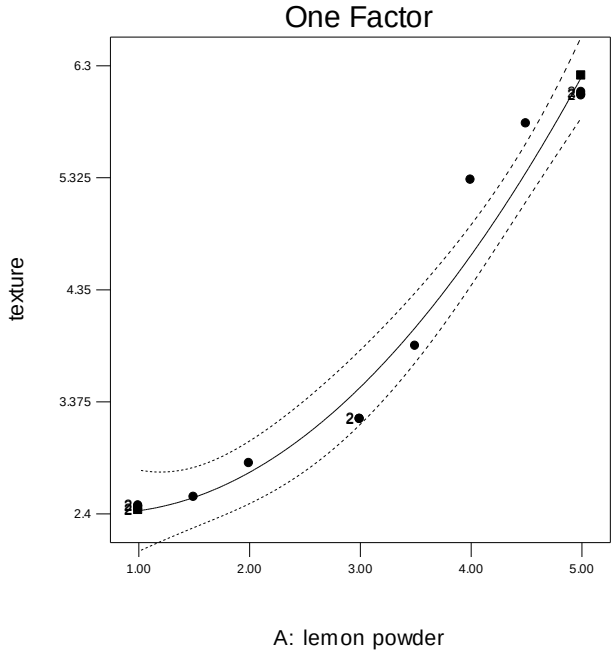

One Factor
texture
3.6
3.1
2.6
2.1
1.6
1.00
2.00
3.00
4.00
5.00
A: ginger

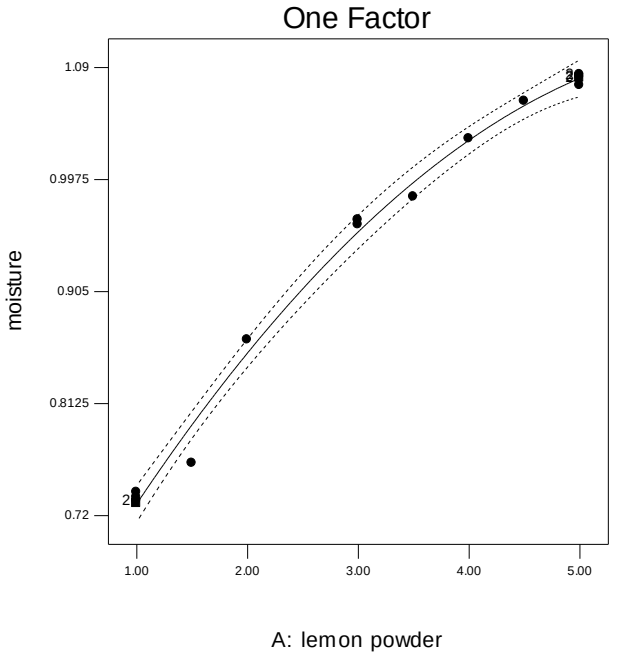


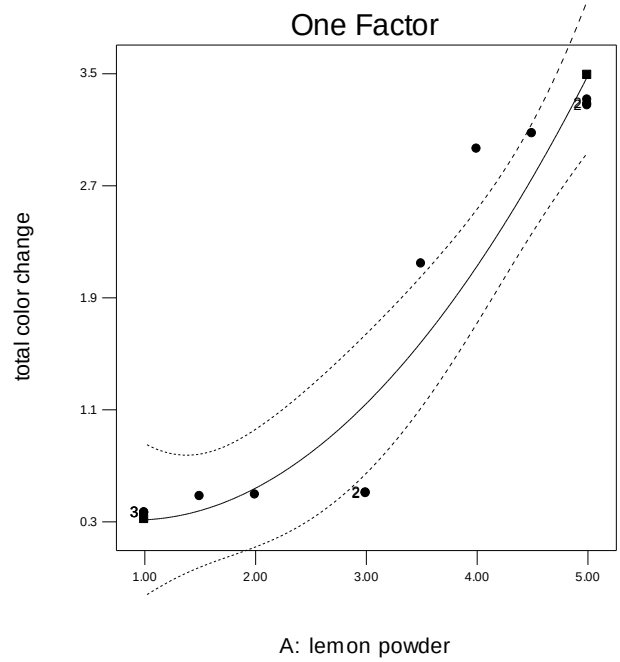


A29. Change in the viscosity, texture, total color and moisture of ginger chocolate

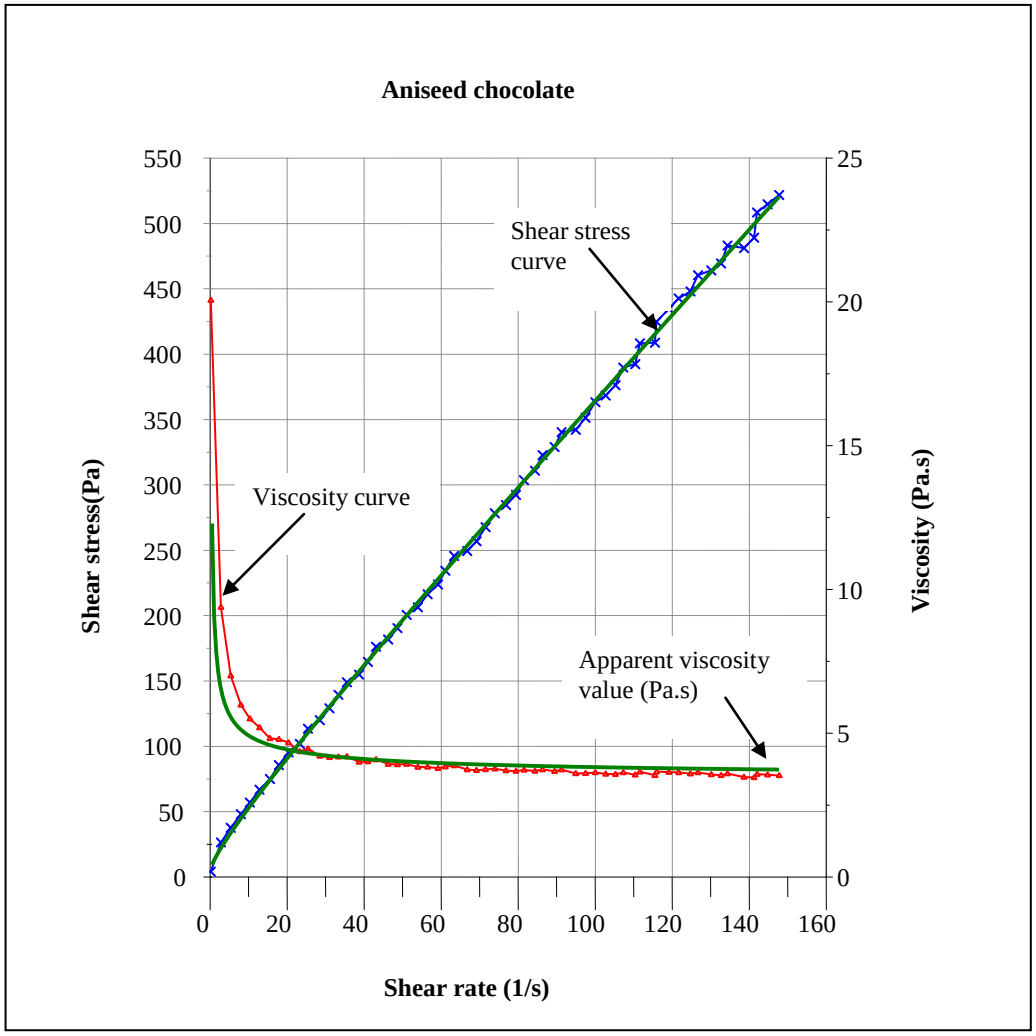

One Factor
viscosity
2.52
2.3775
2.235
2.0925
1.95
1.00
2.00
3.00
4.00
5.00
A: lemon powder


One Factor
texture
6.3
5.325
4.35
3.375
2.4
1.00
2.00
3.00
4.00
5.00
A: lemon powder

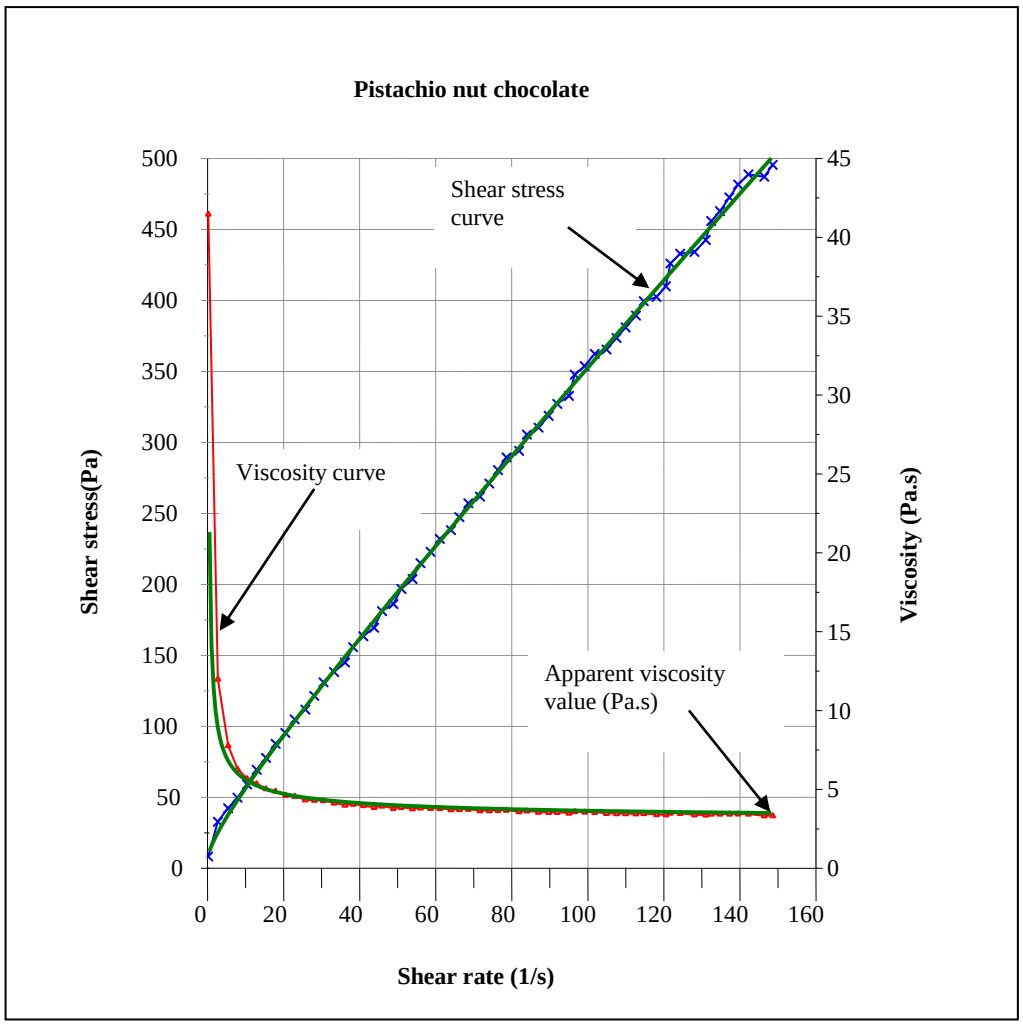




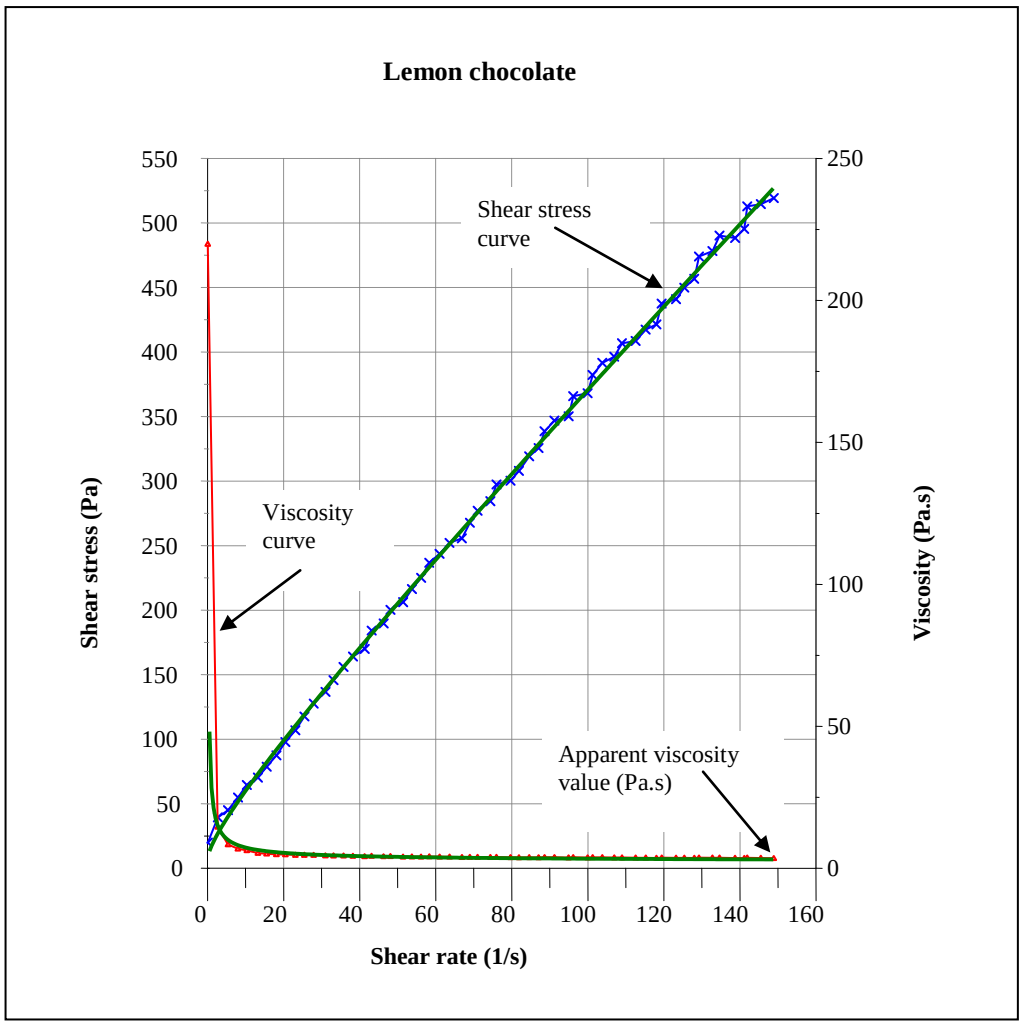
A30. Change in the viscosity, texture, total color and moisture of lemon chocolate



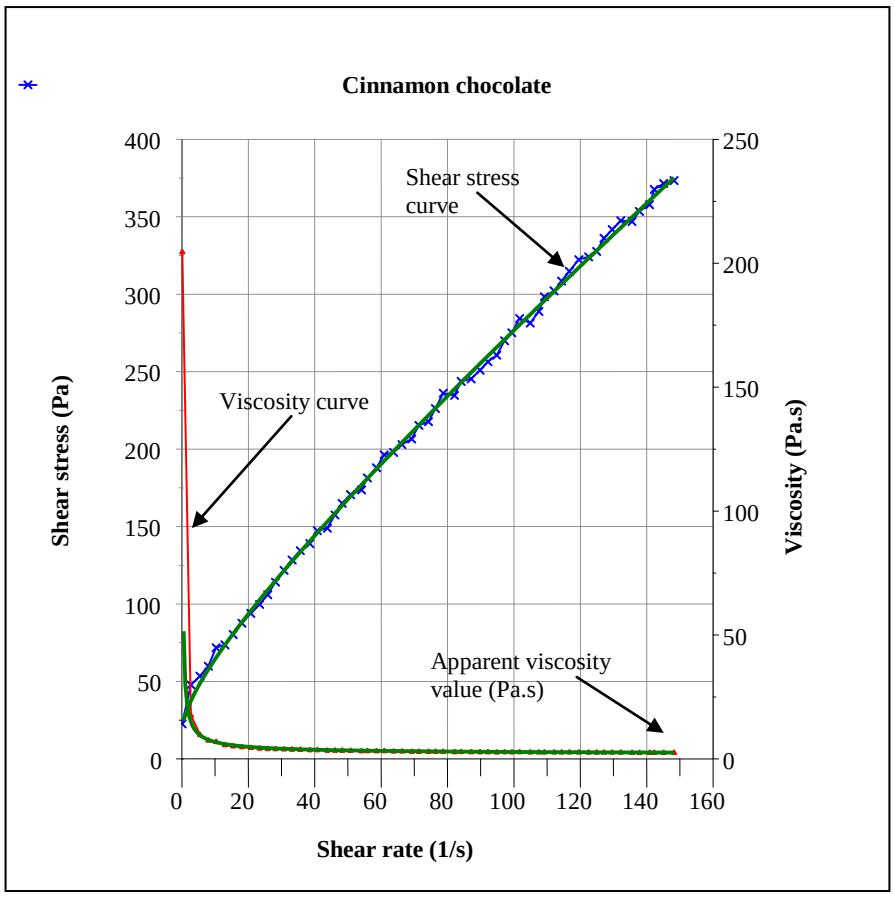
A31. Typical rheology graph illustrating measurement of apparent viscosity and yield stress for aniseed chocolate.



A32. Typical rheology graph illustrating measurement of apparent viscosity and yield stress for pistachio nut chocolate.



A33. Typical rheology graph illustrating measurement of apparent viscosity and yield stress for lemon chocolate.



A34. Typical rheology graph illustrating measurement of apparent viscosity and yield stress for cinnamon chocolate

A35. Panel Test

Taste									
	9	8	7	6	5	4	3	2	1
Bitterness									
Sweetness									
Chocolate									
Aniseed									
Smell									
	9	8	7	6	5	4	3	2	1
Chocolate odour									
Aniseed odour									
Texture									
	9	8	7	6	5	4	3	2	1
Coarseness									
Thickness									
Effort									
Hardness									
Softness									

Attribute	**Definition**
Sweetness	Taste associated with sucrose
Chocolate	Aromatic taste associated with chocolate liquor
Effort	Work required to melt,manipulate and swallow the sample
Coarseness	Perception of particles against the roof of the mouth
Thickness	Perception of the viscosity of the melted chocolate sample in the mouth

9- Super good; 8- Really good; 7-Good; 6-Just a little good; 5-Maybe good or maybe bad 4-Just a little bad; 3-Bad; 2-Really bad; 1-Super bad

CURRICULIM VITAE

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Master	University of Gaziantep	2008
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WORK EXPERIENCE

	Place	**Enrollment**
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FOREIGN LANGUAGE English

PUBLICATION

1. **Albak,F.,** Tekin, A.R. (2015). Effect of cinnamon powder addition during conching on the flavor of dark chocolate mass, *Journal of Food Science and Technology,* **52** Issue (4), 1960-1970.
2. **Albak,F.,** Tekin, A.R. (2014). Development of functional chocolate with spices and lemon peel powder by using response surface method, *Gıda Bilimi ve Teknolojisi Dergisi*, **12 (2),** 19-25.
3. **Albak,F.,** Tekin, A.R. (2014). The effect of addition of ingredient on physical properties of dark chocolate during conching, *Basic Research Journals,* **1(7),** 51-59.
4. **Albak, F.,** Belibağlı, B.(2011). Osmotic dehydration of cherry-part I. using general factorial design, *Journal of Food Science and Engineering,* **1(1)**,62-66.
5. **Albak, F.,** Belibağlı, B. (2010).Ozmotik dehidrasyon tekniğinin sakız kabağında kullanımı, *Gıda Bilimi ve Teknolojisi Dergisi,* **8 (2),** 6-10.
6. **Albak, F.,** Belibağlı, B., (2013). Ozmatik dehidrasyonun kirazın kurutma kinetiği üzerindeki etkisi, *Dünya Gıda Dergisi*,**3(3),** 82-89.

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2. **Albak, F.,** Konçlama boyunca tarçının çikolatanın aroması üzerindeki etkisi. The second international symposium on "Traditional Foods from Adriatic to Caucasus" October 24-23-2013 Struga-Ohrid/Macedonia page -652

3. **Albak, F.,** Baharatlarla aromatik çikolata üretimi. International Food Congress Novel Approaches in Food Industry 26-29 May 2014 kUşadası/Turkey page 127

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6. **Albak, F.,**Belibağlı, B. Part 2 Ozmotik olarak kurutulan kirazların difizyon katsayılarının belirlenmesi. 1. Internatioanal Congress on Food Technology 03-06 November 2010 Antalya/Turkey page320.

7. **Albak, F.,**Belibağlı, B. Yüzey tepki metodu kullanılarak ses üstü dalgalarının ozmotik kurutmaya uygulanması ve kabağın ozmotik olarak kurutulması esnasında kullanınlan sesüstü dalgalarının ve karıştırma işleminin kütle transferi üzerindeki etkisinin karşılaştırılmas Traditioanl Foods from Adriatic to Caucasus 15-17 April Tekirdağ/Turkey page 937.

8. **Albak, F.,**Belibağlı, B. Ozmotik dehidrasyonun kabak kurutulmasında kullanılması. International Food Congress Novel Approaches in Food Industry Nafi 2011 26-29 May 2011 page 1070

PROJECTS

Çikolata konçlama işlemi öncesi ve sonrası tat ve aroma değişimi (2013). BAP projesi (Araştırmacı). Proje Yürütücüsü: Prof. Dr. Ali Rıza Tekin.