DARK CHOCOLATE: EFFECTS OF PARTICLE SIZE DISTRIBUTION AND COMPOSITION ON PHYSICAL QUALITIES AND FLAVOUR VOLATILES RELEASE

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ABSTRACT

Rheological, texture and melting properties of dark chocolates were studied varying particle size distribution (PSD) [D_{90} (90% finer than this size) of 18, 25, 35 and 50 μm], fat (25, 30 and 35%) and lecithin (0.3 and 0.5%) contents. Fat pre-crystallisation systems during tempering and fat bloom development were also investigated, and influences on product microstructure, texture, appearance (colour and gloss) and melting characteristics elucidated. Flavour volatile release from varying product matrices were characterised and quantified. Rheological, textural and melting properties were examined quantitatively using Haake rheometer, texture analyzer and differential scanning calorimetry respectively; surface colour and gloss with a HunterLab Colorimeter and Gloss meter respectively; microstructures by light, stereoscopic binocular and scanning electron microscopy; and flavour volatiles quantified by GC-MS and GC-Olfactometry. Multivariate regression, correlation and principal component analyses were employed to explore interrelationships among the rheological, textural and melting characteristics.

The PSD, fat and lecithin contents significantly affected all rheological and textural parameters, with significant interactions among factors. Increasing particle size reduced all rheological and textural parameters with greatest effects noted with 25% fat and 0.3% lecithin, which reduced with increasing fat and lecithin contents. Statistical analyses revealed that fat exerted the greatest effect on variability in rheological, textural, melting properties and appearance followed by PSD and lecithin. Microstructures revealed wide variations in crystalline network structure and inter-particle interaction strengths related to PSD, fat content and temper regimes. Correlation (r = 0.78-0.99) and regression coefficients (R^2 = 0.59-0.99) suggested rheological, textural and melting index parameters

had inter-relationships predictive of character. Effects of temper regime were noted with varied influences on product character, and hypotheses proposed for mechanisms of bloom development in under-tempered products. Finally, flavour-active volatiles were characterised and their release quantified with data suggesting potential effects of matrix structure and lipophilic-flavour interactions.

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ABBREVIATIONS USED

AEDA	Aroma Extract Dilution Analysis
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
CBE	Cocoa Butter Equivalent
CBR	Cocoa Butter Replacer
CCRD	Central Composite Rotatable Design
cDNA	Complementary Deoxyribonucleic acid
CTU	Chocolate Temper Units
CSD	Crystal Size Distribution
DSC	Differential Scanning Calorimetry
EC	European Commission
EU	European Union
FID	Flame Ionisation Detection
GC	Gas Chromatography
GC-MS	Gas Chromatography-mass Spectrometry
GC-O	Gas Chromatography-Olfactometry
GMS	Glycerol Mono Stearates
GU	Gloss Units
HRGC	High Resolution Gas Chromatography
ICA	International Confectionery Association
ICCO	International Cocoa Organization
IOCCCC	International Office of Cocoa, Chocolate and Confectionery

NCA/CMA	National Confectioners Association/Chocolate Manufacturers Association
PC	Principal Component
PCA	Principal Component Analysis
PGPR	Polyglycerol Polyricinoleate
PS	Particle Size
PSD	Particle Size Distribution
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
SMP	Skimmed Milk Powder
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SPME	Solid Phase Micro Extraction
SSA	Specific Surface Area
TAGs	Triacylglycerols

CHAPTER 1

INTRODUCTION

1.1 HISTORY OF COCOA AND CHOCOLATE

'Cocoa' is a corruption of 'Cacao' taken directly from Mayan and Aztec languages (Awua, 2002). Chocolate is derived from cocoa beans, central to fruit of a tree *Theobroma cacao*. *Theobroma* (food of the gods) are of the family Sterculiaceae with two principal types: Criollo, about 5% world cocoa production; and the more common Forastero, with smaller, flatter and purple beans. A third variety, Trinitario, a more disease-resistant hybrid of Criollo and Forastero is regarded as a flavour bean (Fowler, 1999). *Theobroma cacao* grows between tropics of Cancer and Capricorn varieties originating in forest areas of South America. Forastero, basic, cocoa grows mainly in Brazil and West Africa; flavour cocoas, largely hybrids, in Central and South America. Aztecs in Mexico cultivated cocoa from South America, via Caribbean islands, and Hernandos Cortés, Spanish took cocoa to Spain as a beverage and to Spanish Guinea as crop. Currently West Africa produces >70% world cocoa (Amoye, 2006).

Use of cocoa beans dates back at least 1,400 years (Rössner, 1997), Aztecs and Incas using the beans as currency for trading or to produce so-called "chocolatl", a drink made by roasting and grinding cocoa nibs, mashing with water, often adding other ingredients such as vanilla, spices or honey. In the 1520s the drink was introduced to Spain (Minifie, 1989) although Coe and Coe (1996) emphasized that the Europeans arrivals in the New World, including Christopher Columbus and Herman Cortes were unimpressed with the Mayan beverage, sweetening it with honey. Nevertheless, conquistadors familiarised the chocolate beverage throughout Europe and being expensive, it was initially reserved for consumption by the highest social classes and only in the seventeenth century consumption of chocolate spread through Europe. In the UK in 1847, Joseph Fry was the first to produce a plain eating chocolate bar, made possible by introduction of cocoa butter as an ingredient (Beckett, 2000). Demand for cocoa then sharply increased, and chocolate processing became mechanised with

development of cocoa presses for production of cocoa butter and cocoa powder by Van Houten in 1828, milk chocolate by Daniel Peters in 1876 and the conche process by Rodolphe Lindt in 1880. Chocolate confectionery is now ubiquitous with consumption averaging 8.0 kg/person per annum in many European countries (Nuttall & Hart, 1999; Whitefield, 2005).

1.2 WORLD PRODUCTION AND CONSUMPTION OF COCOA AND CHOCOLATE PRODUCTS

1.2.1 World production and consumption of cocoa

Theobroma cacao originated in the Amazon Bason and optimal conditions for growth are 20 - 30°C (68-86°F), 1,500 - 2,500 mm of annual rainfall and 2,000 hours of sunshine per year. Table 1.1 shows density of production is centred within West Africa, accounting for ~ 71% of world cocoa production in 2005/2006 growing season. West African countries are ideal in climatic terms for growing cocoa as a cash crop. However, as a consequence, natural or man-made problems have potentially a disproportionately large impact upon cocoa trade. Small holders of West Africa have dominated world production since the 1930s. In 1980s, emergence of Malaysia and Indonesia gave more balanced geographical spread of production. However, a period of low prices wiped out Malaysia as a major producer and Brazil as a major exporter increasing share of production of West Africa. In 2007, 71% of world cocoa came from Africa: Cote d'Ivoire - 37.8%; Ghana - 19.9% (ICCO, 2008).

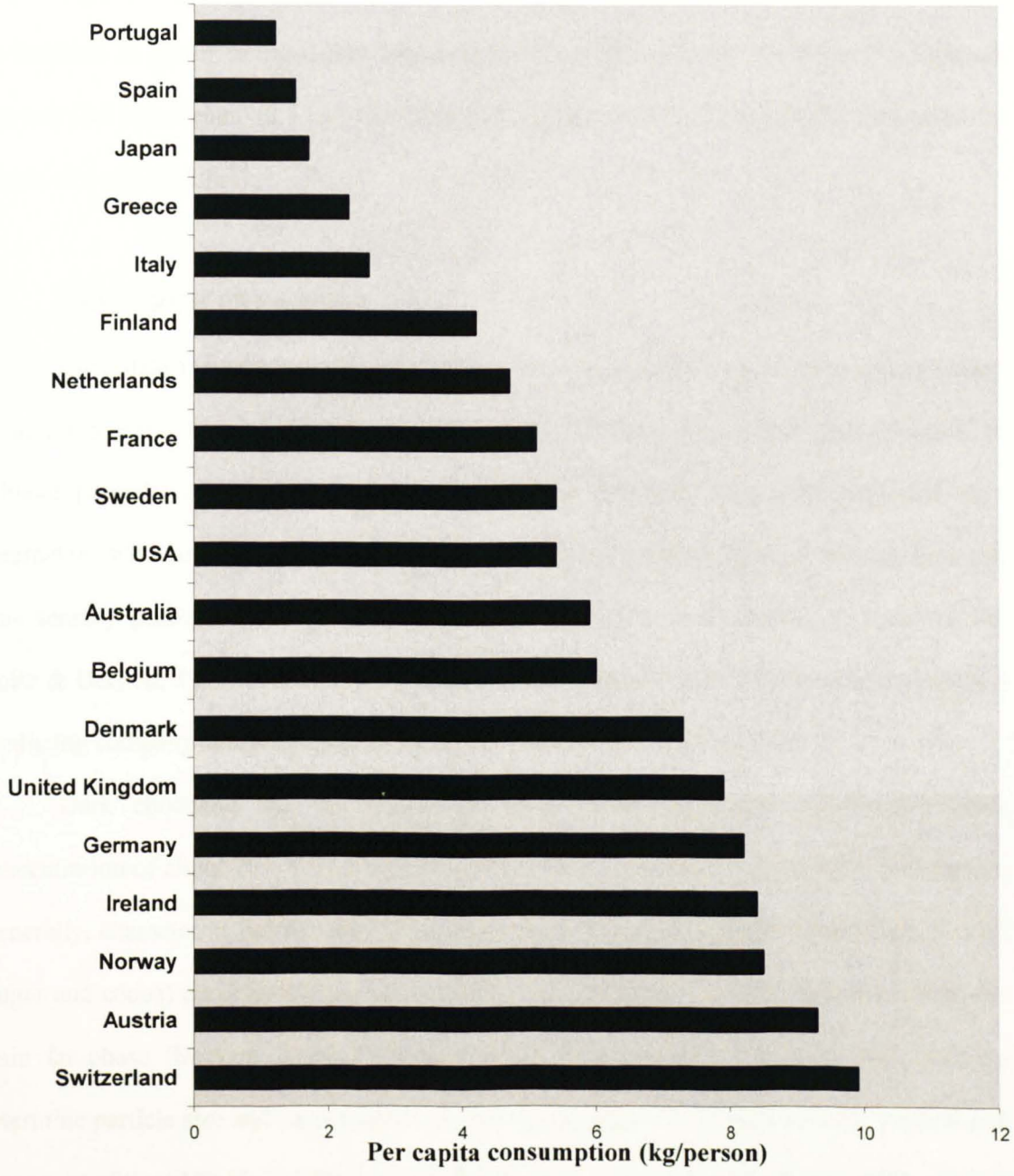
The International Cocoa Organization (2008) reported that the average consumption of cocoa beans in the world is 0.55 kg/person. Europeans consume most at 1.81 kg/person, followed by Americans (1.38 kg/person) with people of Africa and Asia/Oceania consuming only 0.14 and 0.11 kg/person, respectively. Belgium/Luxembourg had the highest *per capita* consumption of cocoa beans with 5.97 kg/person followed by Switzerland (5.28 kg), France

(3.90 kg), Germany (3.76 kg) and the United Kingdom (3.72 kg). Others include USA (2.72 kg), Slovenia (2.66 kg), Australia (2.65 kg), Croatia (2.14 kg), Japan (1.29 kg), Russia (1.24 kg), Brazil (0.53 kg), Cote d'Ivoire (0.49 kg), Ghana (0.47 kg) and China (0.03 kg).

Table 1.1. World Cocoa Production, 2005/2006

Country	'000 Metric Tons	Percentage (%)
World Total	3,724	100
Africa	2,642	71.0
Americas	446	12.0
Asia and Oceania	636	17.1
Cote d'Ivoire	1,408	37.8
Ghana	740	19.9
Indonesia	530	14.2
Nigeria	200	5.4
Cameroon	166	4.5
Brazil	162	4.4
Ecuador	114	3.9
Papua New Guinea	51	1.4
Dominican Republic	42	1.1

Source: International Cocoa Organisation [ICCO] (2008)



Source: International Confectionery Association [ICA] (2007)

Figure 1.1. Per capita consumption of chocolate confectionery by country

1.2.2 World consumption of chocolate products

Figures for consumption of chocolate products in 2006 (Figure 1.1) revealed Switzerland as leader in chocolate consumption at 9.9 kg/head with Austria at 9.3 kg/head, Norway (8.5 kg), Ireland (8.4 kg), Germany (8.2 kg) and the UK (7.9 kg). No African country comes close (ICA, 2007).

1.3 RESEARCH PREAMBLE

Chocolate manufacturing is complex: a number of physical and chemical processes require numerous technological operations and the addition of a range of ingredients, to achieve products of suitable physical and chemical attributes and appearance and taste parameters with prespecified ranges. Rheological characteristics, flavour development and thus sensory perception are all important (Ziegleder, 1997; Tscheuschner & Linke, 1998; Voltz & Beckett, 1997). Chocolate processing differs due to historical development within a producing company and geographical locations in which products are sold.

Dark chocolate can be categorised as a dense suspension of average solids concentration of about 70 – 75% (containing 40 – 60% non-fat solids from sugar and cocoa). Generally, character in finished dark chocolates depends on inherent size distribution of solid (sugar and cocoa) particles and composition of other ingredients, especially cocoa butter, the main fat phase (Beckett, 2000; Ziegleder, 1992). In manufacture, refining and conching determine particle size and suspension consistency and viscosity, to yield specific textural and sensory qualities (Minifie, 1987; Beckett, 2000). Dark chocolates with higher solids content and particle size distribution represent lower costs (savings in fat) for a manufacturer and lower caloric density products for the consumer. However, limits to solids concentration and particle size distribution are effects on rheological and sensory properties. Awua (2002) suggested that particle size distribution (PSD) was important in determining flow

(rheological) properties, but not the only factor influencing rheological characteristics. Research indicates a small proportion of particles up to 65 μm yields a suitable texture for milk chocolate, good dark chocolate requires a maximum of 35 μm. At solids concentrations >61% by volume and PSD exceeding 35 μm, chocolate becomes unacceptable due to high viscosity and poor texture (Minifie, 1989) but such figures are a bit arbitrary - affected by the nature of confection and added ingredient composition which requires further investigation.

To enhance acceptability, solids PSD and ingredients composition can be manipulated to modify flow behaviour and sensory attributes. However, this requires understanding of underlying principles and factors affecting changes in flow behaviour and inter-relationships with character in finished chocolates. General principles should be applicable to all dark chocolates. Requirements include information on system properties and compositional factors that contribute in manufacture to physical (rheological (flow) behaviour) and textural properties, appearance, melting characteristics, tempering (fat crystallisation) behaviours and other effects on product character attributes such as flavour volatiles release. Influences of varying PSD and ingredient composition and roles in determining character of dark chocolates during and after manufacture required in-depth investigation.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

The primary aim was to investigate effects of particle size distribution (PSD) and compositional variations on physical qualities and flavour volatiles release of dark chocolates.

1.4.2 Specific objectives

The study was divided into five broad specific objectives, each with impact on the character and thus quality of dark chocolates from industrial manufacture, namely:

i. Characterise effects of PSD and compositional variations on physical (rheological, textural and melting) properties and relationships with crystalline network microstructure of molten chocolate.

ii. Study tempering behaviour with varying PSD and fat content using response surface methodology.

iii. Evaluate effects of tempering and fat crystallisation behaviours on microstructure and quality attributes (mechanical properties, appearance and melting characteristics).

iv. Investigate structural changes and underlying hypothesis associated with fat bloom formation and development in under-tempered chocolates.

v. Characterise flavour volatiles and matrix effects on flavour release in chocolates varying in PSD and fat content using GC-Mass Spectroscopy and GC-Olfactometry.

1.4.3 Structure of thesis

The thesis has been split into ten chapters:

Chapter 1 introduces research aims and objectives and reviews structure of the thesis.

Chapter 2 reviews critically literature relevant to the research, assessing current information relating to influences of processing strategy, particle size distribution and ingredient composition on rheological and sensory character in chocolates, and how these contribute to texture and certain other sensory qualities in chocolates.

Chapter 3 is a critical review of literature discussing key factors in formation and development of cocoa flavour during post-harvest treatments and establishes development of individual flavour components and contributions to overall flavour character in chocolates.

Chapter 4 discusses results of original research investigating effects of particle size distribution and composition (fat and lecithin) on the rheological, textural, melting properties and appearance, and predictive inter-relationships in chocolates. It also describes crystalline

network microstructure of molten chocolates varying in PSD and fat content and establishes relationships with rheological and mechanical properties of chocolates during manufacture.

Chapter 5 discusses results of original research that studied tempering behaviours in dark chocolates varying in PSD and fat content using response surface methodology.

Chapter 6 describes results of investigations of fat crystallisation behaviours during tempering of dark chocolates, temper regimes attained and effects on quality attributes (mechanical properties, appearance, melting characteristics) and crystalline network microstructure of finished dark chocolates. It also characterises fat-sugar melting profiles of optimally-, over- and under-tempered (bloomed) dark chocolates, and discusses associated structural changes during post-tempering handling and storage of the derived products.

Chapter 7 discusses results of investigations of structural changes associated with fat bloom formation and development in under-tempered products and establishes hypotheses for fat bloom development during post-processing storage of chocolates.

Chapter 8 characterises flavour volatiles in dark chocolates and estimates respective contribution to overall flavour character including matrix effects on volatile release with varying PSD and fat content using GC-Mass Spectroscopy and GC- Olfactometry.

Chapter 9 summarises the conclusions and applications of this original research in the chocolate confectionery industry, and makes recommendations for further studies.

Chapter 10 is a compilation of all references cited in this thesis. All publications and papers presented at conferences from this study are provided as additional information.

CHAPTER 2

FACTORS INFLUENCING RHEOLOGICAL AND TEXTURAL QUALITIES IN CHOCOLATE – A REVIEW

This chapter has been published in *Trends in Food Science & Technology, Volume 18,* Issue 6, June 2007, Pages 290-298. (Afoakwa et al., 2007). doi:10.1016/j.tifs.2007.02.002.

Part of this review was orally presented at the Ghanaian Scholars UK (AGGOSS) Seminar organised by the Office of the Ghana High Commission in London, Highgate Hill, London, June 21, 2008.

2.1 ABSTRACT

Chocolate, a complex emulsion, is a luxury food that during consumption evokes a range of stimuli that activate pleasure centres of the human brain. Central to chocolate quality is an appropriate melting behaviour so that products are solid at ambient temperature and on ingestion melt to undergo dissolution in oral saliva, with a final assessment of texture after phase inversion. Particle size distribution and ingredient composition play important roles in shaping its rheological behaviour and sensory perception but are poorly understood. With opportunities for improvements in quality possible through improved and more transparent supply chain management, plant breeding strategies and new product development, associated with fair trade and development of niche premium quality products, there is a need for greater understanding of variables.

Table 2.1 Definition of some technical words used

Technical word used	Definition
Bloom	Fat or sugar on the surface of chocolate giving it white sheen or sometimes individual white blobs.
Cocoa Butter Equivalent (CBE)	Vegetable fats totally compatible with cocoa butter and can be mixed with it in proportions stipulated by regulation.
Cocoa Butter Replacer (CBR)	Vegetable fats that may be mixed with cocoa butter but only in a limited proportion by regulation.
Cocoa nib	Cocoa cotyledon, bean with shells removed.
Cocoa liquor, cocoa mass	Roasted, ground cocoa nibs
Origin liquor	Cocoa mass manufactured in country of bean origin.
Non-Newtonian liquid	A liquid such as molten chocolate whose viscosity varies according to rate of stirring (shear).
Plastic viscosity	Amount of energy required to keep a non-Newtonian liquid moving once motion has been initiated.
Yield value	Amount of energy required to initiate motion in a non-Newtonian liquid, e.g. molten chocolate.
Casson equation	$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} \cdot \sqrt{\dot{\gamma}}$ Variable definitions: τ: yield stress; τ_{CA}: Casson yield stress; μ_{CA}: Casson viscosity; and $\dot{\gamma}$: shear rate

2.2 INTRODUCTION

Chocolates are semi-solid suspensions of fine solid particles from sugar and cocoa, about 70% total, in a continuous fat phase. Cocoa solids are derived from beans obtained from the fruit of *Theobroma cacao*, with world production dominated by Forastero types, made up of small, flattish and purple beans. Another type, Criollo, is presently rare in production; Trinitario, a disease-resistant hybrid of Criollo and Forastero, regarded as a flavour bean (Awua, 2002), is about 3% of world production. Growth of Forastero, in the trade name basic or bulk cocoa, occurs mainly in West Africa and Brazil. Criollo (flavour cocoa), is largely grown in Central and South America. West Africa now produces over 70% of world cocoa (Amoye, 2006). New demand for Fairtrade and premium products has stimulated improvements in quality assurance that make possible single variety and origin chocolates (Table 2.2).

Primary chocolate categories are dark, milk and white that differs in content of cocoa solid, milk fat and cocoa butter. The outcome is varying proportions of carbohydrate, fat and protein (Table 2.2). Chocolate manufacturing processes (Beckett, 2000; Awua, 2002; Whitefield, 2005) differ due to variation in national consumer preferences and company practices.

Table 2.2 Dark, milk and white chocolate: major constituents

Product	Carbohydrate (%)	Fat (%)	Protein (%)
Dark chocolate	63.5	28.0	5.0
Milk chocolate	56.9	30.7	7.7
White chocolate	58.3	30.9	8.0

Source: Chan et al. (1994)

Central to sensory character is continuous-phase lipid composition, which influences mouthfeel and melting properties. Chocolate triglycerides are dominated by saturated stearic (34%) and palmitic (27%) fatty acids and monounsaturated oleic acid (34%). Chocolates are solid at ambient (20-25 °C) and melt at oral temperature (37 °C) during consumption giving a smooth suspension of particulate solids in cocoa butter and milk fat (Beckett, 1999; Whitefield, 2005). This constrains lipid composition. The oral epithelia are also sensitive to gradations of smoothness which selects for desirable lipid crystal forms.

Despite high lipid and sugar contents, chocolate consumption makes a positive contribution to human nutrition through provision of antioxidants, principally polyphenols including flavonoids such as epicatechin, catechin and notably the procyanidins. White chocolates differ from milk and dark through the absence of cocoa nibs containing antioxidants, reducing product shelf-life (Beckett, 2000; Whitefield, 2005). Chocolates also contain minerals, specifically potassium, magnesium, copper and iron (Holland et al., 1991). Differences in the sensory characters of chocolate can be attributed to: use of different cocoa types, variations in ingredient proportions, use of milk crumb instead of milk powder, blending techniques and processing methods. Specifications depend on type of chocolate and its intended use (Jackson, 1999).

As chocolates melt in the mouth the continuous fat phase inverts into the oral continuous aqueous phase mixing with saliva that dissolves the sugar particles. Lipids and cocoa solids coat oral epithelial surfaces. Oral particle dissolution influences perception of coarseness and solvation at rates corresponding to size and work input such as mastication, tongue compression and swallowing (Lee & Pangborn, 1986). Particle size distribution and ingredient composition therefore influence perception of primary taste (gustation) and oral volatiles release with retronasal flavour characters in magnitude and temporal profile.

Rheological properties of chocolate are important in manufacturing process for obtaining high-quality products with well-defined texture (Servais et al., 2004). Chocolates with high viscosity have a pasty mouthfeel, persisting in the mouth (Beckett, 2000). Viscosity relates to composition, processing strategy and particle size distribution. Apparent viscosity in aqueous solutions influences flavour 'by-mouth' and taste intensity during consumption (Denker et al., 2006), thus rheological measurements often give information related to sensory character of chocolate.

This paper assesses current information relating processing strategy, particle size distribution and ingredient composition to rheological and sensory qualities in chocolates.

2.3 The initial stages of the chocolate manufacturing processes

In cocoa pods, 30 to 50 white pulp-covered seeds reach maturity after 4 to 6 months and contain two cotyledons (nibs) that yield cocoa mass for chocolate manufacture or when pressed, cocoa butter and cocoa powder (Fowler, 1999; Whitefield, 2005). Chemical reactions during processing for cocoa solids are complex contributing to final flavour and textural properties and have been reviewed (Beckett, 2000; Awua, 2002; Minifie, 1989).

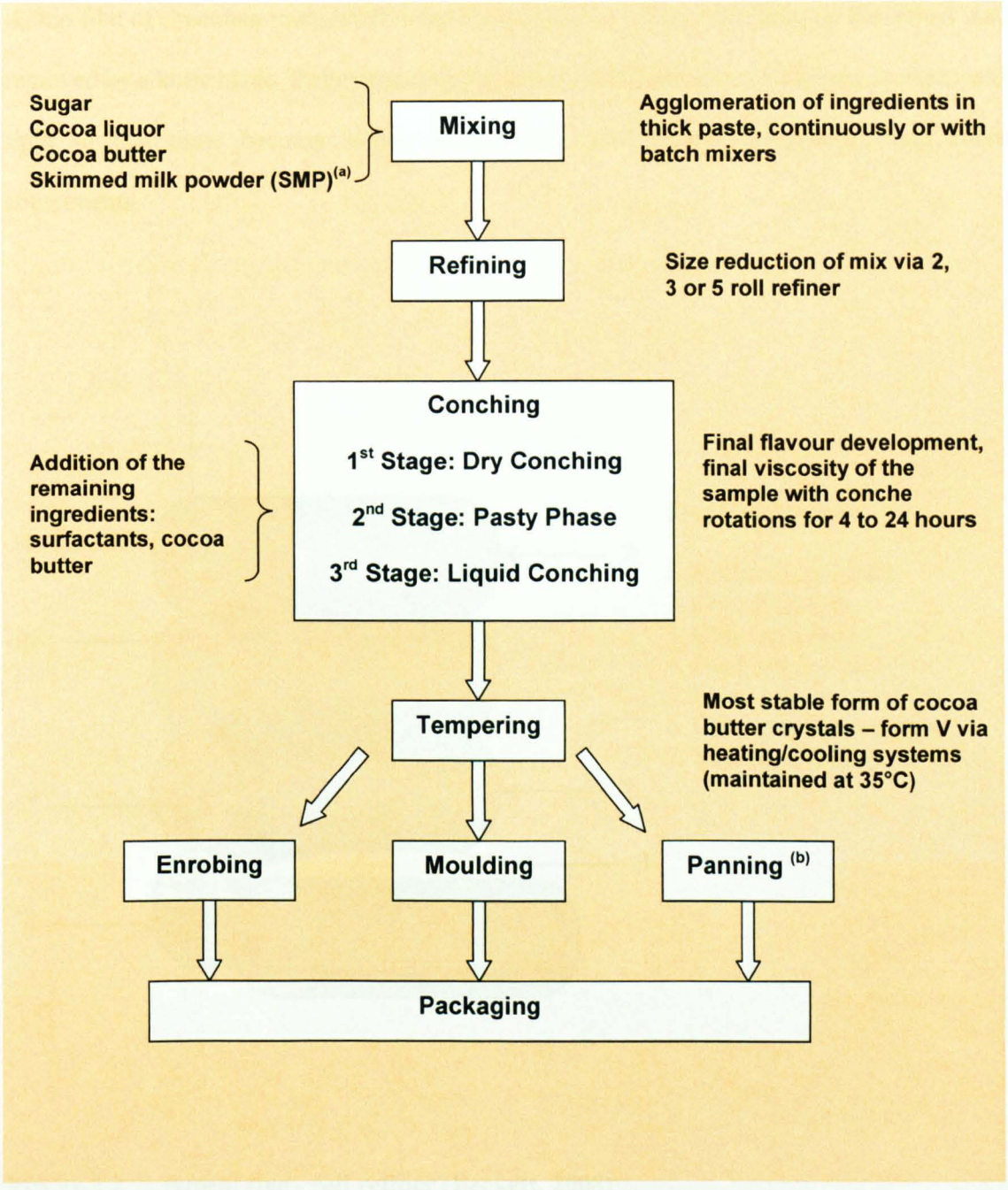
Enzymic and microbial fermentations after harvest induce physical and chemical changes in beans over 5 to 7 days (Fowler, 1999) with key browning reactions of polyphenol with proteins (ca.12–15% total) and peptides giving colours characteristic of cocoa. Fermentation reactions have been reviewed by Fowler (1999) and Beckett (2000). Drying limits mould growth during transportation and storage, reducing bean moisture content from 60% to 8%. Sun drying is favoured for flavour development and can be carried out above or on hard surfaces, with differences in air flow (Amoye, 2006) and final moisture content (Fowler, 1999). Beans are transported under controlled storage conditions to chocolate manufacturing

sites, or processed in the origin country to add value with requirements for traceability in quality assurance (Whitefield, 2005).

Beans are roasted before or after winnowing with shells broken by high-speed impact against metal plates. Heat transfer in nib roasting is facilitated by grinding of cotyledons. Liquor roasting (Awua, 2002) uses a prior liquefaction. In roasting moisture contents fall to <3% and Maillard reactions of amino acids, from fermentation protease activities, yield flavour-active aldehydes with chocolate notes. Fermentation-derived volatile acids are also removed influencing astringency and taste. Roasting temperature (90 – 170°C) and time influence nib composition, as does rate of moisture loss and whether moist or dry roasted (Awua, 2002; Whitefield, 2005). Grinding of nib cells releases cocoa butter into liquor with particle size up to 30 μm and for production of cocoa powder, fine grinding is particularly important. Typically ca. 78-90% cocoa butter is collected by pressing; residual lipids may be removed by supercritical fluid extraction (Beckett, 2000).

Chocolate manufacturing processes generally share common features (Figure 2.1) such as mixing, refining and conching of chocolate paste. The outcome sought is smooth textures of products considered desirable in modern confectionery and elimination of oral perceptions of grittiness.

Chocolates contain cocoa liquor, sugar, cocoa butter, milk fat and milk powder (depending on product category). A mix of sugar, milk solids and cocoa liquor at an overall fat content of 8-24% is refined to particle size <30 μm normally using a combination of two and five-roll refiners (Beckett, 1999; Beckett, 2000). Final particle size critically influences the rheological and sensory properties. A five-roll refiner (Figure 2.2) consists of a vertical array of four hollow cylinders, temperature controlled by internal water flow, held together by hydraulic pressure.



Note: (a) Skimmed milk powder is only used in milk chocolate manufacture;
(b) Panning means that the chocolate is used as coating for hard centres such as nuts.

Figure 2.1. Processing steps for chocolate manufacture

A thin film of chocolate is attracted to increasingly faster rollers, travelling up the refiner until removed by a knife blade. Roller shearing fragments solid particles, coating new surfaces with lipid so that these become active, absorbing volatile flavour compounds from cocoa components.

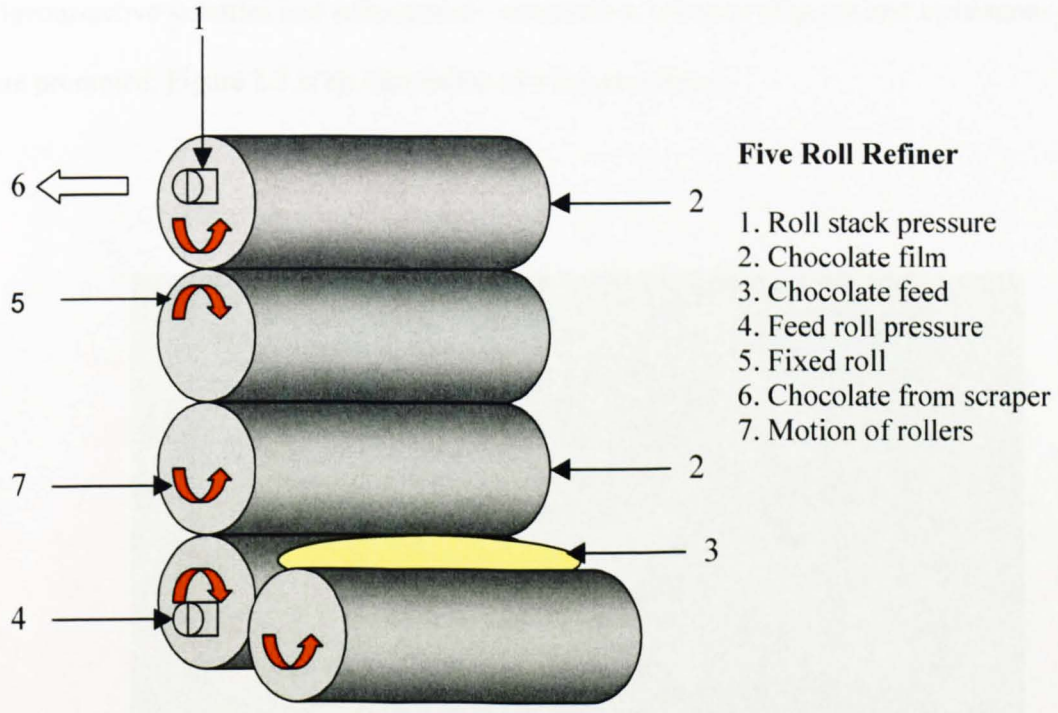


Figure 2.2. A typical five - roll refiner (Beckett, 2008)

Texture in milk chocolate appears improved by a bimodal distribution of particles with a small proportion having sizes up to 65 μm. Optimum particle size for dark chocolate is lower at <35 μm although values are influenced by product and composition (Awua, 2002).

Refiners, in summary, not only effect particle size reduction, and agglomerate breakdown but distribute particles through the continuous phase coating each with lipid.

Refined mixtures then move into conching, a process that contributes to development of viscosity and final texture and flavour. This is the endpoint for manufacture of bulk chocolate. Conching is normally carried out by agitating chocolate at >50°C for some hours (Beckett, 2000). In the early stages moisture is reduced with removal of certain undesirable flavour-active volatiles and subsequently interactions between disperse and continuous phase are promoted. Figure 2.3 is an illustration of a Frisse conche.

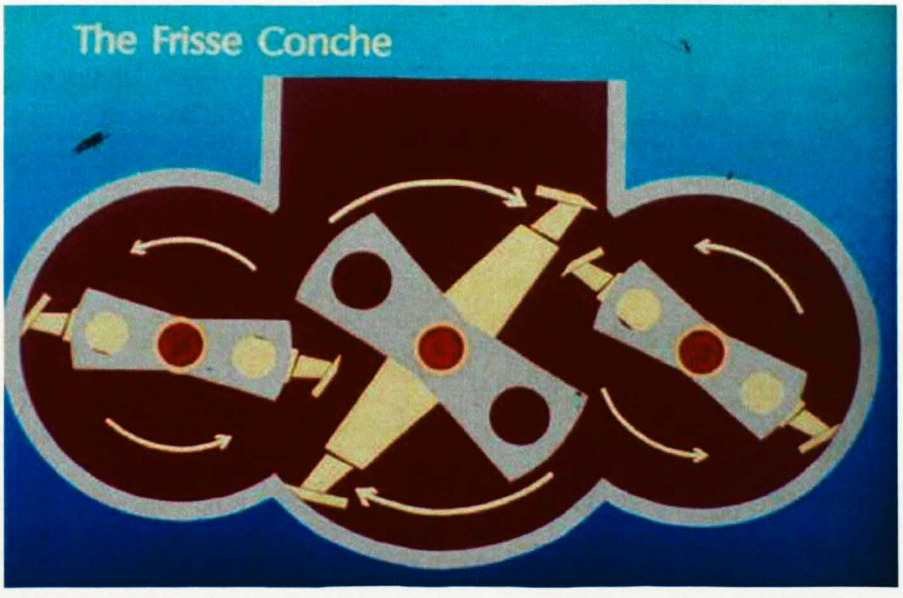


Figure 2.3. Frisse conche (Beckett, 2008)

Conching times and temperatures vary (Awua, 2002) typically: for milk crumb - 10 to 16 h at 49 - 52°C; with milk powder products, 16 to 24 h at up to 60°C; and with dark chocolates at 70°C and continue up to 82°C. Replacing full-fat milk powder with skimmed milk powder and butter fat, temperatures up to 70°C may be used (Awua, 2002). To give

chocolate a suitable viscosity, additional cocoa butter and lecithin can be added towards the end of conching to thin chocolate prior to tempering (Beckett, 2000; Whitefield, 2005).

2.4 Lipid crystallisation and continuous phase character during chocolate manufacture

Cocoa butter can crystallise in a number of polymorphic forms as a function of triglyceride composition, with fatty acid composition influencing how liquid fat solidifies (Awua, 2002). Cocoa butter has six polymorphic forms (I – VI), the principals being α, β and β' (Table 2.3). Form V, a β polymorph, is the most desirable form (in general) in well-tempered chocolate, giving a glossy appearance, good snap, contraction and resistance to bloom (Beckett, 2000).

Table 2.3. Melting point and chain packing of the polymorphic forms of cocoa butter

Polymorphic forms of cocoa butter		Melting point (°C)	Chain packing
Form I	β'_2	16-18	Double
Form II	α	21-22	Double
Form III	Mixed	25.5	Double
Form IV	β_1	27-29	Double
Form V	β_2	32-34	Triple
Form VI	β'_1	34-36	Triple

Source: Talbot (1999)

If chocolate is poorly tempered, the outcome is the β Form IV which rapidly transforms into Form V. This influences colour as reflected light is disoriented by unstable, disorganised crystal growth (Hartel, 2001). Untempered chocolate is soft and not effectively demoulded. In cocoa butter Forms V and VI are the most stable forms. Form VI is difficult to generate although formed on lengthy storage of tempered chocolate accompanied by fat bloom. In addition Form VI has a high melting temperature (36°C), and crystals that are large and gritty on the tongue. The unstable Form I has a melting point of 17°C and is rapidly converted into form II that transforms more slowly into III and IV. Polymorphic triglyceride forms differ in distance between fatty acid chains, angle of tilt relative to plane of chain end methyl group and manner in which triglycerides pack in crystallisation (Talbot, 1999).

Polymorphic form is determined by processing conditions. Fatty acids crystallise in a double- or triple-chain form depending on triglyceride composition and positional distribution. Form IV crystallises in a double-chain form, Form V in a triple-chain system that enables closer packing and greater thermodynamic stability. Unstable lower polymorphic forms (II and III) transform into higher melting, more stable forms, with closer packing and lower volume. These changes can be observed in terms of overall contraction of the chocolate, appearance, or undesirable fat bloom formation at rates dependent on relative stabilities of the polymorphic forms and temperature (Talbot, 1999). For chocolate to be in an appropriate polymorphic form, tempering is crucial, influencing final quality characteristics such as colour, hardness, handling, finish and shelf-life characteristics.

Tempering involves pre-crystallisation of a small proportion of triglycerides, with crystals forming nuclei (1 – 3% total) for remaining lipid to set in the correct form. Tempering has four key steps: melting to completion (at 50°C), cooling to point of crystallisation (at 32°C), crystallisation (at 27°C), and conversion of any unstable crystals (at 29-31°C) (Talbot, 1999) (Figure 2.4). Tempering sequence is a function of recipe, equipment, and the final

purpose. Before the use of tempering machines, chocolate used to be hand-tempered, and this method is still occasionally used by Chocolatiers, who produce relatively small quantities of hand-made confections. Current tempering machines consist of multistage heat exchangers through which chocolate passes at widely differing rates making it difficult to identify optimum conditions. Time-temperature combinations are of paramount importance in process design and in continuous tempering, molten chocolate is usually held at 45°C then gently cooled to initiate crystal growth.

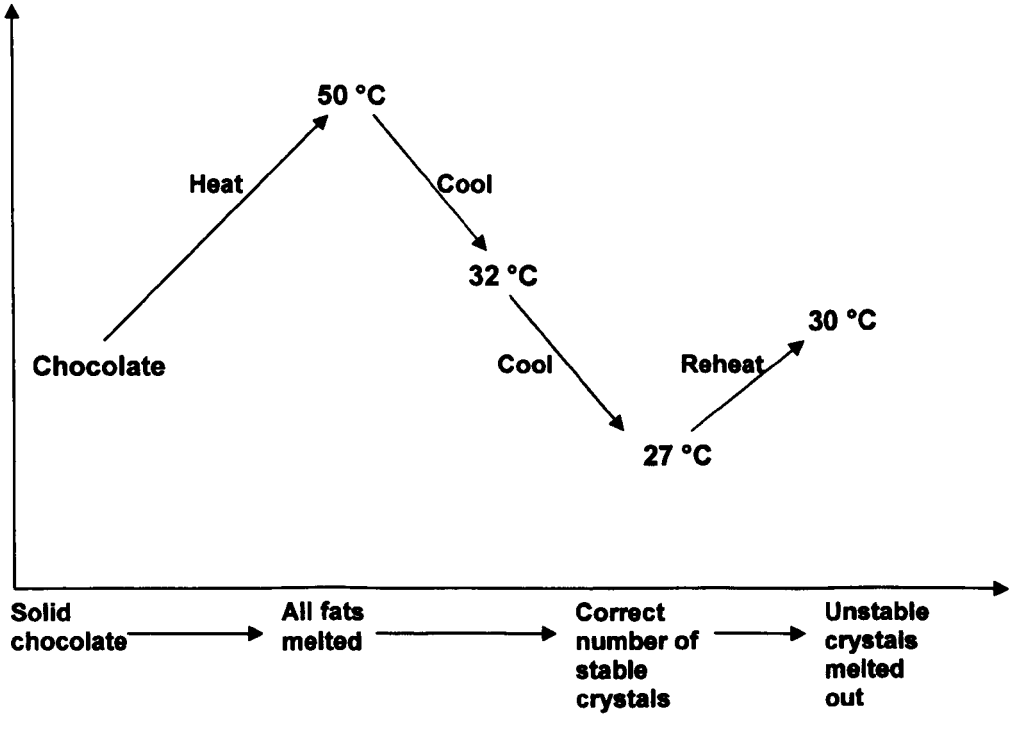


Figure 2.4. Tempering sequence during lipid crystallisation in chocolates

Working with the Buhler "Masterseeder", Windhab (ETH Zurich, Switzerland) and Mehrle (Buhler AG, Uzwil, Switzerland) found that high shear seed tempering can be beneficial as the kinetics of fat crystal nucleation and polymorphic transformations ($\alpha \rightarrow \beta_2 \rightarrow \beta'_1$) are strongly accelerated by shear forces acting in high shear flow fields: overall quality of products was better, as fat bloom was reduced (Windhab et al., 2002). During tempering, the temperatures are precisely controlled and the agitation provided enhances nucleation rates. As the viscosity increases, the chocolate is reheated again in the third stage to prevent runway solidification. In the fourth stage, crystals are matured.

Chocolate can also be tempered by the use of high pressure (Yaseda & Mochizuki, 1992) with molten chocolate compressed to 150 bar. This increases chocolate melting point and causes it to solidify into solid crystals of all polymorphic forms. When pressure is released, lower polymorphic forms melt leaving behind tempered chocolate. Subsequent batches can be seeded with stable fat crystals.

A well tempered chocolate will have the following properties; good shape, colour, gloss, contraction from the mould, better weight control, stable product - harder and more heat resistant (fewer finger marks during packaging) and longer shelf-life. The tempering regime for milk chocolate slightly differs from that for dark due to the influence of milk fat molecules on crystal lattice formation (Haylock & Dodds, 1999). Milk chocolate contains a proportion of butter fat that causes an eutectic effect, which prevents bloom formation, results in a lower melting point, softening of texture and lowering of temperature to obtain crystal seed for the tempering process (around 29.4°C compared to 34.5°C for plain chocolate). Cocoa butter equivalents (CBEs) and replacers (CBRs) may also find application in the chocolate industry. While cocoa butter equivalents are compatible with cocoa butter, cocoa butter replacers (CBRs), which do not require tempering, can only be used if almost all the cocoa butter is

replaced. These CBRs melt in the same temperature range as cocoa butter, but crystallise only in the β' form (Talbot, 1999; Whitefield, 2005).

More recently, the effect of shear on chocolate or cocoa butter tempering has been studied in a number of different flow geometries, for example, scraped surface heat exchanger with cocoa butter and chocolate (Bolliger et al., 1999), Couette geometry with milk chocolate (Stapley et al., 1999) and cocoa butter (Mazzanti et al., 2003), cone and plate system with cocoa butter (MacMillan et al., 2002; Dhonsi & Stapley, 2006), parallel plate viscometer with milk chocolate (Briggs & Wang, 2004), and a helical ribbon device with cocoa butter (Toro-Vazquez et al., 2004).

2.5 Particle size distribution in chocolate

Particle size distribution is a key determinant of the flow (rheological) properties in chocolates with a direct influence on sensory perception. Beckett (2000) concluded that the largest particles are important for mouth-feel with respect to grittiness, but the smaller ones are more important with respect to chocolate flow properties. Traditionally, continental European chocolate has been described as having a fineness of 15-22 μm particle diameter, and that in North America 20-30 μm (Jackson, 1999). However, with increased globalisation of the industry, traditional differences have began to blur with specifications becoming much more product specific.

Particle size distribution has been used as a tool to control consistency of solid-liquid mixtures to aid pumping and mixing of molten milk chocolate (Mongia & Ziegler, 2000), transportation, atomisation, and grinding of foods of high solid content in milk suspensions (Saeseaw et al., 2005), and D-limonene (Soottitantawat et al., 2005). Malvern Instrument identified applicability of their laser diffraction instrument for nearline chocolate process control, indicating the importance of particle size distribution for fluidity control.

Understanding and control of factors influencing fluid performance during high solid content processing is necessary with increasing competitiveness in modern chocolate manufacturing processing (Servais et al., 2002).

Optimisation of particle size distribution in chocolate requires consideration of palate sensitivity. For example, there is a maximum particle size of 30 μm, or a product is perceived as 'gritty or coarse' in the mouth. Particle size affects viscosity as well as texture, and a chocolate milled to a maximum particle size of 20 μm will have a creamier taste and texture than that with 30 μm. Particle size distribution plays clear roles in process fluidity, but is generally restricted to experienced-based empirical knowledge (Beckett, 2000). Several clear examples of particle size distribution optimisation show improvement in process efficiency and/or yield in food manufacture. In apple sauces (Missaire et al., 1990) and in mustard (Aguilar et al., 1991) bimodal particle size distribution promoted viscosity reduction and better mixing yielding improvements in final product shear, time and temperature stability. Villagran et al. (1996) patented a process for reduced fat nut spreads. The process results in bimodal particle size distribution and reduction of spread viscosity, allowing the low fat spread to display the "desirable fluidity, texture and flavour".

A widely appreciated example of a solid suspension is chocolate, a polydisperse suspension of sugar, cocoa and/or milk solids in a Newtonian fluid (fat phase), hence the applicability of Casson's equation (Table 2.1) can model chocolate flow behaviour (Beckett, 2000) where solid content varies from 65% to 75%. Many chocolate products have bimodal and trimodal particle size distributions. A typical particle size distribution of commercial enrobing mass is given in Figure 2.5. In bimodal distributions, minima are generally located around 15-25 μm.

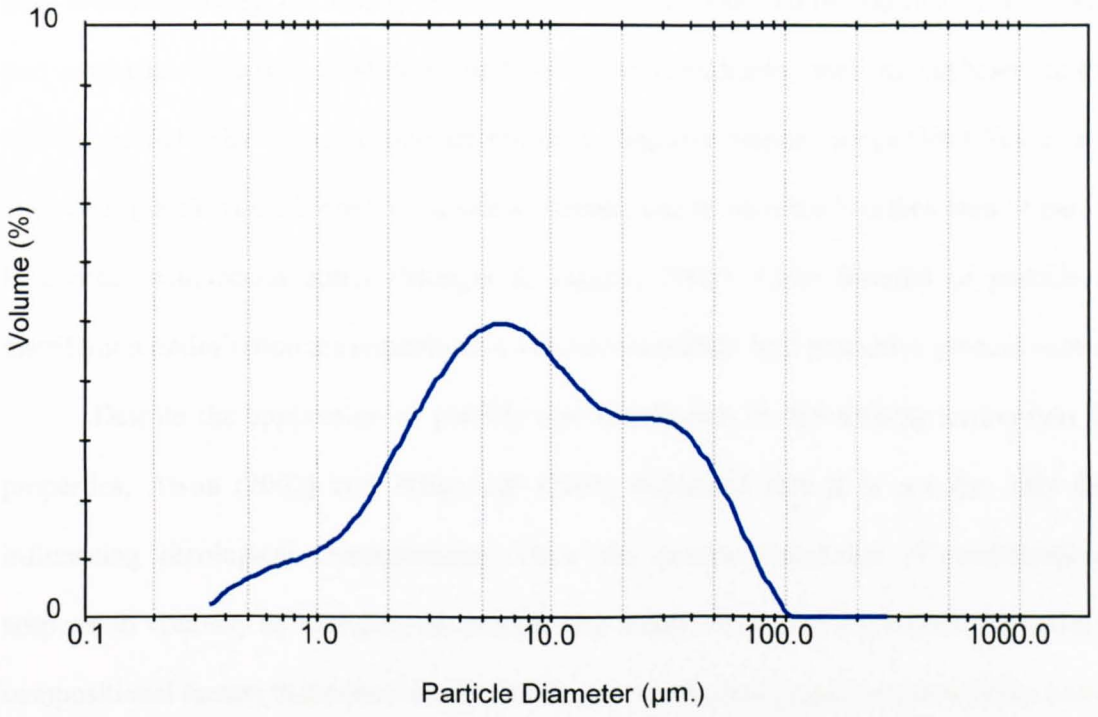


Figure 2.5. Particle size distribution of commercial enrobing mass during chocolate manufacture (Afoakwa et al., 2008a)

Particle size distribution influences chocolate rheology (Chevalley, 1999), with specific surface area and mean particle size influencing yield stress (Beckett, 2000). Bouzas and Brown (1995) noted that "a chocolate with particles sized according to the infinite modal distribution may give the lowest plastic viscosity". Aguilar and Ziegler (1995) employed a bimodal particle size distribution for a controlled reduction in viscosity. Servais et al. (2002) reported that in blends of chocolates of fine ($d_{4,3}$ = 8.5 µm) and coarse ($d_{4,3}$ = 17.0 µm) particles, varying the blend ratio, influenced relationship between packing fraction and the shear viscosity with yield value closely related to mean particle diameter and particle specific surface area but not packing fraction. A ratio of 60% coarse particles to 40% fine particles

gave lowest viscosity. Generally, chocolate viscosity is controlled by addition of cocoa butter and expensive viscosity modifiers (surface active ingredients, such as soybean lecithin). Smaller particle sizes in chocolate are known to improve sensory properties (Ziegler et al., 2001) but plastic viscosity and yield stress increase due to increased surface area of particles in contact with cocoa butter (Mongia & Ziegler, 2000). Clear benefits of particle size distribution optimisation are reductions in viscosity modifiers and predictive process control.

Despite the application of particle size distribution in determining suspension flow properties, Awua (2002) and Whitefield (2005) explained that it is not the only factor influencing rheological characteristics. Thus, the general principles of modification of suspension viscosity by changing the particle size require review of a system's properties and compositional factors that contribute to the changes in physical properties, flow behaviour and sensory character of chocolate.

2.6 Compositional effects on rheological and textural qualities in chocolate

2.6.1 The role of fats

Cocoa nibs consist of about 55% butter which constitutes around 30% of the final chocolate. Cocoa butter triglycerides have saturated fatty acids at the 1,3-positions and oleic acid at the 2-position. Fatty acid contents are around oleic (35%), stearic (34%) and palmitic acid (26%) with in addition polar lipids, sterols, and tocopherols (Talbot, 1999) each depending on factors such as growing conditions and origin. The simple glyceride composition makes chocolate melt over the temperature range of 23 to 37°C. The lipid crystal Form V (β_2) is the desirable form in chocolate production and dominant in well tempered chocolate (Beckett, 2000; Whitefield, 2005).

Some vegetable fats are similar to cocoa butter in triglyceride composition and such cocoa butter equivalents (CBEs) can be added in any proportion to chocolate without causing

a significant effect on texture. Legally such vegetable fats are permitted up to 5% in the EU for a product to be sold as chocolate (Cocoa and Chocolate Products Regulations, 2003). Cocoa butter replacers (CBRs) such as the lauric fats, palm kernel or coconut oils, crystallise only in one crystal form, β', in a very different way and are used to totally replace cocoa butter (Talbot, 1999). Low-caloric fats such as caprenin, which contain fatty acids different from cocoa butter, and are poorly absorbed by the gut, also find application as CBRs. With non-lauric fats, some cocoa butter can be used (Babin, 2005) and the mix can be tempered normally.

Most chocolates contain between 25% and 35% fat, although ice-cream coatings are much higher and some special products like cooking chocolate and vermicelli pieces are lower in fat. The actual level present will depend on the process being used and this affects the texture of the finished chocolate, so a high quality tablet of chocolate is likely to have a higher fat content and a lower particle size than a chocolate that is used to coat biscuit (Beckett, 2000). The effect of an extra 1% of fat upon the viscosity depends upon the amount already present and the viscosity parameters being considered. Above fat content of 32% there is very little change in viscosity with any further additions. A 1% increase to a 28% fat content has a really dramatic effect especially on the plastic viscosity, which is almost halved. The change becomes more dramatic at even lower fat contents as 'chocolates' below 23% fat are normally a paste rather than a liquid (Beckett, 2000).

The effect of fat is proportionately much higher for the plastic viscosity than the yield value. Beckett (2000) explained that this phenomenon is not surprising as the extra fat only adds to the free moving fat that aids particles when they flow past each other. The majority of the fat is 'wetting' fat, which is partially tied to the particle surfaces. This free fat has a large effect on lubricating the flow when it takes place and so the plastic viscosity decreases dramatically. The yield value is more pronounced with the forces between the solid particles,

which in turn are connected with the absolute distance between them, hence their less effect with fat additions.

2.6.2 The role of sugar

Sugar is considered an inert ingredient in chocolate with regard to subtleties of flavour, contributing "only" to sweetness. A change of 1-2% in sugar content has a great effect on costs and other economic factors, and at 5% change large flavour changes become apparent (Beckett, 1999). Fine crystalline sucrose is utilized at up to 50% in chocolate confectionery (Krüger, 1999). Lactose, in milk solids, is present at lower levels in an amorphous form and in its glassy state holds a proportion of milk fat (Beckett, 2000), influencing chocolate flavour and flow properties. Lactose enhances the browning by participating in Maillard reactions (Krüger, 1999; Bolenz et al., 2006). Monosaccharides, glucose and fructose, are rarely used in chocolate as they are difficult to dry. Consequently, the additional moisture present in chocolate would increase interactions between sugar particles, and increase viscosity. Dextrose and lactose can successfully replace sucrose in milk chocolate (Müller, 2003; Bolenz et al., 2006).

In recent years, sucrose-free chocolates have become popular among consumers and manufacturers because of reduced calorific values, and the fact that these are both non-cariogenic and suitable for diabetics (Zumbe & Grosso, 1993; Olinger, 1994; Olinger & Pepper, 2001; Sokmen & Gunes, 2006). Sugar alcohols, including xylitol, sorbitol, mannitol and lactitol are used for the manufacture of lower-calorie or sugar-free products. Replacement of sucrose with sugar alcohols however affects rheological properties and thus the processing conditions and quality of chocolates (Zumbe & Grosso, 1993; Sokmen & Gunes, 2006; Wijers, & Sträter, 2001). Sokmen and Gunes (2006) noted that maltitol results in similar rheological properties of chocolate to sucrose, and thus may be recommended as a good

alternative to sucrose in chocolate formulations. These authors also observed that chocolate with isomalt resulted in higher plastic viscosity while xylitol causes higher flow behaviour index. Polydextrose may be added as an edible carbohydrate and intense sweeteners used. The EU limits consumption of sugar alcohols to 20 g per day due to laxative effects (Krüger, 1999).

2.6.3 The role of milk and other dairy components

As water binds sugar particles, milk solids rather than liquid milk is added to chocolate contributing about 12-25 %. Milk contains about 5% lactose, 5% milk fat, 3.5% protein and 0.7% minerals. Milk fat triglycerides, dominated by saturated fatty acids, exhibit a different crystalline structure although present are adequate amounts of palmitic, stearic and oleic acid, the main fatty acids found in cocoa butter (Haylock & Dodds, 1999). Milk fat is mainly liquid (15-20% solid) at ambient, and softens chocolate texture, slows setting, and is used at up to 30% of the total fat content (German & Dillard, 1998), inhibiting fat bloom. Milk fat is prone to oxidation and influences shelf-life (Haylock & Dodds, 1999).

Milk proteins add to the perceived creaminess of milk chocolate and at 80% caseins and 20% whey proteins, the casein fraction act as surfactants and reduces viscosity of chocolate, whey proteins in contrast increase viscosity (Haylock & Dodds, 1999). Milk solids added as spray-dried skimmed milk powder or full cream milk powder contribute to flavour, texture and liquid flow properties dependent on heat treatment and drying conditions. Milk fat is free to react with the cocoa butter when mixed with skimmed milk powder but strongly bound in full cream milk powder. Skimmed milk powder softens cocoa butter to an extent (Haylock & Dodds, 1999) and addition of milk solids in the form of chocolate crumb is preferred in certain European countries. Chocolate crumb, developed when cocoa liquor is mixed with sugar-milk mass and vacuum dried, is characterised by a brown colour and

slightly cooked flavour. Crumb has a longer shelf-life than milk powder as the chocolate liquor provides natural antioxidants – flavonoids (Holland et al., 1991), stabilising it against rancidity (Haylock & Dodds, 1999; Beckett, 2000). Chocolate flavours vary depending on the crumb processing conditions. Whey and lactose powders can be used to reduce sweetness in some chocolate confectionery. Demineralised whey powder is preferred to avoid off-flavour generation (Haylock & Dodds, 1999).

2.6.4 The role of surfactants in modern chocolate confectionery

Chocolate has a continuous fat phase in which sugar, being hydrophilic and lipophobic will not dissolve so surfaces have to be coated with fat. This does not occur readily and a surface active agent is beneficial and allows the fat content of the chocolate to be reduced while maintaining desirable flow properties. Choice of natural surfactant - gums, lecithin, soluble polysaccharides or synthetic (carboxymethyl cellulose) depends upon function in the end-product (Schantz & Rohm, 2005).

Lecithin, a by-product of soya-oil production is a mixture of natural phosphoglycerides (Minifie, 1989). In chocolate the most surface active component of crude lecithin (mainly oleic C18:1 and palmitic acid C16:0), is believed to be phosphatidylcholine (Vernier, 1998). Lecithin addition dramatically changes yield value and plastic viscosity and when added at between 0.1-0.3% reduces chocolate viscosity and enhances toleration of higher moisture levels. At more than 0.5%, yield value increases while plastic viscosity continues to fall (Chevalley, 1999; Rector, 2000; Schantz & Rohm, 2005). Increase in yield value is linked to micelle formation in the continuous phase possibly as multi-layers around sugar, which hinders flow. Alternatively, reverse micelles may form in the continuous phase and interact with fully covered sugar particles, consequently increasing yield value (Vernier, 1998). Thickening depends on the particle size distribution as smaller particles require more lecithin

to coat sugar surfaces. Lecithin can only be added up to 1% but will always be present in chocolate as traces from both cocoa and milk.

Polyglycerol polyricinoleate (PGPR), obtained by polycondensation of castor oil and glycerol, is a complex mixture with polyglycerol component dominated by di-, tri and tetraglycerols (Vernier, 1998). Legally approved within the EU, PGPR can be used in cocoa-based confectionery at up to 0.5% (Rector, 2000). It does not have large effects on plastic viscosity but can reduce yield value by 50% at 0.2% or remove it at about 0.8% (Rector, 2000; Schantz & Rohm, 2005), turning chocolate into a Newtonian liquid so that it flows more readily and settles rapidly. A similar outcome can be achieved by adding more cocoa butter at greater cost as PGPR coats solid particles, displacing cocoa butter to the continuous phase, decreasing yield value. Rector (2000) reported that chocolate with 35 % cocoa butter content has a similar yield value to that containing 32 % cocoa butter and 0.1 % PGPR. PGPR coats solid particles and with higher molecular weight, extends further into the lipid continuous phase, producing a better steric stabilization (Vernier, 1998). In contrast to lecithin, PGPR in chocolate does not structure within the suspension, but increases the continuous phase volume fraction and binds residual water in chocolate, making it unavailable to hydrate and swell the solid particles (Rector, 2000; Schantz & Rohm, 2005).

In recent developments, many chocolate manufacturers use PGPR and lecithin in combination for a desirable yield value and plastic viscosity - balancing out viscosity-reducing effects (Vernier, 1998; Schantz & Rohm, 2005). Adding PGPR to chocolate containing 0.5% of lecithin, gives a further decrease in yield value and only slight increase in plastic viscosity (Rector, 2000). Increases in plastic viscosity at lecithin concentrations above 0.5% are uncontrolled, effects on yield value reduction by adding PGPR have greater influence on the flow properties of chocolate (Rector, 2000). PGPR seems less effective in inhibiting bloom formation (Walter & Cornillon, 2001).

Glycerol mono stearates (GMS) widely used in confectionery industries, are formed by the incomplete esterification of hydroxyl groups of glycerol using a single fatty acid (Heath, 1982). Vernier (1998) reported glycerol fatty acid esters were inefficient at reducing yield value and increased plastic viscosity through less efficient coverage of sugar particles, thus leading to greater friction. A mixture of sorbitan and glycerol esters of fatty acids give yield values similar to lecithin but higher plastic viscosity (Vernier, 1998; Rousset et al., 2002).

2.7 Moisture and chocolate flow

Molten chocolate typically has moisture contents of 0.5-1.5%, mainly in the cocoa solids, that does not affect chocolate flow. Greater moisture aggregates sugar particles to form gritty lumps and moisture at sugar particle surfaces increases friction and apparent viscosity. Beckett (2000) stated that for every 0.3% of extra moisture left within the chocolate at the end of conching, the manufacturer must add an extra 1% fat, and because fat is by far the most expensive major component in chocolate, it is important that as much 'free' water is removed as possible. Water at 3-4% increases viscosity and yield value of chocolate markedly (Chevalley, 1999) and viscosity increases up to 20% moisture, after which an aqueous phase is formed (Beckett, 2000).

2.8 Conclusion and further research

The physical properties, rheological behaviour and sensory perception of chocolate are influenced largely by its processing techniques, particle size distribution and ingredient composition. To enhance chocolate texture, solid particle size distribution and ingredient composition can be manipulated to modify the physical properties, rheological behaviour and sensorial attributes. Several improvements have been made in recent years on chocolate

quality using varying processing strategies and ingredient composition. However, the use of particle size distribution and ingredient composition as tools to modify the rheological behaviour and sensory properties of chocolate still require a greater understanding of underlying principles and factors affecting changes in flow behaviour. Thus, factors shaping chocolate character during and after its manufacture require much in-depth investigation.

CHAPTER 3

FLAVOUR FORMATION AND CHARACTER IN COCOA AND CHOCOLATE - A CRITICAL REVIEW

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3.1 ABSTRACT

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

Keywords: Theobroma cacao; genetic origin; cocoa fermentation; roasting; conching; chocolate flavour.

3.2. INTRODUCTION

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

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

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

The chemistry of cocoa beans in fermentations is still under study (Buyukpamukcu et al., 2001; Luna et al., 2002; Misnawi, et al., 2003; Schwan & Wheals, 2004; Kyi et al., 2005) as are contributions from roasting and alkalisation (Gill et al., 1984; Oberparleiter & Ziegleder, 1997; Jinap & Dimick, 1991; Dimick & Hoskin, 1999; Stark et al., 2005; Ramli et

al., 2006; Granvogl et al., 2006; Stark et al., 2006a; Reineccius, 2006) and conching (Pontillon, 1995; Plumas et al., 1996 Awua, 2002; Beckett, 2000; Reineccius, 2006). Key flavour compounds in chocolate have been identified (Cerny, & Fay, 1995; Cerny, & Grosch, 1994; Schnermann & Schieberle, 1997; Schieberle & Pfnuer, 1999; Counet et al., 2002; Taylor, 2002; Taylor & Roberts, 2004; Reineccius, 2006). However, the biochemical and chemical processes leading to chocolate flavour formation and development, and their relationships to the final character and perceptions of quality are not fully understood.

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

3.3. BEAN COMPOSITION AND FLAVOUR PRECURSOR FORMATION

The shell (testa) represents 10-14% dry weight of the cocoa bean. The kernel or cotyledon, most of the remaining 86-90% (Table 3.1), confers characteristic flavours and aromas of chocolate (Rohan & Stewart, 1967; Osman et al., 2004) and is composed of two types of parenchyma storage cells. Polyphenolic cells (14-20% dry bean weight) contain a single large vacuole filled with polyphenols and alkaloids including caffeine, theobromine and theophylline (Osman et al., 2004). The pigmented polyphenols, when undisturbed, confer deep purple colour to fresh Forastero cotyledons. Lipid-protein cells, on the other hand, have cytoplasms tightly packed with multiple small protein and lipid vacuoles and other components such as starch granules - all of which play roles in defining cocoa flavour and aroma characters (Kim & Keeney, 1984; Nazaruddin et al., 2001).

Reineccius et al. (1972) reported fresh unfermented cocoa beans contained 15.8 mg/g sucrose and trace amounts of fructose, sorbose, mannitol and inositol. Berbert (1979) suggested sucrose content at 24.8 mg/g unfermented beans formed about 90% of total sugars (27.1 mg/g).

Table 3.1. Bean composition of unfermented West African (*Forastero*) cocoa

Constituents	Dried beans (%)	Fat-free materials (%)
Cotyledons	89.60	-
Shell	9.63	-
Germ	0.77	-
Fat	53.05	-
Water	3.65	-
Ash (Total)	2.63	6.07
Nitrogen		
Total nitrogen	2.28	5.27
Protein nitrogen	1.50	3.46
Theobromine	1.71	3.95
Caffeine	0.085	0.196
Carbohydrates		
Glucose	0.30	0.69
Sucrose	1.58	3.86
Starch	6.10	14.09
Pectins	2.25	5.20
Fibre	2.09	4.83
Pentosans	1.27	2.93
Mucilage and gums	0.38	0.88
Polyphenols	7.54	17.43
Acids		
Acetic (free)	0.014	0.032
Oxalic	0.29	0.67

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

The reducing sugars, fructose and glucose form about 6% (0.9 and 0.7 mg/g, respectively) and others (including mannitol and inositol) at <0.50 mg/g. Differences have been attributed to method and time of harvesting, type and origin of cocoa beans (Reineccius et al., 1972). Tissue components remain compartmentalized, separating flavour constituents that may interact with cell membrane and wall breakdown during the subsequent fermentation.

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

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

reactions with sugar and amino acids contribute flavour and colour to cocoa beans, and alkaloids to the bitterness (Lehrian & Patterson, 1983).

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

and proteolytic degradation products in five popular genotypes (Forastero, Criollo, Trinitario, SCA 12, and UIT1) concluded character in chocolate may vary, but all genotypes had potential for abundant aroma content in raw cocoa (Amin et al., 2002).

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

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

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

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

Proteases effect multiple cellular processes in plants, such as protein maturation and degradations associated with tissue restructuring and cell maintenance (Callis, 1995). Key aspartic proteinases (EC 3.4.23) have been characterised in a number of *Theobroma cacao* gymnosperms (Mutlu & Gal, 1999) and activity in seeds of *Theobroma cacao* extensively studied by Biehl et al. (1993). Partially purified aspartic proteinase had activity optima at 55 °C and pH 3.5. Subsequently, Voigt et al. (1995) purified *Theobroma cacao* seed aspartic

proteinase into a heterodimer of 29 and 13 kDa polypeptides that efficiently hydrolysed *Theobroma cacao* seed vicilin and (less effectively) trypsin inhibitor into peptides (Voigt et al., 1994a).

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

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

Significant differences in enzyme activities exist between cocoa genotypes but simple and general relationships have not been established between genotype flavour potential and key enzyme activities in unfermented beans. Therefore, how enzymatic processes are

regulated, and substrates and products that relate to desirable flavours, and limiting factors for the enzymatic contribution to fermentation processes remain unclear.

3.4 EFFECT OF GENOTYPE ON COCOA BEAN FLAVOURS

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

Each bean variety has a unique potential flavour character. But growing conditions such as climate, amount and time of sunshine and rainfall, soil conditions, ripening, time of harvesting, and time between harvesting and bean fermentation all contribute to variations in final flavour formation. Table 3.2 summarises how differences in genetic origin, cocoa variety

and duration of fermentation influence flavour profile but different conditions may lead to significant differences in flavour from a single cocoa variety.

Table 3.2. Origin, cocoa variety and fermentation duration effects on flavour character

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

A good example is the difference in flavour profile between a single *Forastero* variety produced originally in Ghana and now grown in Malaysia (Clapperton, 1994), arising possibly through geographic, climatic conditions and duration and/or method of fermentation (Table 3.2).

Bulk cocoas typically show strong flavour characters, fine cocoas are perceived as *aromatic* or *smoother* (Kattenberg & Kemming, 1993; Jinap et al., 1995; Luna et al., 2002). Clapperton et al. (1994) noted consistent differences in flavour attribute specifically overall cocoa flavor intensity, *acidity, sourness, bitterness*, and *astringency.* Bean origins include the West African Amelonado variety (AML), four Upper Amazon clones [Iquitos Mixed Calabacillo 67 (IMC67), Nanay 33 (NA33), Parinari 7 (PA7), and Scavina 12 (SCA12], and Unidentified Trinatario (UIT1) grown in Sabah, Malaysia. Flavour characters in UIT1 differed from West African Amelonado, characterised by intense *bitterness* and *astringency* associated with caffeine and polyphenol contents. Fermented beans from Southeast Asia and the South Pacific are characterised by a higher *acidity* (more lactic and acetic acids) than West African beans (Clapperton et al., 1994) due to varietal differences, box fermentation and rapid artificial drying.

Cocoa liquors differ in sensory character. The West African group (Ghana, Ivory Coast and Nigeria) are generally considered sources of standard (benchmark) cocoa flavour with a balanced but pronounced cocoa character with subtle to moderate *nutty* undertones. Cameroon liquors are renowned for *bitterness*, those from Ecuador for *floral-spicy* notes. American and West Indian varieties range from *aromatic* and *winy* notes from Trinidad cocoa to the floral or *raisin-fruity* notes of Ecuadorian stocks making unique contributions to blends. Asian and Oceanian beans exhibit a range of flavour profiles ranging from *subtle* cocoa and *nutty/sweet* notes in Java beans to the intense *acid* and *phenolic* notes of Malaysian (De La Cruz et al., 1995). Counet et al. (2004) reported fine varieties with short fermentation processes had high

contents of procyanidins, while *Trinatario* from New Guinea and *Forastero* beans were specifically higher in total aroma. Aroma compounds formed during roasting were found to vary quantitatively directly with fermentation time and inversely with procyanidin content of cocoa liquors.

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

3.5 CHOCOLATE FLAVOUR DEVELOPMENT - POST-HARVEST TREATMENTS

3.5.1 Fermentation processes

Fermentation is essential for development of appropriate flavours from precursors. After pod harvest, beans and adhering pulp are transferred to heaps, boxes, or baskets for fermentations lasting from 5 to 6 days for *Forastero* beans but for *Criollo* only 1 to 3 days. In the first day, the adhering pulp liquefies and drains off, with steady rises in temperature. Under anaerobic conditions micro-organisms produce acetic acid and ethanol that inhibit germination and contribute to structural changes such as removal of the compartmentalisation of enzymes and substrates with movements of cytoplasmic components through the cocoa cotyledon generally between 24-48 h of bean fermentation. By the third day, the beans mass

will have heated typically around 45°C, remaining at 45-50°C until fermentation is complete (Lehrian & Patterson, 1983; Schwan et al., 1995; Kealey et al., 2001; Fowler, 1999).

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

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

Sucrose and proteinaceous constituents are partially hydrolyzed, phenolic compounds oxidized and glucose is converted into alcohols, oxidized to acetic and lactic acids during

fermentation. Beans subsequently undergo an anaerobic hydrolytic phase, followed by aerobic condensation. Timing, sequence of events and degree of hydrolysis and oxidation varies between fermentations. Concentration of flavour precursors is dependent on enzymatic mechanisms. Colour changes also occur with hydrolysis of phenolic components by glycosidases accompanied by bleaching, influencing final flavour character (Lopez & Quesnel, 1973; Biehl et al., 1990; Lopez & Dimick, 1991b; Lopez & Dimick, 1995).

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

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

Important flavour-active components produced during fermentation include: ethyl-2-methylbutanoate, tetramethylpyrazine and certain pyrazines. Bitter notes are evoked by theobromine and caffeine, together with diketopiperizines formed from roasting through

thermal decompositions of proteins. Other flavour precursor compounds derived from amino acids released during fermentations include 3-methylbutanal, phenylactaldehyde, 2-methyl-3-(methyldithio)furan, 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine (Taylor, 2002). Immature and unfermented beans develop little *chocolate* flavour when roasted and excessive fermentation yields unwanted *hammy* and *putrid* flavours (Fowler, 1999; Beckett, 2000; Zaibunnisa et al., 2000; Reineccius, 2006).

3.5.2 Drying

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

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

Table 3.3. Dominant odour-active volatiles in cocoa mass

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

Sources: [a] Belitz & Grosch (1999); [b] Schnermann & Schieberle (1997).

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

3.6 FLAVOUR DEVELOPMENT DURING COCOA PROCESSING

3.6.1 Effect of roasting

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

Maillard reactions, central to cocoa flavour development are important in roasting, and free amino acids, peptides and reducing sugars all participate (Rohan & Stewart, 1967). Voigt et al. (1993, 1994a) noted the hydrophobic amino acids leucine, alanine, phenylalanine and

tyrosine, released by proteinase activities in fermentation, are important contributors (Mohr et al., 1976,Voigt et al., 1993, 1994a), as are reducing sugars fructose and glucose derived from sucrose hydrolysis (Lopez et al., 1978).

Maillard reactions (Fig. 3.1) require heating at pH values above 3, in the presence of water, a reducing sugar such as glucose, and an amino group, generally from protein.

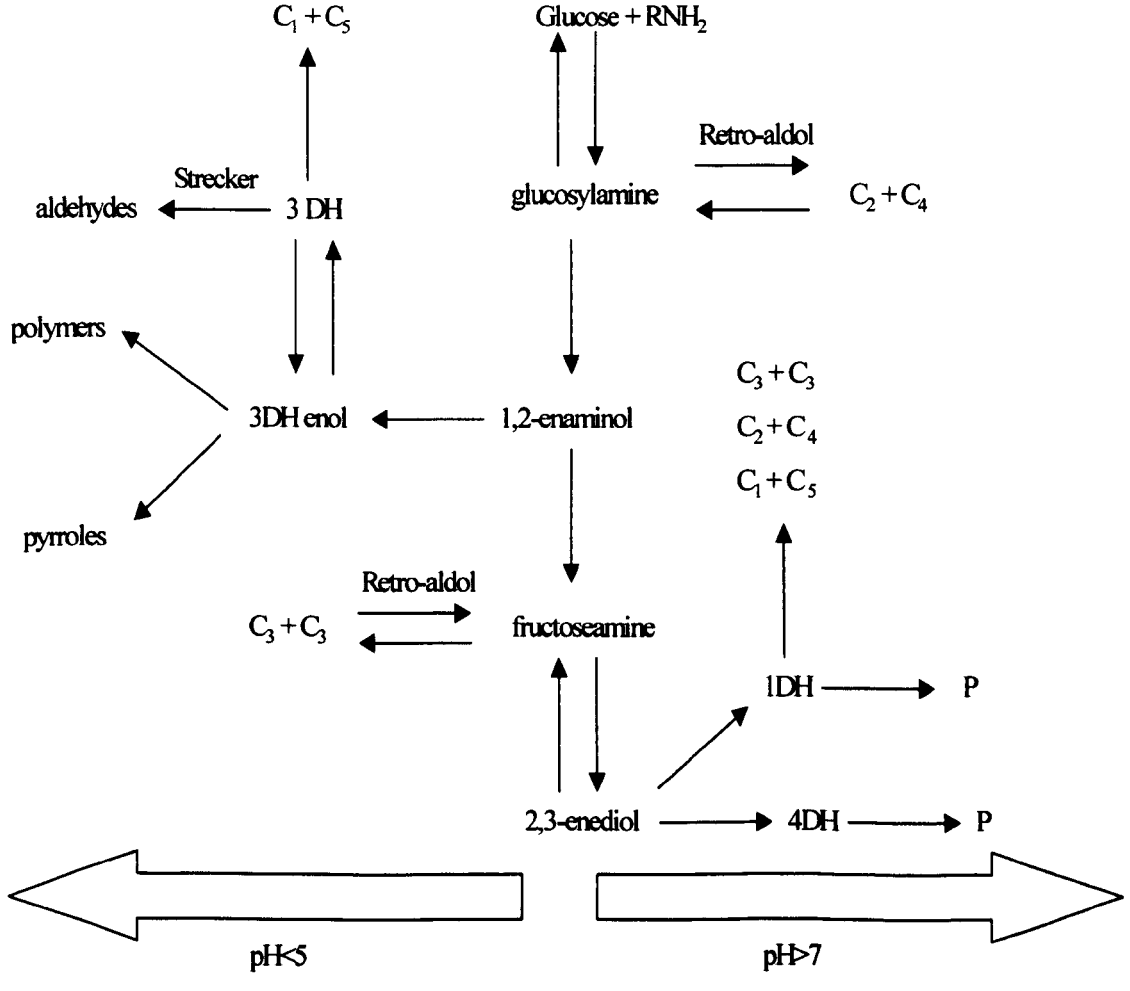


Figure 3.1. Model of Maillard reaction

Reactions to the left of Figure 3.1 yield flavours, to the right colour formation. The 1-DH, 3-DH and 4-DH intermediates are 1-, 3- and 4-deoxyhexosuloses respectively, all dicarbonyl compounds. Initial amine-assisted degradation of a reducing sugar proceeds by a sugar-amine condensation forming a Schiff base (Fig. 3.2), tautomerizing to a 1,2-enaminol (Fig. 3.3). The link between glucose C 1 and fructose C 2 in sucrose prevents ring opening and Schiff base formation blocking participation in Maillard reactions. Reaction intermediates can act as catalysts or inhibitors for other reactions contributing to flavour (Beckett, 2000; Ramli et al., 2006; Granvogl et al., 2006).

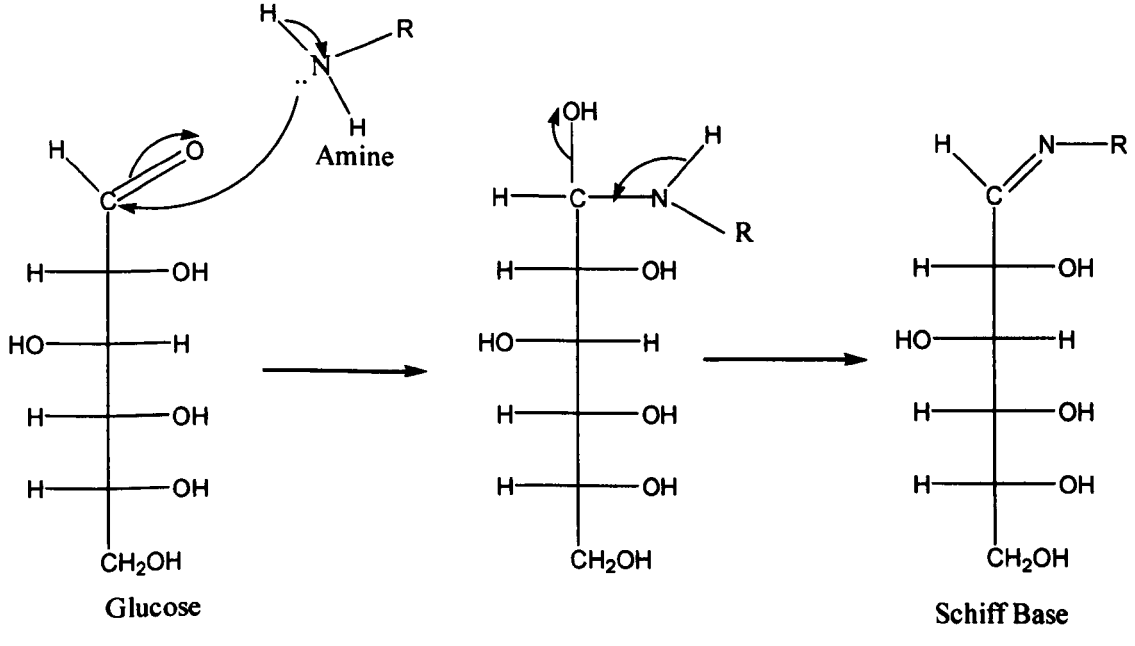


Figure 3.2. Mechanism of sugar-amine condensation to form a Schiff Base

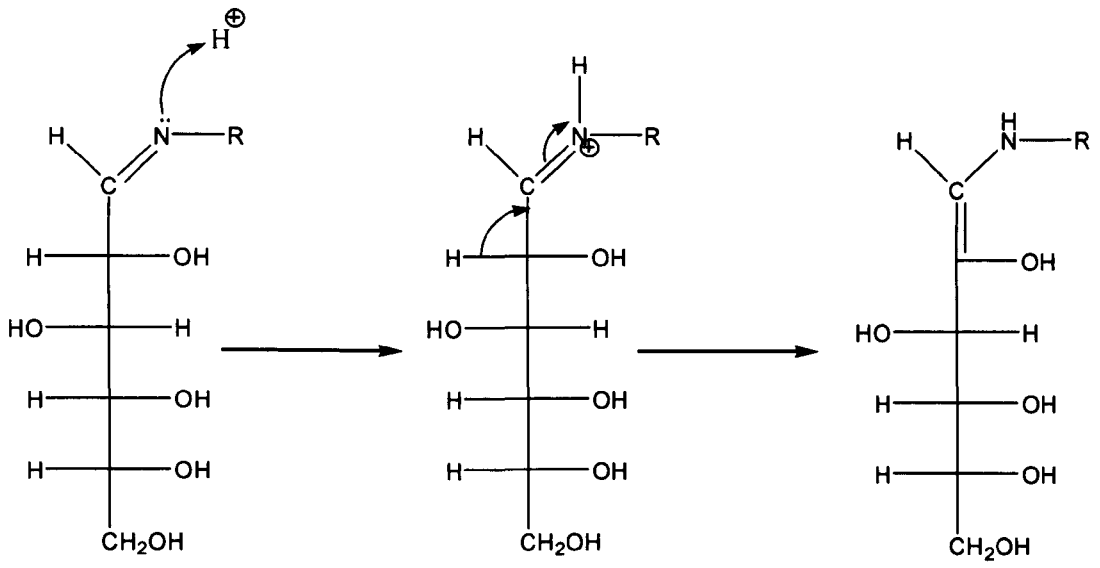


Figure 3.3. Mechanism for the formation of 1,2-enaminol from Schiff Base

Reducing sugars and amino acids form addition compounds, such as glucosylamines or fructosylamines with rearrangement of glucosylamines into isomerization products. At this point, the reaction pH influences the intermediates formed: acidic conditions favour 3-deoxyhexuloses (3-DH); basic or neutral pH favour formation of dehydroreductone intermediates (1-DH). Central to flavour formation are intermediates that have lost amino groups (1-DH compounds), the nature of the amine does not influence ultimate aroma character but may be important for overall reaction rate (Williams, 2000; Stark et al., 2006a; Granvogl et al., 2006). Transformed compounds are not detectable by colour or flavour changes that may be reversible at this stage, but isomerized products are key substrates for subsequent reactions. The 1-DH compounds are dehydrated, fragmented and transaminated yielding smaller dicarbonyl molecules, or contributing to Strecker degradation reactions, depending on temperature and pH (Dimick & Hoskin, 1999; Williams, 2000; Ramli et al.,

2006; Granvogl et al., 2006). Strecker degradation reactions, central to the appropriate flavours for chocolate, involve interactions of numerous compounds, leading to the structure derived from amino acids being split into three parts (Fig. 3.4).

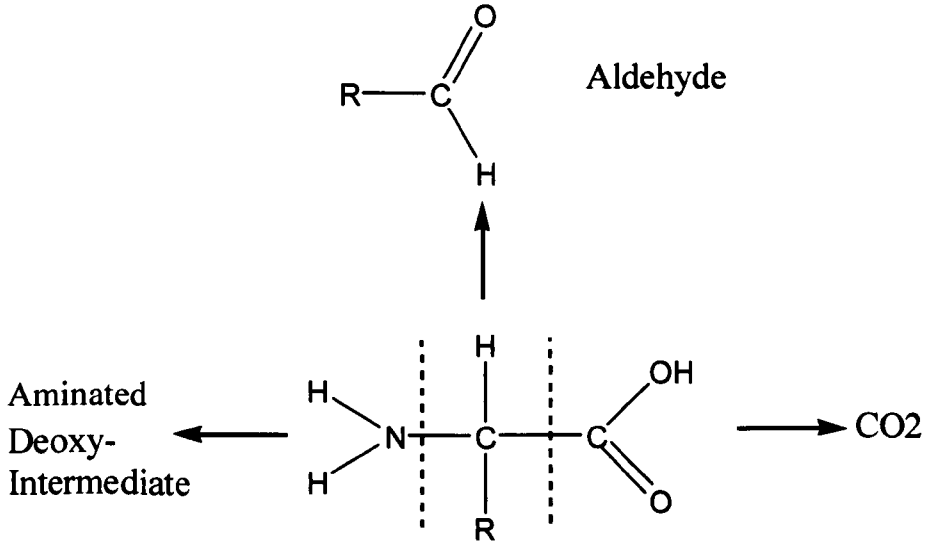


Figure 3.4. Formation of amino acid specific aldehydes through Strecker degradation reaction

The nature of the amine component is crucial to chocolate flavour formation as not only are these aldehydes themselves flavour-active but further reactions yield heterocyclic compounds important to final character. Leucine and glucose yield aroma notes described as '*sweet chocolate*', threonine and glutamine and glucose give '*chocolate*' notes when heated to 100°C and valine and glucose, heated to 180°C a note described as '*penetrating chocolate*' (Dimick & Hoskin, 1999). Such aroma notes indicate reactions have proceeded past the initial

stage. Strecker degradation reactions and subsequent formation of a model pyrazine are summarised in Figures 3.5 and 3.6.

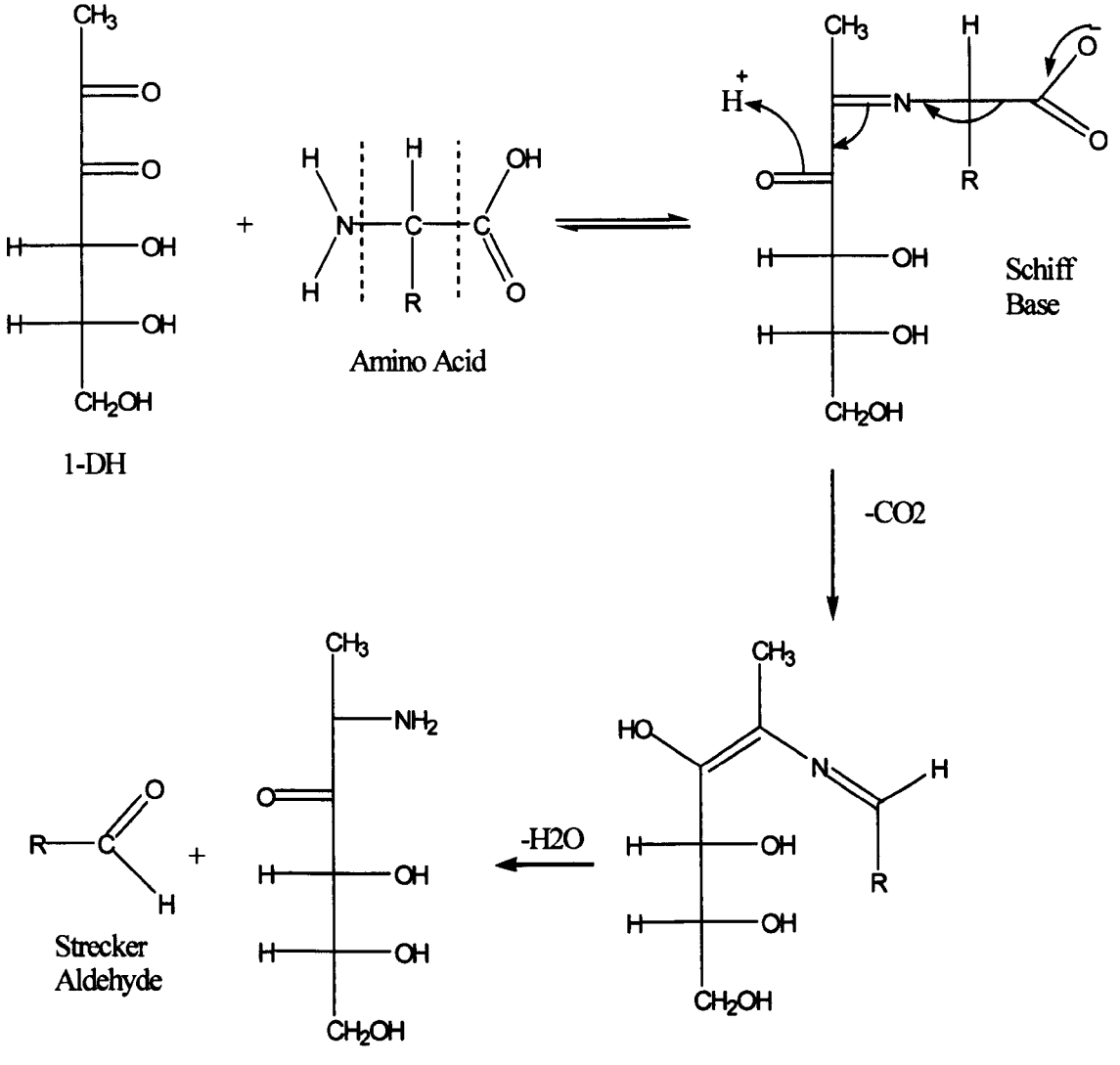


Figure 3.5. Mechanism of a Strecker degradation reaction

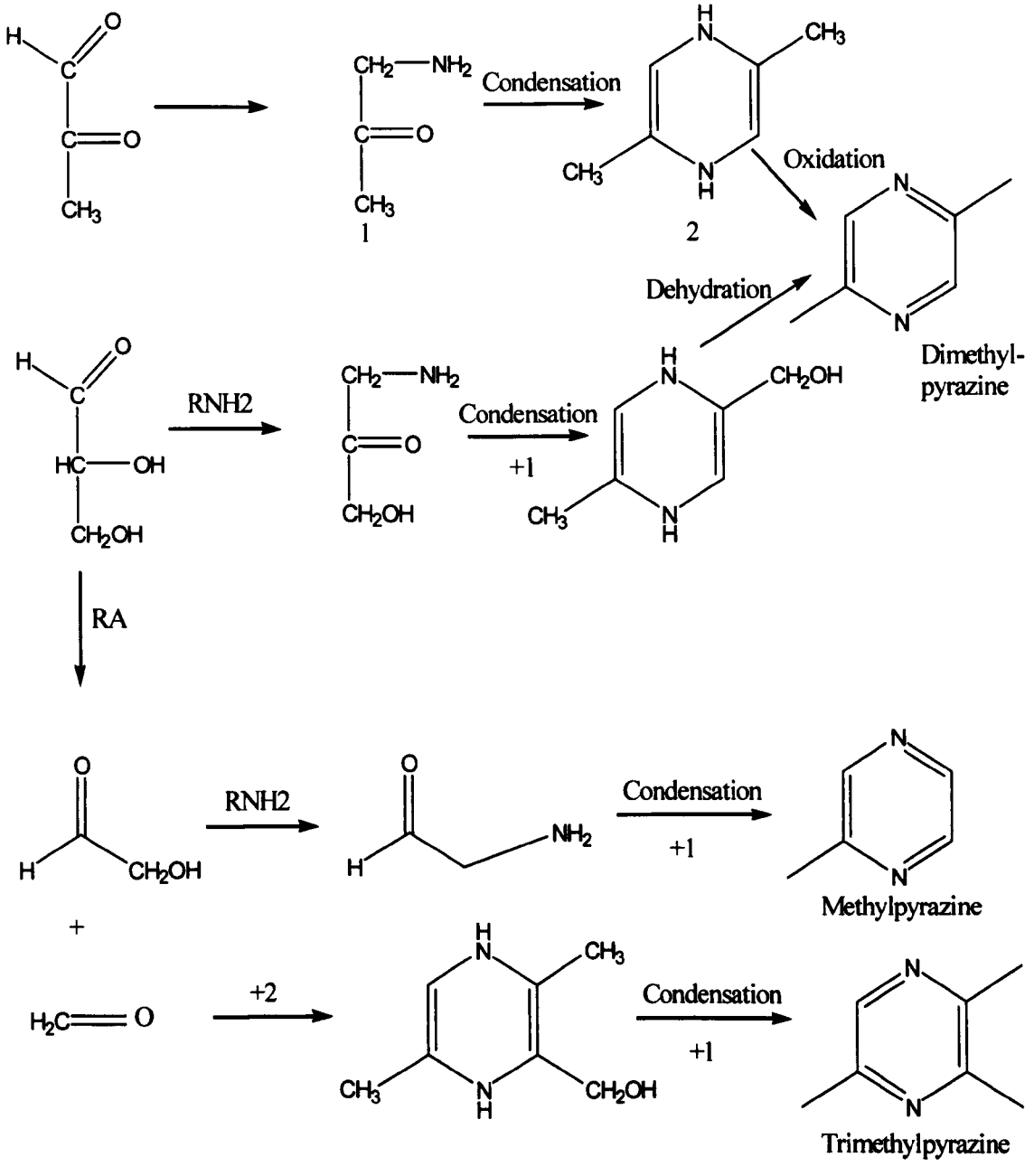


Figure 3.6. Formation of pyrazines through the reaction of deoxy intermediates with amino acids

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

3.6.2 Mailard reactions - Aldol Condensation, Polymerisation and Cyclization

These final stages of Maillard reactions are probably least understood but it is generally accepted that aldol condensation and cyclization lead to formation of heterocyclic aroma volatiles such as pyrazines (Fig. 3.6) whilst polymerisation produces melanoidin pigments. Dimick and Hoskin (1999) concluded specific pyrazine structure is dictated by side groups on dioxo compounds. Pyruvaldehyde and valine, for example, yield end products of 2-methyl-propanal and 2,5-dimethyl pyrazine, contributing *nutty* flavours. Precursors make chocolates rich in pyrazines, with at least 80 contributing significantly to overall flavour (Counet et al., 2002; Stark & Hofmann, 2005), but total concentrations in roasted beans vary: Ghanaian cocoas typically have 698 μg per 100g; Mexican beans as low as 142 μg (Reineccius, 2006). Nitrogenous component contents are a source of flavour differentiations.

Simple degradation products of amino acids in cocoa products are summarised in Table 4. However there are >500 compounds identified from volatile and non-volatile chocolate fractions - hydrocarbons, alcohols, aldehydes, ketones, esters, amines, oxazoles, sulphur compounds (Heinzler & Eichner, 1991; Dimick & Hoskin, 1999; Taylor, 2002; Taylor & Roberts, 2004; Reineccius, 2006; Stark et al., 2006b). Aldehydes from amino acids

play important roles in chocolate flavour balance. Aldehydes from Strecker degradation of amino acids, also produces pyrazines. The amino acid structure dictates the resulting aldehyde and also the amine and acid that can be produced from the amino acid degradation (Table 3.4).

Table 3.4. Degradation products of amino acids found in cocoa products

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

Source: Dimick & Hoskin (1999)

3.6.3 Effects of alkalisation

Alkalisation (treatment of cocoa nibs or liquor with solutions of alkali) is carried out primarily to change colour but also influence flavour of cocoa powder. Alkalising is common for cocoa products such as drinks to enhance solubility or in baking or coatings (Minifie,

1989; Awua, 2002; Whitefield, 2005). Dimick and Hoskin (1999) suggested that cocoa nibs from Malaysia and Brazil are characterised by high acidity and low chocolate flavour, limiting possible character developments in processing and Sharif (1997) showed improvements in quality of cocoa nibs and liquors from these origins could be achieved by alkali treatments reducing acidity before nib roasting or thin film processing. Sharif (1997) noted alkalising Malaysian cocoa nibs to pH 6.0 did not significantly ($p \leq 0.05$) change flavour relative to a control but chocolates from nibs alkalised to pH of 7.2 and 8.1 were significantly different and dark chocolate prepared from Ivory Coast, Malaysian and Brazilian cocoa had their *sour, bitter, fruit* and *mouldy* notes significantly changed by alkali treatment. The conclusion was that chocolates from alkalised and thin-film processed cocoa liquor had better flavours than non-alkalised nib-roasted chocolate (Sharif, 1997). Alkalisation reduces acidity as well as astringency with aspects like typical cocoa and bouquet enhanced and intensified. Reductions in astringency are effected by further polymerisations of flavonoids during alkali treatments (ADM Cocoa Manual, 2006).

3.7 FLAVOUR DEVELOPMENT DURING CHOCOLATE MANUFACTURE

3.7.1 Conching

Conching is regarded as essential for final flavour development and appropriate texture. This is the final stage in chocolate manufacture, whether dark or milk. Residual volatile acids and moisture are removed, angular sugar crystals and viscosity are modified and the colour changed due to emulsification and oxidation of tannins (Awua, 2002; Beckett, 2003; Reineccius, 2006; Afoakwa et al., 2007). Generally a two-stage process, the first stage converts flake or powder into a paste by mechanical or heat energy, driving off moisture and undesirable volatiles, effect oxidations and distribute lipids through a continuous fat phase. Beckett (2000) suggested oxidations modify precursors developed in fermentation and

roasting processes to achieve final *cooked* flavour and eliminates undesirable *astringent* and *acidic* notes. The second stage converts the thick paste into a free flowing liquid through addition of cocoa butter and lecithin.

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

The air spaces surrounding a conche in operation have an odour of acetic acid, suggesting an initial loss of short-chained volatile fatty acids, such as acetic acid, the end products of fermentation. This was confirmed by quantitative studies (Dimick & Hoskin, 1999; Beckett, 2000). Volatile phenols show 80% reductions in headspace concentrations within a few hours of conching (Beckett, 2000). Hoskin and Dimick (1984) reported phenols decreased from 21.3 μg/100g to 10.9 μg/100g after 44 h in low roast chocolate, and 10.3 μg/100g to 6.0 μg/100g after 24 h in high roast in conching. In a later paper, Dimick and Hoskin (1999) concluded that polyphenols, through oxidation and enzymatic mechanisms, form complexes with amino acids, peptides and proteins. The outcome is withdrawal of flavour-active volatiles from headspaces and reductions in perceptions of astringency through irreversible phenol interactions, and more "mellow" final flavours.

Hoskin and Dimick (1983) suggested that in conching of dark chocolate, amino acid concentrations do not fall as temperature and/or the concentrations of amino acids and sugars are below thermal thresholds for Maillard reactions. Heinzler and Eichner (1991), however,

reported that Amadori compounds formed in drying and roasting decrease during conching and Pontillon (1995) proposed caramelizations of lactose and Maillard reactions with milk proteins (in milk chocolate). A consensus is that chocolates show marked decreases in overall off-flavours after conching (Pontillon, 1995; Hoskin & Dimick, 1983; Plumas et al., 1996; Beckett, 2003; Counet et al., 2002).

Counet and his coworkers (Counet et al., 2002) concluded key dark chocolate odourants were present prior to conching, during which Strecker aldehydes were partially lost through evaporation and/or chemical reactions. On the other hand, 2-phenyl-5-methyl-2-hexenal content was increased through aldol condensation of phenylacetaldehyde and 3-methylbutanal followed by dehydration (Counet et al., 2002). Schnermann and Schieberle (1997) noted furaneol and maltol (Table 3.5) were also generated during conching. Of heterocycles, only concentrations of the least volatile compounds were increased, notably polysubstituted ethyl-, isobutyl-, and isopentylpyrazines, tri- or tetramethylpyrazine, furans and acetylpyrrole (Table 3.5).

3.8. KEY FLAVOUR COMPOUNDS IN MILK CHOCOLATE

Analytical studies have identified >600 volatile compounds in cocoa and chocolate products (Schieberle & Pfnuer, 1999; Taylor, 2002; Taylor & Roberts, 2004; Reineccius, 2006), primarily pyrazines, esters, amines and amides, acids, and hydrocarbons. Schnermann and Schieberle (1997) identified as key neutral/basic flavour-active components of milk chocolate: 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine, 1-octen-3-one, 2-ethyl-3,6-dimethyl pyrazine, 2,3-diethyl-5-methylpyrazine, (*Z*)-2-nonenal, 2-methyl-3-(methyldithio)furan, (*E*,*E*)-2,4-nononadienal, (*E*,*E*)-2,4-decadienal, and *R*-δ-decalactone (Table 3.5).

Table 3.5. Flavour Compounds Identified in Milk Chocolates

No.	Compound	RI		Odour description
		FFAP	SE-54	
A.	***NEUTRAL/BASIC FRACTIONS***			
1	3-methylbutanal [a,p]	920	651	*malty*
2	2,3-butandione (diacetyl) [b,p]	984	592	*buttery*
3	hexanal [c,p]	1083	801	*green*
4	1-hexen-3-one [p]	1101	775	*linseed oil-like*
5	unknown [p]	1195	-	*gerarium-like*
6	(*Z*)-4-heptenal [p]	1246	899	*sweet, biscuit-like*
7	5-methyl-(*E*)-2-hepten-4-one [p]	1287	972	*hazelnut-like*
8	1-octen-3-one [p]	1304	980	*mushroom-like*
9	dimethyl trisulfide [d,p]	1384	968	*sulfurous*
10	nonanal [c,p]	1400	1093	*soapy*
11	trimethylpyrazine [ep]	1406	1000	*earthy*
12	unknown [p]	1422	-	*fruity, waxy*
13	2-methoxy-3-isopropylpyrazine [p]	1428	1097	*earthy, beany*
14	(*E*)-2-octenal [p]	1433	1060	*fatty, waxy*
16	2-ethyl-3,6-dimethylpyrazine [f,p]	1445	1079	*nutty, earthy*
17	unknown [p]	1454	-	*tallowy*
18	2-ethyl-3,5-dimethylpyrazine [e,p]	1461	1083	*potato chip-like*
20	2,3-diethyl-5-methylpyrazine [g,p]	1490	1158	*potato chip-like*
21	(*Z*)-2-nonenal [p]	1513	1148	*green, fatty*
23	(*E*)-2-nonenal [p]	1528	1161	*green, fatty*
24	(*E,Z*)-2,6-nonadienal [p]	1579	1154	*cucumber-like*
25	(*Z*)-2-decenal [f,p]	1601	1250	*tallowy*
27	(*E*)-2-decenal [p]	1647	1262	*fatty, green*
28	phenylacetaldehyde [e,p]	1652	1047	*sweet, honey-like*
30	2-methyl-3-(methyldithio)furan [p]	1667	1170	*cooked meat-like*
31	(*E,E*)-2,4-nonadienal [p]	1703	1215	*fatty, waxy*
33	ethyl phenylacetate [p]	1724	-	*sweet, waxy*
34	(*E,E*)-2,4-decadienal [p]	1812	1318	*fatty, waxy*
35	phenylethyl acetate [h,p]	1821	1244	*fruity, sweet*

37	2-phenylethanol [e,p]	1915	1118	*sweet, yeast-like*
40	*R*-δ-octenolactone (99%) [p]	2009	1261	*sweet, coconut-like*
42	*R*-γ-nonalactone (80%) [i,p]	2038	1663	*sweet, coconut-like*
43	ethyl cinnamate [i,p]	2125	1469	*sweet, cinnamon-like*
44	γ-decalactone [p]	2155	1470	*sweet, peach-like*
46	*R*-δ-decalactone (84%) [j,p]	2208	1469	*sweet, peach-like*
47	*R*-δ-decenolactone (99%) [p]	2241	1477	*sweet, peach-like*
49	3-methylindol (skatole) [p]	2494	1388	*mothball-like*
B.	***ACIDIC FRACTIONS***			
15	acetic acid [k,p]	1439	600	*sour*
19	unknown [p]	1478	-	*waxy*
22	unknown [p]	1522	-	*green*
26	butanoic acid [k,p]	1610	821	*buttery, rancid*
29	2- and 3-methylbutanoic acid [l,p]	1652	873	*sweaty*
32	pentanoic acid [l,p]	1721	911	*sweaty, pungent*
36	hexanoic acid [l,p]	1829	1019	*sweaty, pungent*
38	unknown [p]	1936	-	*sour*
39	3-hydroxy-2-methylpyran-4-one (maltol) [l,p]	1961	1111	*caramel-like*
41	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) [m,p]	2022	1070	*caramel-like*
45	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) [p]	2182	1110	*seasoning-like, spicy*
48	3-hydroxy-5-ethyl-4-methyl-2-(5*H*)-furanone (abhexon) [p]	2250	1198	*seasoning-like, spicy*
50	phenylacetic acid [n,p]	2254	1262	*sweet, flowery*
51	3-methoxy-4-hydroxybenzaldehyde (vanillin) [o,p]	2577	1406	*vanilla-like*

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

In acidic volatiles, 14 components were identified as contributing to flavour (Table 5) with vanillin (*vanilla*), added also in manufacture, followed by 2- and 3-methylbutanoic acid (*buttery, rancid*) and sotolon (*fenugreek/maple syrup/caramel*) showing highest odour intensity values. Although 1-octen-3-one and (*E,E*)-2,4-decadienal have been reported as primary odourants of milk products (Widder et al., 1991; Schieberle et al., 1993), these and, in addition, dimethyl trisulphide and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone may also be generated in conching although this is experimentally unproven. In essence key flavour components of milk chocolate appear to primarily originate in the roasted cocoa mass.

3.9 KEY FLAVOUR COMPOUNDS IN DARK CHOCOLATE

In a more recent analytical study of dark chocolate (Counet et al., 2002), a similar aroma extract dilution analysis (AEDA) approach to that of Schieberle and his colleagues was used to identify key flavour-active components and effects of conching on flavour. Of 60 compounds - nitrogen and oxygen heterocycles, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles, and sulphides (Table 3.6) - 10 had not previously been identified as chocolate constituents: 1-pentanol (1), 3-(methylthiol)-propionaldehyde, methylbenzene, pyrazine, ethenylpyrazine, pyridine, 2-methylpyridine, 1-(2-furanylmethyl)-1*H*-pyrrole, 1*H*-indole, and dimethyl disulfide (Table 3.6). Two others, benzyl alcohol and dihydro-2-methyl-3(2*H*)-furanone, had only been reported in milk chocolates. Specific nitrogen heterocycles, from Maillard reactions, were concluded as important: 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine, 3,5-(or 6)-diethyl-2-methylpyrazine, acetylpyrrole and furfurylpyrrole (Table 3.2) all with *praline* and *chocolate* notes. Ethyl group in two pyrazine compounds suggests key roles for alanine and/or its Strecker aldehyde, acetaldehyde, in chocolate flavour synthesis (Cerny, & Fay, 1995; Cerny, & Grosch, 1994).

Table 3.6. Flavour Compounds Identified in Dark Chocolates

No.	Compound	RI[a]	Odour description
A.	***NEUTRAL/BASIC FRACTIONS***		
	Alcohol		
1	1-pentanol [g]	757	
2	2-heptanol [g]	879	
3	benzyl alcohol [f,g]	1010	
4	3,7-dimethyl-1,6-octadien-3-ol (*linalool*) [b,e,g]	1086	*flowery*
5	2-phenylethanol [c,d,e,f,g]	1090	
	Aldehydes		
6	2-methylpropanal (*isobutanal*) [e,g]	566	*chocolate*
7	3-methylbutanal [b,c,d,e,g]	633	*chocolate*
8	2-methylbutanal [e,g]	643	*chocolate*
9	2-methyl-2-butenal [b,g]	764	
10	3-(methylthio)propionaldehyde (*methional*)	866	*potato*
11	Heptanal [e,g]	877	
12	Benzaldehyde [b,e,f,g]	935	
13	Phenylacetaldehyde [c,d,e,f,g]	1015	*flowery, honey*
14	Nonanal [b,d,e,g]	1082	
15	2-phenyl-2-butenal [f,g]	1242	*cocoa, sweet, roasted, rum*
16	2-phenyl-5-methyl-2-hexenal [b,f,g]	1485	
	Esters		
17	ethylbenzoylformate [g]	1039	
18	ethylbenzoate [b,f,g]	1156	
19	ethyloctanoate [e,g]		
20	2-phenylethylacetate [b,c,d,g]	1233	
	Furans		
21	dihydro-2-methyl-3(2*H*)-furanone [f,g]	781	
22	furancarboxaldehyde (*furfural*) [b,e,f,g]	805	
23	furfuryl alcohol (*furfurol*) [b,e,f,g]	827	

24	1-(2-furanyl)ethanone (*acetylfuran*) [b,e,f,g]	884	
25	5-methyl-2-furancarboxaldehyde [b,e,f,g]	931	
26	5-ethenyltetrahydro-R,R,5-trimethyl-*cis*-2-furanmethanol (*linalool oxide*) [g]	1076	
27	3-phenylfuran [g]	1208	*cocoa, green, mint*
	Hydrocarbons		
28	methylbenzene (*toluene*) [g]	767	
	Ketones		
29	2,3-butanedione (*diacetyl*) [c,d,e,f,g]	578	*buttery*
30	2-heptanone [e,f,g]	868	
	Nitrogen Compounds		
31	Benzonitrile [b,f,g]	951	
	Pyrans		
32	3,4-dihydro-8-hydroxy-3-methyl-1*H*-2-benzopyran-1-one [g]	1517	
	Pyrazines		
33	pyrazine [g]	731	*hazelnut, green*
34	methylpyrazine [b,g]	803	
35	2,5-dimethylpyrazine [b,f,g]	889	*green, ether, rum*
36	Ethylpyrazine [b,g]	895	*hazelnut, roasted*
37	2,3-dimethylpyrazine [b,g]	899	
38	ethenylpyrazine	907	
39	2-ethyl-5(or 6)-methylpyrazine [b,f,g]	973	*cocoa, roasted, green*
40	Trimethylpyrazine [b,c,d,f,g]	980	
41	2-ethyl-3-methylpyrazine [b,g]	983	*hazelnut, roasted*
42	2-ethenyl-6-methylpyrazine [g]	992	*roasted, smoky, praline,*
43	3(or 2),5-dimethyl-2(or 3)-ethylpyrazine [c,d,f,g]	1057	*rum*
44	tetramethylpyrazine [b,f,g]	1065	*milk coffee, mocha,*

			roasted, green
45	2-isopropyl-3-methoxypyrazine [c,d,g]	1081	*garden peas, green,*
46	2,3-diethyl-5-methylpyrazine [c,d,g]	1135	*hazelnut*
47	3,5(or 6)-diethyl-2-methylpyrazine [f,g]	1137	*cocoa, chocolate, rum, sweet, roasted*
48	3,5(or 6)-diethyl-2-methylpyrazine [f,g]	1139	
49	2,5(or 6)-dimethyl-3-(2-methylpropyl)pyrazine [g]	1184	*hazelnut*
50	2,5-dimethyl-3-(3-methylbutyl)pyrazine [g]	1296	*roasted, sweet, green*
	Pyridines		
51	pyridine [g]	724	
52	2-methylpyridine [g]	800	
53	1-(2-pyridinyl)-1-propanone [g]	1114	
	Pyrroles		
54	2-carboxaldehyde-1*H*-pyrrole [g]	986	
55	1-(1*H*-pyrrol-2-yl)ethanone *(acetylpyrrole)* [b,f,g]	1030	*cocoa, chocolate, hazelnut, roasted*
56	3-ethyl-2,5-dimethyl-1*H*-pyrrole [g]	1119	*cocoa, hazelnut, coffee, roasted*
57	1-(2-furanylmethyl)-1*H*-pyrrole *(furfurylpyrrole)* [g]	1166	*roasted, chocolate, green*
58	1*H*-indole [g]	1276	
	Sulphur Compounds		
59	dimethyl disulphide [g]	743	
60	dimethyl trisulphide [c,d,g]	969	*onion, cabbage, sweaty*

B.	***ACIDIC FRACTIONS***		
	Alcohols		
61	2,4-hexadien-1-ol [g]	831	
	Aldehydes		
7	3-methylbutanal [b,c,d,g]	633	*chocolate (low intensity)*
8	2-methylbutanal [g]	643	*chocolate (low intensity)*
	Furans		
23	furfuryl alcohol (*furfurol*) [b,e,f,g]	827	
62	2,5-dimethyl-4-hydroxy-3(2*H*)furanone (*Furaneol*) [c,d,g]	1023	*caramel-like, sweet*
	Hydrocarbons		
28	methylbenzene (*toluene*) [g]	772	
	Ketones		
29	2,3-butanedione (*diacetyl*) [c,d,e,f,g]	578	*buttery (low intensity)*
63	4-methylcyclohexanone [g]	998	
64	3,4,4-trimethyl-2-cyclopenten-1-one [g]	1064	
	Phenols		
65	phenol [f,g]	961	
66	4-methylphenol [g]	1031	
67	2-methoxyphenol (*guaiacol*) [g]	1063	*smoked, sweet (low intensity)*
68	4-hydroxy-3-methoxybenzaldehyde (*vanillin*) [c,d,e,f,g]	1366	*vanilla-like*
	Pyrazines		
51	2,5-dimethyl-3-(3-methylbutyl)pyrazine [g]	1289	

	Pyridines	
69	2-pyridinamine [g]	803
	Pyrones	
70	3-hydroxy-2-methyl-4-pyrone (*maltol*)[d,g]	1086
	Pyrroles	
71	2,3-dimethyl-1*H*-pyrrole [g]	804
	Thiazoles	
72	4,5-dihydro-2-methylthiazole [g]	1151

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

Four other heterocycles; 2,3-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2-isopropyl-3-methoxypyrazine were identified (Table 3.6). Tetramethylpyrazine, the most abundant pyrazine in dark chocolate at >6 ppm, exhibited *milk coffee-mocha-roasted* notes.

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

3.10 CONCLUSION

Chocolate flavour resides in not only a volatile aromatic fraction of flavour-active components but also in non-volatile compounds influencing taste perception. Its complex composition depends on the cocoa bean genotype specifically on contents of bean storage proteins, polysaccharides and polyphenols. The inheritance and regulation of such flavour origins remains an area for advanced research. Following, critical review of the entire process, a summary of the parameters important for chocolate flavour generation has been developed (Fig. 3.7). An appropriate starting composition can be converted through controlled post-harvest treatments and subsequent processing technologies to a high quality flavour character. Cocoa bean fermentation is crucial not only to the formation of key volatile fractions (alcohols, esters, and fatty acids) but also provision of flavour precursors (amino acids and reducing sugars) for important notes contributing to chocolate characters. Drying reduces levels of acidity and astringency in cocoa nibs by decreasing the volatile acids and total polyphenols. Maillard reactions in roasting convert flavour precursors formed during fermentation into two main classes of flavour-active component: pyrazines and aldehydes. Although no new key odourants are synthesized during conching, levels of 2-phenyl-5-methyl-2-hexenal, furaneol, and branched pyrazines significantly increase and form key odourants in both milk and dark chocolates, while Strecker aldehydes are lost by evaporation.

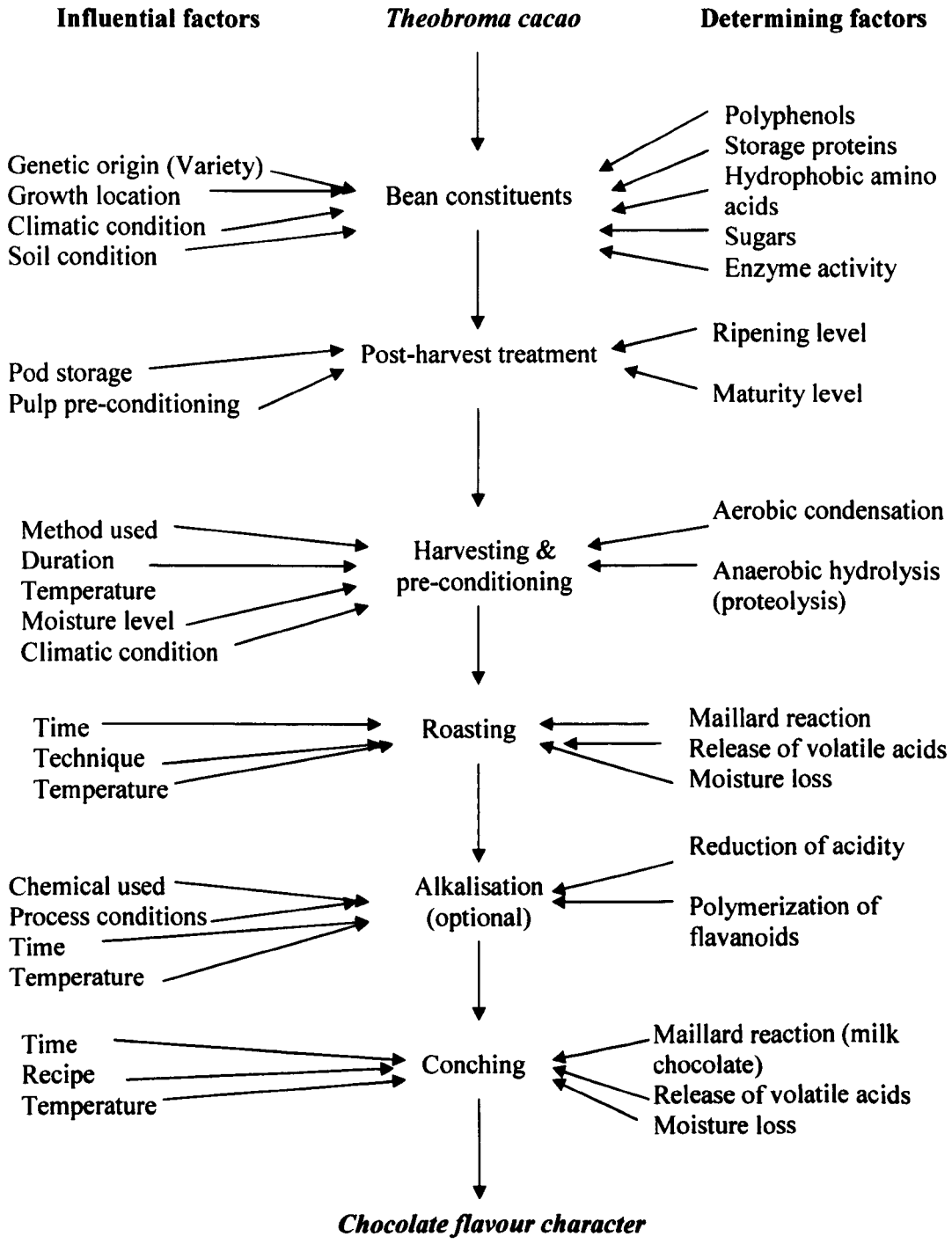


Figure 3.7. Mechanism of chocolate flavour formation and development process

These processes suggest an important role of conching in chocolate manufacture in determining final flavour characters. Direct relationships are thus observed between the initial composition and post-harvest treatments (fermentation and drying) of cocoa beans and subsequent processing (roasting and conching) and technological effects on the flavour formation, development and character in chocolate.

CHAPTER 4

EFFECTS OF PARTICLE SIZE DISTRIBUTION AND COMPOSITIONAL VARIATIONS ON THE PHYSICAL (RHEOLOGICAL, TEXTURAL AND MELTING) PROPERTIES AND THEIR RELATIONSHIPS WITH MICROSTRUCTURE IN DARK CHOCOLATE SYSTEMS

Results from this chapter have been published in six research articles, namely:

1. Effects of particle size distribution and composition on rheological properties of dark chocolate. *European Food Research and Technology* (2008) 226: 1259-1268. doi.10.1007/s00217-007-0652-6. (Afoakwa et al., 2008).

2. Comparative evaluation of rheological models used for evaluating dark chocolate viscosity. *International Journal of Food Science and Technology,* (Published Online, July 2008). doi:10.1111/j.1365-2621.2008.01710.x. (Afoakwa et al., 2008).

3. Particle size distribution and compositional effects on textural properties and appearance of dark chocolates. *Journal of Food Engineering, 87,* 181 - 190. doi:10.1016/ j.jfoodeng.2007.11.025. (Afoakwa et al., 2008).

4. Microstructure and mechanical properties related to particle size distribution and composition in dark chocolate. *International Journal of Food Science and Technology.* (Published Online, July 2008). doi:10.1111/j.1365-2621.2007.01677.x. (Afoakwa et al., 2008).

5. Characterization of melting properties in dark chocolates from varying particle size distribution and composition using Differential Scanning Calorimetry. *Food Research International* 41 (2008) 751–757. doi:10.1016/j.foodres.2008.05.009. (Afoakwa et al., 2008).

6. Relationships between rheological, textural and melting properties of dark chocolates as influenced by particle size distribution and composition. *European Food Research and Technology, 227* (4) 1215-1223. doi. 10.1007/s00217-008-0839-5. (Afoakwa et al., 2008).

Parts of this chapter have been presented at two conferences:

1. Comparative evaluation of rheological models used for evaluating dark chocolate viscosity. Paper presented at the First International Chester Conference of Food Science and Technology, Chester, UK. April 10-13, 2007 (Oral presentation).

2. Relationship between rheological and textural properties of dark chocolates as influenced by particle size distribution and composition. Paper Presented at the Annual Meeting of the Institute of Food Technologists, McCormick Place Convention Center, Chicago, Illinois, USA. July 28 – August 1, 2007 (Poster presentation).

4.1 ABSTRACT

Effects of PSD and composition on rheological, textural and melting properties of dark chocolates were investigated using multivariate statistics to explore interrelationships. Levels were: PSD [D_{90} (90% finer than this size)] 18, 25, 35 and 50 μm; fat – 25, 30 and 35%); and lecithin - 0.3 and 0.5%. Instruments utilised included a shear rate-controlled rheometer, TA.HD Plus Texture Analyzer and differential scanning calorimeter (DSC). Surface colour was evaluated in terms of CIELAB parameters L*, C* and h° using a HunterLab Miniscan Colorimeter and microstructure of products determined using light microscopy. Levels of PSD, fat and lecithin content significantly affected all rheological parameters, with significant interaction among factors. Increasing particle size gave significant reductions in all rheological and textural properties with greatest effect noted at 25% fat and 0.3% lecithin, then inversely related to fat and lecithin contents. PSD and fat concentration also influenced melting characteristics and colour (L*, C* and h°). Micrographs revealed PSD and fat level induced wide variations in sugar crystalline network structure and inter-particle interaction: 25% fat yielded more crystal agglomerates, well flocculated with greater particle-to-particle interaction strengths than those with higher (30% and 35%) fat contents. Increasing PSD to 35-50 μm resulted in particles becoming coarser at all fat levels. Fat showed the greatest effect on the variability in each property followed by PSD and lecithin. Analyses showed high correlation (r = 0.89-1.00) and regression coefficients (R^2 = 0.84-1.00). The newer ICA technique gave higher correlation and regression coefficients than the Casson model but was highly related and either could effectively quantify dark chocolate viscosity parameters. High correlation (r = 0.78-0.99) and regression coefficients (R^2 = 0.59-0.99) were observed among rheological, textural and melting index. As PSD, fat and lecithin could be manipulated to control dark chocolate rheology, texture, appearance and melting character it would be possible to influence quality whilst reducing production cost.

4.2 INTRODUCTION

Dark chocolate is a dense suspension of solid particles on average 65-75% sugar and non-fat cocoa solids, dispersed in a fat continuous phase, mostly of cocoa butter. During manufacture, refining and conching determine particle size (PS) and suspension consistency and viscosity, to yield specific textural and sensory qualities (Beckett, 2000; Afoakwa et al., 2007).

Rheologically, molten chocolates behave as non-Newtonian liquids with yield stress (minimum amount of energy to initiate fluid flow) and plastic viscosity (energy to keep fluid in motion), dependent on processing. Quality in finished chocolates is highly dependent on inherent size distribution of solid particles from sugar, milk and cocoa, composition of fat phase and emulsifiers (Beckett, 2003; Ziegleder, 1992). Rheological properties determine efficiency of mixing, pumping and transportation of finished products during processing. Servais et al. (2004) concluded that the control of chocolate rheology is important for quality and exact weight control during enrobing, shell making and molding processes. Processing parameters such as conching, particle size distribution (PSD), fat content, emulsifiers (lecithin and polyglycerol polyricinoleate [PGPR]), temper, vibrations and temperature all influence rheological properties and production cost (Tscheuschner & Wunsche, 1979; Beckett, 1999; Vavreck, 2004; Schantz & Rolm, 2005).

Of techniques for characterising rheological properties, the International Confectionery Association (ICA, previously IOCCC) suggests use of rotational viscometers with concentric cylinders (bob and cup geometry) and the Casson equation (International Confectionery Association, 2000; Bouzas et al., 1995; Sokmen & Gunes, 2006; IOCCC, 1973), with measurement of stress and viscosity at shear rates between 2 s^{-1} and 50 s^{-1} using up and down curves, preceded by a pre-shear at 5 s^{-1} of >5 min (Servais et al., 2004).

Important are the rheological models of Herschel-Bulkley, Casson and Bingham (Chevalley, 1999; Beckett, 2000; Sokmen & Gunes, 2006), following the equations:

Herschel-Bulkley: $\tau = \tau_0 + \eta_{pl} \cdot (\dot{\gamma})^n$ ..... (Eqn. 4.1)

Casson: $\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\eta_{CA}} \cdot \sqrt{\dot{\gamma}}$ ..... (Eqn. 4.2)

Bingham: $\tau = \tau_0 + \eta_{pl} \cdot \dot{\gamma}$ ..... (Eqn. 4.3)

(τ: shear stress; τ_0: yield stress; η_{pl} : plastic viscosity; τ_{CA}: Casson yield value; η_{CA}: Casson plastic viscosity; $\dot{\gamma}$: shear rate; η: viscosity of the suspension; n: flow viscosity index). Since 1973, the International Confectionery Association (ICA) has accepted rheological measurement of molten chocolate using rotational viscometers with concentric cylinders (bob and cup geometry) and Casson equation calculation of the parameters (IOCCC, 1973; Bouzas & Brown, 1995). In 2000 ICA recommended measurement of stress and viscosity at shear rates between 2 s^{-1} and 50 s^{-1} using up and down curves in shear rate, preceded by a pre-shear at 5 s^{-1} lasting $\geq$ 5 min (ICA, 2000).

The basis for change in 2000 was results from an inter-laboratory study (Aeschlimann & Beckett, 2000), that concluded that the Casson's mathematical model employing only a small set of parameters was limited in accuracy as, at lower shear rates, rheology data does not fit the Casson equation well. The outcome was a low degree of repeatability in inter-laboratory analyses, and ICA thus recommended use of interpolation data for chocolate viscosity. Servais et al. (2004) noted this strategy was simple, accurate and readily applicable to different systems, given a basis of relevant information. In the USA, the current National Confectioners Association/Chocolate Manufacturers Association (NCA/CMA) method for chocolate rheological properties is to extrapolate concentric cylinder flow data using the Casson equation (Baker et al., 2006). This conflicts with the ICA quantification strategy (ICA, 2000) and therefore requires an understanding of their inter-relationships.

PSD, central to rheological properties, has a direct influence on sensory character. The largest particles (D_{90}) are important for mouth-feel notably grittiness, but smaller particles influence flow properties (Beckett, 2000, Beckett, 2003; Mongia & Ziegler, 2000; Ziegler et al., 2001). A small proportion of particles up to 65 μm give an improved texture for milk chocolate. Good dark chocolate requires a maximum PS of 35 μm (Awua, 2002), and at solids > 61 % by volume and PSD > 35 μm, the quality becomes unacceptable due to high viscosity and poor texture (Beckett, 1999). Limit values are determined by targets for character and product composition. Generally, chocolate viscosity is controlled by addition of cocoa butter and expensive viscosity modifiers (surface active ingredients *e,g.* soy lecithin and PGPR). The optimum for average sugar particle size is cultural, in the US 30–33 μm with a maximum of 50 μm and in Europe 20–23 μm and 35–40 μm, respectively (Jeffery, 1993). Benefits of PSD optimisation include reductions in viscosity modifiers. There is no general agreement on the central role of PSD in suspension flow properties, with Awua (2002) and Whitefield (2005) arguing other factors influence rheology. Modification of suspension viscosity by changing PSD merits further investigation together with compositional factors that contribute to rheological properties during manufacture.

Chocolate texture and appearance are key attributes in consumer choice and acceptability even though flavour is frequently judged important in product identification (Beckett, 2003; Whitefield, 2005). Although texture perception is a dynamic oral process before and during mastication, individuals also perceive texture through vision, touch, and hearing (Heath & Prinz, 1999; Kilcast, 1999; Wilkinson et al., 2000). Chocolate texture can also be evaluated by instrumental measurements often rationalized as cheap, efficient and objective replacements or complements for sensory evaluations (Lawless & Heymann, 1998) with statistically significant correlations (Mohamed et al., 1982; Christensen, 1984; Meullenet et al., 1997; Rosenthal, 1999; Bourne, 2002). Visual information characterizing objects,

including gloss, colour, shape, roughness, surface texture, shininess and translucency, is summarized into appearance attributes. Briones et al. (2006) concluded that these emerge from complex interactions of incident light, optical characteristics and human perception. Relevant information can be acquired from modern technologies such as computer vision and calibrated colour imaging analysis, HunterLAB and CIELAB models (Lawless & Heymann, 1998; Jahns et al., 2001; Hatcher et al., 2004; Briones & Aguilera, 2005). Such LAB-based models provide close descriptions of colour attributes (Lawless & Haymann, 1998; Taylor & Hort, 2004) although Thai and Shewfelt (1991) found that L (lightness), C (chroma) and H (hue angle) from HunterLAB data, were better correlated. Given that chocolates should meet prior acquired consumer expectations, appearance attributes can have significant commercial implications.

Microstructure is a fundamental variable influencing transport phenomenon and physical properties of foods determining perceived quality in terms of mechanical and sensorial attributes (Kulozik et al., 2003). Consequently, microstructure is important for manipulation or regulation of texture and related to composition and physical forces influencing mechanical properties (van Marle et al., 1997; Afoakwa & Sefa-Dedeh, 2002). Varela et al. (2007) noted that successful delivery in new product development requires understanding of factors that influence texture. Improvement in the quality of existing foods and new product formulations require interventions at microscopic level. Most elements that critically participate in transport properties, physical and rheological behaviours, textural and sensorial characters are <100 μm in diameter (Aguilera, 2005). Bourne (2002) concluded texture is derived from food structure and relationship between microstructure, and rheological and textural properties have been studied (Remeuf et al., 2003; Pereira et al., 2003; Kulozik et al., 2003; Sandoval-Castilla et al., 2004; Braipson-Danthine & Deroanne, 2004; Christiansen et al., 2006; Baixauli et al., 2007). However, there is limited information

on relationship between microstructure and mechanical properties of finished dark chocolates specifically on how PSD and composition affect the microstructure and mechanical properties.

During chocolate manufacture, the crystalline state and the proportion of solid fat present are important in determining the melting character in finished products. Differential scanning calorimetry (DSC) has been used to characterise changes in chocolate melting profiles and measures the relative amounts of each crystalline state (Tabouret, 1987; Ziegleder & Schwingshandl, 1998; Walter & Cornillon, 2001; Walter & Cornillon, 2002); and peaks corresponding to latent heat, are observed in temperature ranges related to melting of specific polymorphs (McFarlane, 1999). Such information is relevant to sensory character and impacts on mechanical and rheological properties of chocolate and confectionery shelf life (Hartel, 2001).

As demand for dark chocolate products is increasing in global markets, understanding the factors influencing the rheological, textural and melting properties as well as appearance would be of value in predicting changes in quality. The acquired information would impact on process improvement and new product development in dark chocolate manufacture. The objectives of this work were to;

i. investigate effects of PSD and composition (fat and lecithin) on rheological behaviour of molten dark chocolate characterised using steady shear measurements.

ii. study the relationship between the two models (Casson's model and the new ICA recommendations) for estimating dark chocolate rheological parameters.

iii. evaluate effects of particle size distribution and compositional variations on textural properties and appearance of dark chocolates.

iv. determine the influence of PSD and fat content on dark chocolate microstructure and how it relates to the rheological and mechanical properties of dark chocolates.

v. characterise the effects of particle size distribution, fat and lecithin content on the crystallinity and melting profiles of finished dark chocolates.

vi. explore relationships between rheological, textural and melting properties of dark chocolate as influenced by PSD of solids and composition during processing.

4.3 MATERIALS AND METHODS

4.3.1 Materials

Cocoa liquor of Central West African Origin was obtained from Cargill Cocoa Processing Company (York, UK). Sucrose (pure fine granulated) from British Sugar Company (Peterborough, UK); pure prime pressed cocoa butter and soy lecithin from ADM Cocoa Limited (Koog aan de Zaan, Netherlands) and Unitechem Company Ltd. (Tianjin, China) respectively.

4.3.2 Preparation of chocolate samples

Dark chocolate formulations (Table 4.1) from sucrose, cocoa liquor, cocoa butter and emulsifier (lecithin) used total fat contents between 25% (w/w) and 35% (w/w) (from cocoa liquor and cocoa butter) and a minimum of 35% total cocoa solids, to ensure conformation with standard of identity for dark chocolate of the Codex Revised Standard (2003) on Chocolate and Chocolate Products, and European Commission Directive 2000/36/EC on Cocoa Products and Chocolate (European Commission Directive, 2000).

Experimental samples (5 kg batch for each formulation) were produced by mixing sucrose and cocoa liquor in a Crypto Peerless Mixer (Model K175, Crypto Peerless Ltd, Birmingham, UK) at low speed for 2 min and then high speed for 3 min, refining using a 3-roll refiner (Model SDX 600, Buhler Ltd., CH-9240 Uzwil, Switzerland) to pre-determined

particle sizes (D_{90} :18 μm ± 1 μm, 25 μm ± 1 μm, 35 μm ± 1 μm & 50 μm ± 1 μm) confirmed by particle size analysis.

Table 4.1. Recipes used for the formulation of the dark chocolate

Ingredient	25% Fat [% (w/w)]		30% Fat [% (w/w)]		35% Fat [% (w/w)]	
Sucrose (%)	58.8	59.0	49.7	49.9	40.7	40.8
Cocoa liquor (%)	35.9	35.5	45.0	44.6	54.0	53.7
Cocoa butter (%)	5.0	5.0	5.0	5.0	5.0	5.0
Lecithin (%)	0.3	0.5	0.3	0.5	0.3	0.5

Refined chocolates were stored in plastic containers and conditioned at ambient temperature (20-22 °C) for 24 h prior to conching in a Lipp Conche (Model IMC-E10, Boveristr 40-42, D-68309, Mannhein, Germany) at low speed for 3.5 h at 60 °C. Lecithin and cocoa butter were then added and mixtures conched at high speed for further 30 min for mixing and liquefaction. Samples were stored in sealed plastic containers at ambient temperature (20 ± 2 °C) and moisture and fat contents determined using Karl Fischer and Soxhlet methods (ICA, 1988) and (ICA, 1990) respectively.

4.3.3 Determination of particle size distribution

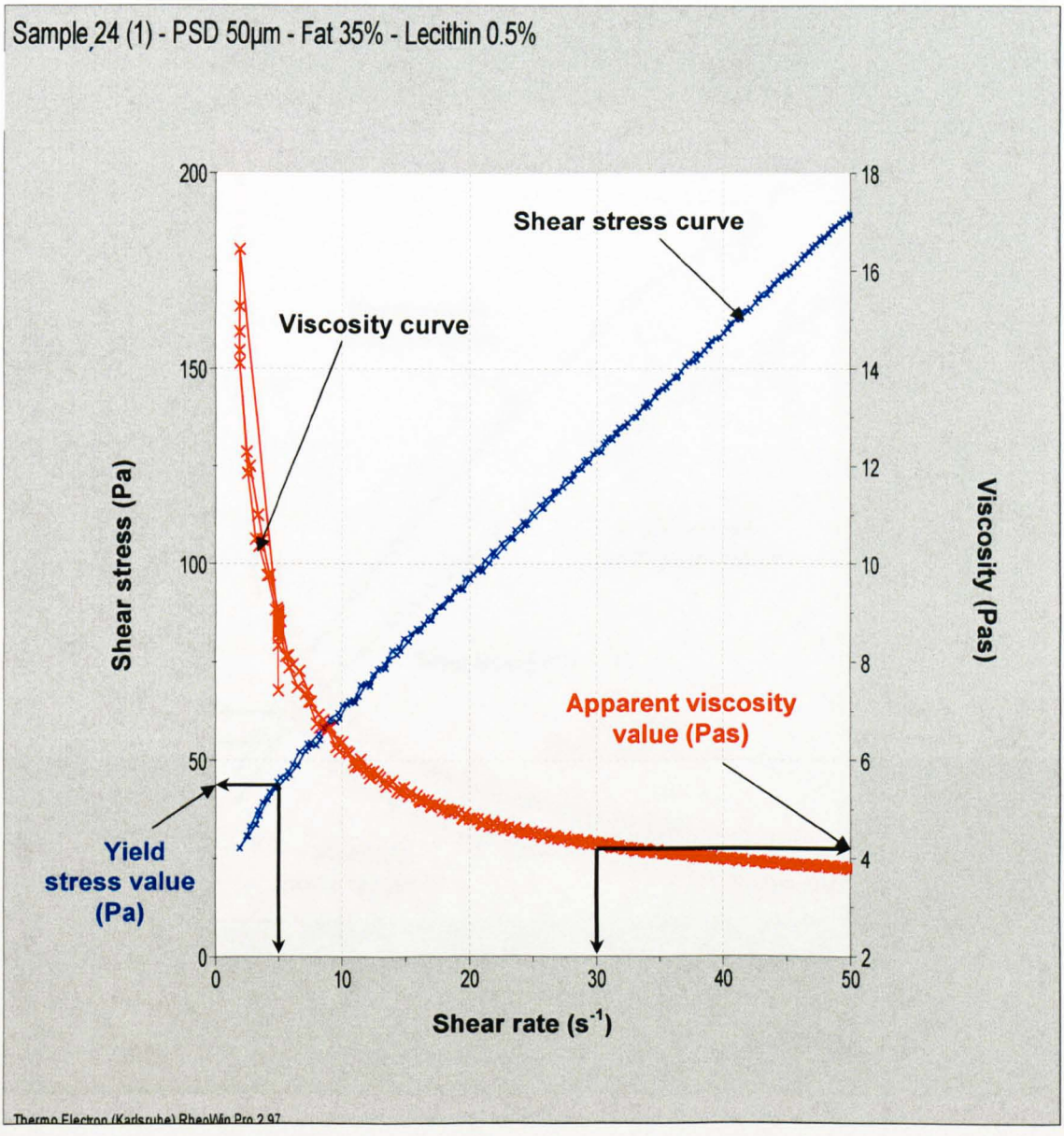
A MasterSizer® Laser Diffraction Particle Size Analyzer equipped with MS 15 sample presentation unit (RI 1.590) (Malvern Instrument Ltd., Malvern, England) was used. About 0.2 g of refined dark chocolate was dispersed in vegetable oil (RI 1.450) at ambient temperature (20 ± 2 °C) until an obscuration of 0.2 was obtained. Ultrasonic dispersion for 2 min to ensure particles were independently dispersed was maintained by stirring. Size

distribution was quantified as relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Micro Software v 2.19). PSD parameters obtained included specific surface area, largest particle size (D_{90}), mean particle volume (D_{50}), smallest particle size (D_{10}), Sauter mean diameter (D[3,2]) and mean particle diameter (D[4,3]). The four PSD used for the study is as shown (Table 2).

4.3.4 Rheological measurements

The rheological behaviour of molten dark chocolate was characterised using steady shear measurements. All measurements were carried out in shear rate-controlled rheometer (Thermo Haake ViscoTester 550 (VT 550), Thermo Electron Corp., Karlsruhe, Germany) using bob and cup (Recessed end) geometry (Sensor SVI and SVII), as for IOCCC method (Aeschlimann & Beckett, 2000) with a ratio of inner to outer radius of 0.92 in the concentric cylinder system. Samples were incubated at 50 °C for 75 min for melting and transferred, pre-sheared at 5 s^{-1} rate for 15 min at 40 °C, before measurement cycles. Shear stress was measured at 40°C as function of increasing shear rate from 5 to 50 s^{-1} (ramp up) within 120 s, then decreasing from 50 to 5 s^{-1} (ramp down), within each ramp 50 measurements were taken. Viscosity was also measured as a function of increasing shear rate from 5 to 50 s^{-1} (ramp down) within 120 s, then decreasing from 50 to 5 s^{-1} (ramp up), within each ramp 50 measurements were taken. Temperature of the chocolates samples was controlled during the experiment using Haake K20 Thermo-regulator (Thermo Electron Corp., Karlsruhe, Germany). Mean value and standard deviation of triplicate readings were recorded. Casson plastic viscosity and Casson yield values were calculated using Casson model (Equation 4.2) and interpolation data from the viscosity and shear stress graphs (Fig. 4.1) respectively using ThermoHaake RheoWin Pro 297 Software by the least square method.

a.


Sample 24 (1) - PSD 50µm - Fat 35% - Lecithin 0.5%
Shear stress curve
Viscosity curve
Apparent viscosity value (Pas)
Yield stress value (Pa)
Shear stress (Pa)
Viscosity (Pas)
Shear rate (s-1)
200
150
100
50
0
18
16
14
12
10
8
6
4
2
0
10
20
30
40
50
Thermo Electron (Karlsruhe) RheoWin Pro 2.97

b.

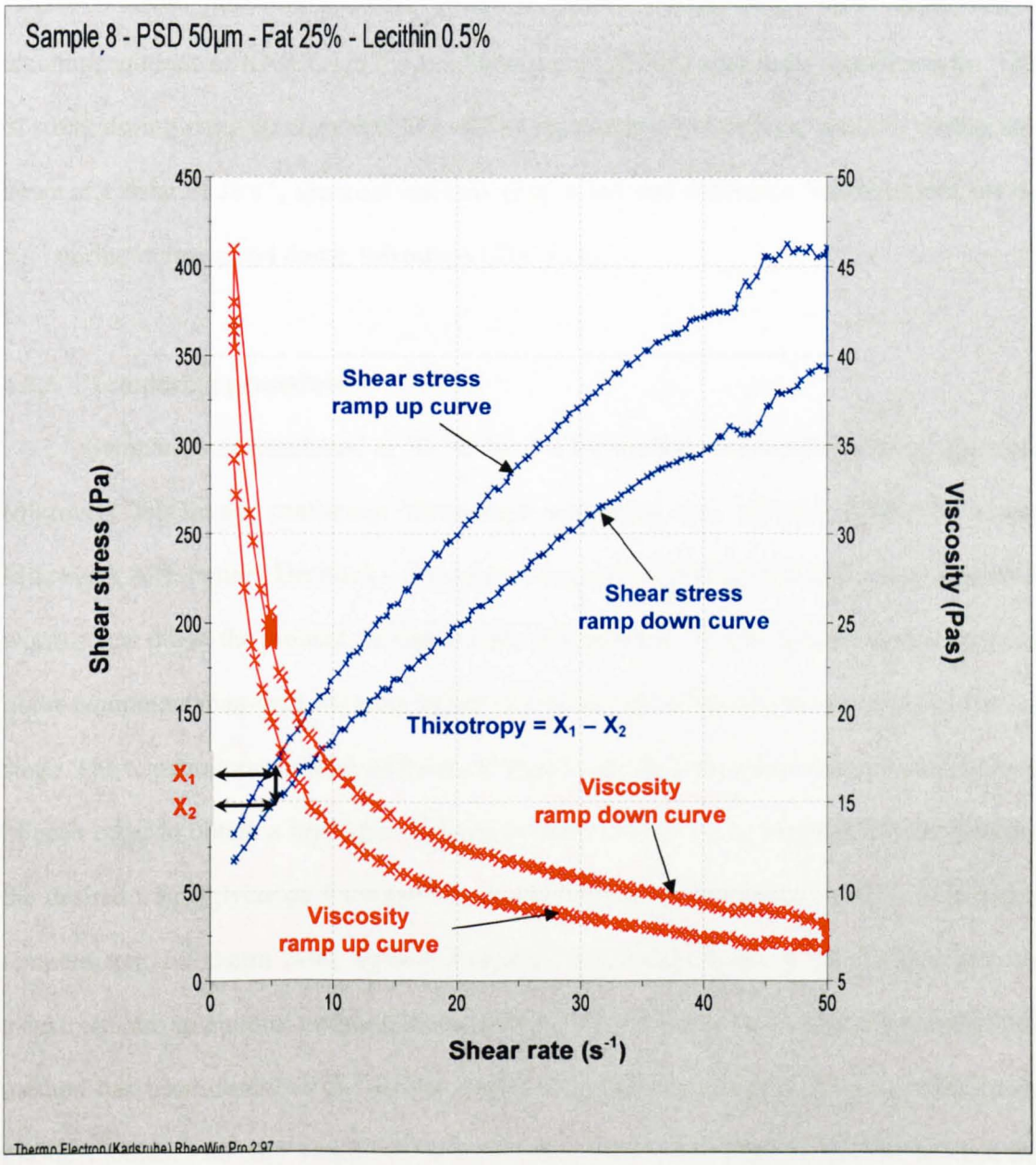


Figure 4.1. Typical rheology graph illustrating measurement of (a) apparent viscosity and yield stress (b) thixitropy from two dark chocolates containing (a) 50 µm PS, 35% fat and 0.5% lecithin, (b) 50 µm PS, 25% fat and 0.5% lecithin

Figure 4.1 shows how the new ICA rheological parameters (yield stress and apparent viscosity) and thixotropy were deduced from interpolation data according to recommendations of IOCCC (1973) and Servais et al. (2004) with some modifications. Value of stress during ramp up at a shear rate of 5 s^{-1} represented yield stress, viscosity during ramp down at a shear of 30 s^{-1}, apparent viscosity (Fig. 4.1a); and difference between yield stress at 5 s^{-1} during ramp up and down, thixotropy (Fig. 4.1b).

4.3.5 Tempering procedure

Samples were incubated at 50 °C for 4 h for melting and tempered using an Aasted Mikrovert laboratory continuous three-stage tempering unit (Model AMK 10, Aasted Mikroverk A/S, Farum, Denmark). Chocolate was pumped through the multi-stage units and a worm screw drove the product through the heat exchangers. Sensors located at specific points in the equipment measured the temperature of both the chocolate and the coolant fluid at each stage. The temperatures of each of the three stages were thus set and controlled independently of each other to obtain a final chocolate temperature of ~ 27 °C to promote crystal growth of the desired triacylglyceride fractions. Pre-crystallisation was measured using a computerised tempermeter (Exotherm 7400, Systech Analytics, SA, Switzerland). A built-in algorithm was used to ensure an optimal temper regime of Slope 0 ± 0.3 (5.0 ± 1 CTU). The principle of this method has been described by Nelson (1999). The tempered chocolate was moulded using plastic moulds: 80 mm length; 20 mm breadth; and 8 mm height, allowed to cool in a refrigerator (12 °C) for 2 h before de-moulding onto plastic trays and conditioned at 20 ± 2 °C for 14 days before analysis.

4.3.6 Texture measurements

Texture of molten chocolates was evaluated using a TA-HD Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, England) with back extrusion rig and a 35 mm diameter compression disk attached to an extension bar using 50 kg load cell (Fig. 4.2a). Samples melted at 50°C for 75 min were quickly transferred to a standard back extrusion container (50 mm diameter) and work done in back extruding 100 mL chocolate determined by measuring force in compression. Eight replications were made at a pre-test 1.0 mm/s, test of 0.5 mm/s and post-test 10.0 mm/s at 50 mm above sample surface, penetrating 30 mm for 60 sec, then returning to the start position. Mean values were used to obtain a force-time graph (XT.RA Dimension, Exponent 32 software; Stable Micro Systems) as shown on Figure 4.3a by calculating as texture parameters:

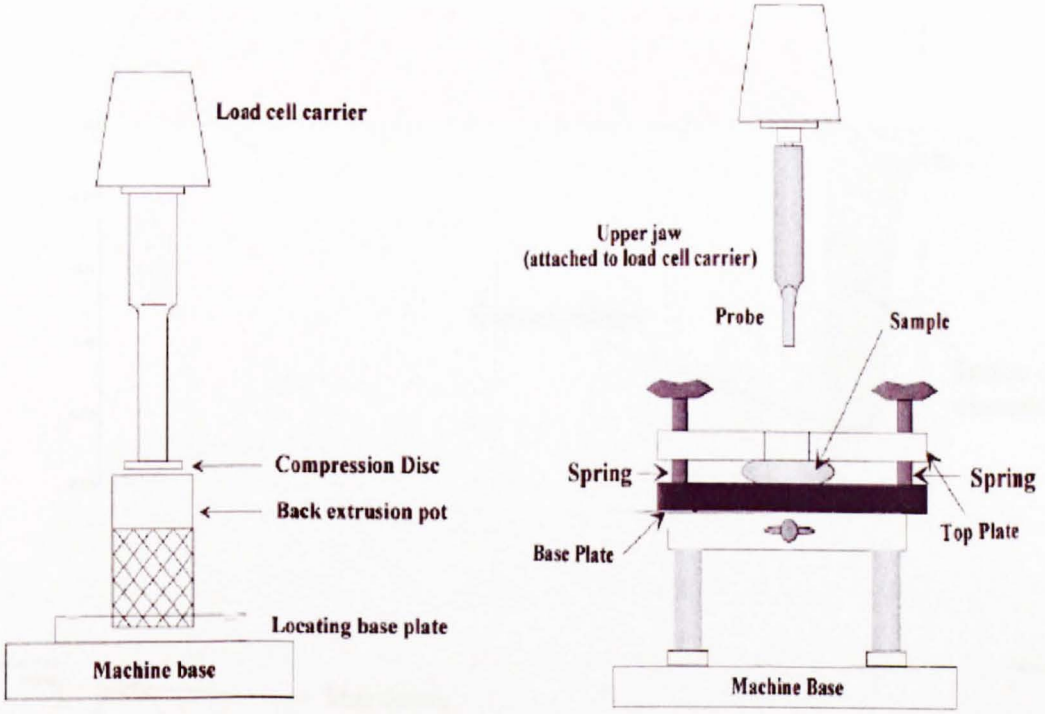
i. Firmness = maximum compression force in extrusion thrust into sample (g).

ii. Consistency = area within curve during extrusion thrust (g.s).

iii. Cohesiveness = maximum compression force during withdrawal of probe from sample (g).

iv. Index of viscosity = area within negative region of curve during probe withdrawal (g.s).

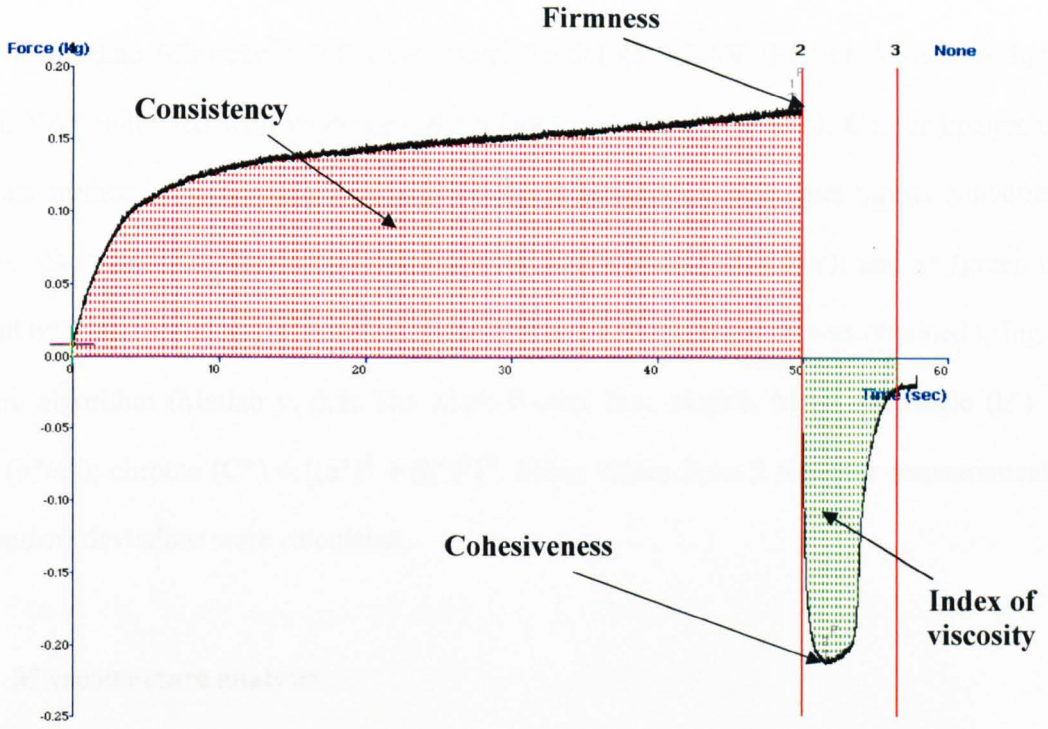
Hardness of solid tempered chocolate was measured using TA-HD Plus Texture Analyzer with a penetration probe (needle P/2) attached to an extension bar and a 50 kg load cell and a platform (Fig. 4.2b). Maximum penetration force through a sample (80 x 20 mm, depth 8 mm) was determined with 8 replications at a pre-speed of 1.0 mm/s, test of 2.0 mm/s, post speed of 1.0 mm/s, penetrating 6 mm for 3 sec at 20°C, converting mean values into hardness data on a force-time graph using XT.RA Dimension, Exponent 32 software (Stable Micro Systems, Godalming, Surrey, UK) as shown on Figure 4.3b.



a b

Figure 4.2. Back extrusion rig (a) and puncture test rig (b) used for texture measurements of molten and solid chocolates respectively.

a.



b.

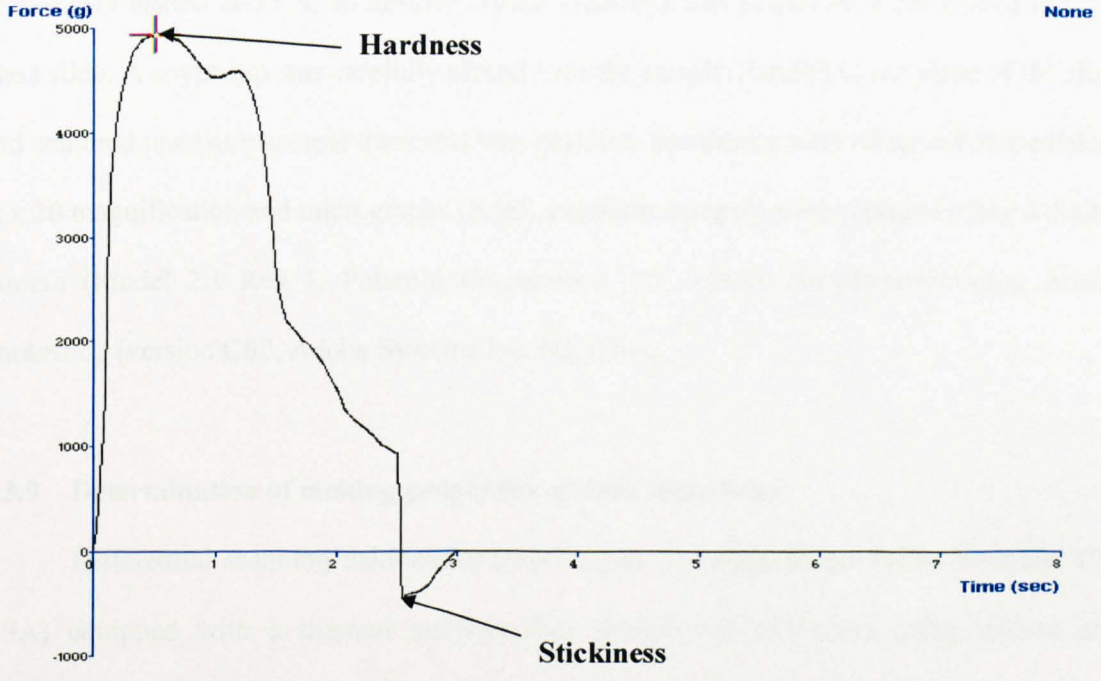


Figure 4.3. Typical (a) back extrusion curve and (b) penetration probe curve used for the measurement of molten and solid dark chocolates respectively.

4.3.7 Colour measurements of solid dark chocolate

HunterLab Miniscan^{TM} XE Colorimeter Model 45/0 LAV (Hunter Associates Inc., Reston, VA) calibrated with white ceramic reference standard was used. Colour images of chocolate surfaces were converted into XYZ tristimulus values, which were further converted to CIELAB system: L*, luminance ranging from 0 (black) to 100 (white); and a* (green to red) and b* (blue to yellow) with values from -120 to +120. Information was obtained using a software algorithm (Matlab v. 6.5; The Math-Works, Inc., Natick, MA): hue angle (h°) = arctan (b*/a*); chroma (C*) = $[(a^*)^2 + (b^*)^2]^{½}$. Mean values from 5 replicate measurements and standard deviations were calculated.

4.3.8 Microstructure analysis

Microstructures were observed using a high resolution polarized light microscope (Olympus Optical U-PMTVC, Tokyo, Japan). One drop (10 µl) of molten chocolate (previously heated at 55 °C to destroy crystal memory) was placed on a pre-heated (55 °C) glass slide. A cover slip was carefully placed over the sample, parallel to the plane of the slide and centered to ensure sample thickness was uniform. Specimens were observed immediately at x 20 magnification and micrographs (black and white images) were captured using a digital camera (Model 2.1 Rev 1, Polaroid Corporation, NY, USA) and observed using Adobe Photoshop (version CS2, Adobe Systems Inc. NJ, USA).

4.3.9 Determination of melting properties of dark chocolates

Differential scanning calorimeter (DSC Series 7, Perkin Elmer Pyris, Norwalk, CT, USA) equipped with a thermal analysis data station was calibrated using indium and octadecane at a scan rate of 5 °C/min using an aluminium pan as reference. Samples (~ 5 mg) were loaded into 40 µl capacity pans with holes and sealed with lids using a sample press.

Pans were heated at 5 °C/min from 15-55 °C in a N_2 stream. Onset temperature (T_{onset}), peak temperature (T_{peak}), end temperature (T_{end}) and enthalpy of melting (ΔH_{melt}) were calculated automatically by the software. Melting index (T_{index}) was computed as (T_{end} - T_{onset}), as described by Vasanthan and Bhatty (1996). Each sample was analyzed in triplicate and mean values and standard deviations reported.

4.3.10 Experimental design and statistical analysis

Three key experimental variables were PSD, fat and lecithin contents with other variables including refiner temperature and pressure, conching time and temperature, and cocoa butter (5% (w/w)) held constant. A 4 x 3 x 2 factorial experimental design was used with principal factors: particle size distribution (D_{90}): 18, 25, 35 and 50 μm; fat content: 25, 30 and 35% (w/w); lecithin content: 0.3 and 0.5% (w/w).

Statgraphics Plus 4.1 (Graphics Software System, STCC, Inc, Rockville, USA) was used to examine the rheological properties (Casson plastic viscosity and yield values, apparent viscosity, yield stress and thixotropy), textural properties (firmness, consistency, cohesiveness, index of viscosity of molten chocolate and hardness of tempered chocolate), colour [lightness (L*), chromaticity (C*) and hue angle (h°)] and melting properties (melting onset (T_{onset}), melting end (T_{end}), melting index (T_{index}) and peak melting (T_{peak}) and melting enthalpy (ΔH_{melt}) using two-way analysis of variance (ANOVA) and multiple range tests to determine effects of PSD, fat and lecithin contents and their interactions. Tukey multiple comparisons at 95% significance level were conducted to determine differences between factor levels. Multivariate statistical techniques comprising regression, correlation and principal component analyses were used to evaluate the relationships between the rheological, textural and melting parameters. All process treatments and analysis were conducted in three replicates and the mean values reported.

4.4 RESULTS AND DISCUSSION

4.4.1 Particle size distribution of molten dark chocolate

The PSD parameters from the four particle sizes are presented in Table 4.2. Wide variations in PSD were produced with intervals ranging between 18 μm, 25 μm, 35 μm and 50 μm using D_{90}, (90% finer than this size). The D_{90} value was used as it has been reported to correlate fairly on sensory character with micrometer measurements made of the biggest particles (Beckett, 2000). Figures 4.4 a – d show volume histograms of samples with size distributions of narrow bimodal distribution for 18 μm PS (Fig. 4.4a), wide bimodal distribution for the 25 μm PS (Fig. 4.4b), narrow multimodal distribution for 35 μm PS (Fig. 4.4c), and a wide multimodal distribution for 50 μm (Fig. 4.4d). Such PSD, ranging from fine (18 μm) to coarse particles (50 μm), cover optimal minima and maxima (Beckett, 2000, 2003; Ziegler & Hogg, 1999).

Data from the PSD parameters (Table 4.2) showed variations in specific surface area, mean particle volume D(v,50) Sauter mean diameter (D[3,2]) and mean particle diameter (D[4,3]) with increasing D_{90} particle sizes. Increasing D_{90} from 18 to 50 μm led to significant ($p \leq 0.05$) reduction in specific surface area, with increases in Sauter mean and mean particle diameter (Table 4.2) that indicate that the largest PS (D_{90}) is directly proportional to the D_{10}, D_{50}, Sauter mean diameter (D[3,2]) and mean particle diameter (D[4,3]), and inversely proportional to specific particle surface area.

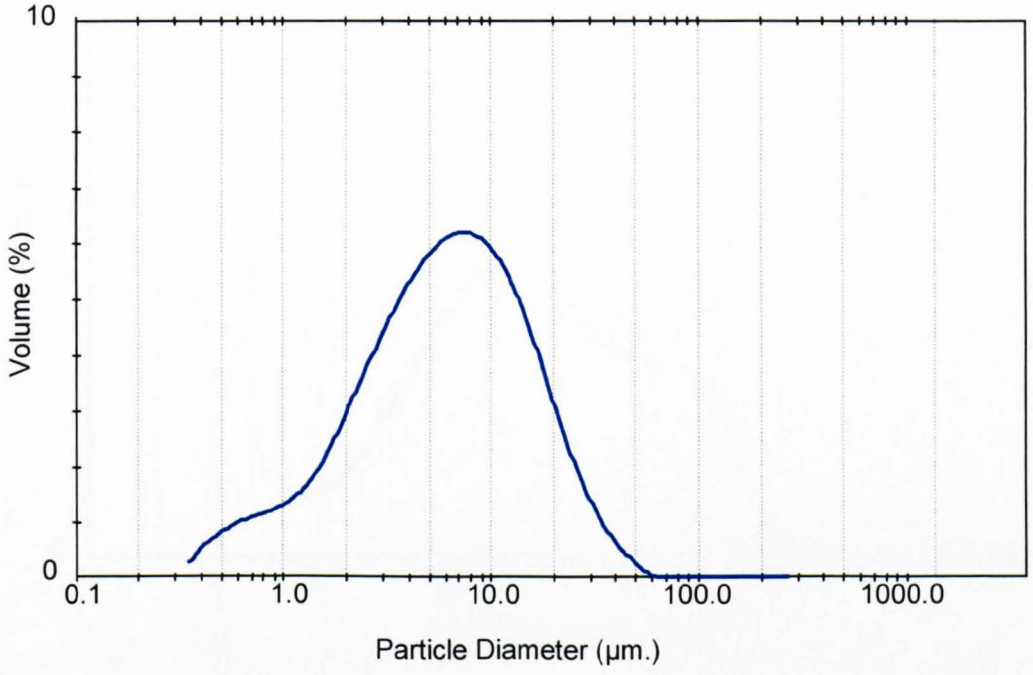
Similarly, increasing fat content from 25% to 35% led to significant ($p<0.05$) reduction in specific surface area with increases in other PSD parameters (Table 4.3), suggesting fat content at refining had a direct influence on PSD. Reduction in sugar levels with increased fat content, influence PSD.

Table 4.2. Particle size distribution of the dark chocolates

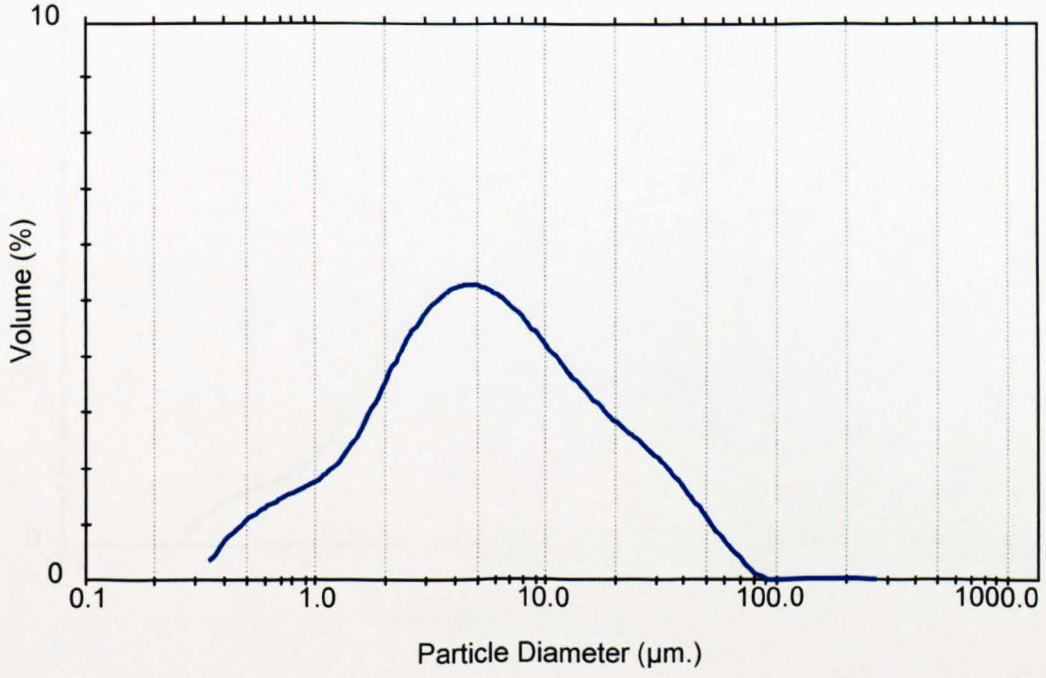
Particle size (PS) $d(0.9)^a$ (μm)	Fat content (%)	Particle size distribution					
		Specific surface area (m^2g^{-1})	D(v,0.1) [a] (μm)	D(v,0.5) [a] (μm)	D[3,2] [a] (μm)	D[4,3] [a] (μm)	D(v,0.9) [a] (μm)
18 ± 1.0	25	1.97 ± 0.04	1.09 ± 0.03	4.72 ± 0.05	2.61 ± 0.04	7.80 ± 0.06	18.71 ± 0.19
	30	1.89 ± 0.03	1.04 ± 0.03	4.89 ± 0.05	2.63 ± 0.04	8.02 ± 0.05	18.72 ± 0.46
	35	1.54 ± 0.03	1.39 ± 0.04	6.03 ± 0.06	3.17 ± 0.05	8.44 ± 0.03	18.60 ± 0.24
25 ± 1.0	25	1.65 ± 0.06	1.22 ± 0.04	5.62 ± 0.04	2.92 ± 0.04	10.28 ± 0.07	25.60 ± 0.04
	30	1.58 ± 0.02	1.24 ± 0.02	5.79 ± 0.06	3.02 ± 0.03	10.32 ± 0.14	25.53 ± 0.65
	35	1.45 ± 0.02	1.45 ± 0.05	6.63 ± 0.06	3.43 ± 0.06	10.39 ± 0.08	25.06 ± 0.32
35 ± 1.0	25	1.46 ± 0.04	1.40 ± 0.04	6.59 ± 0.07	3.36 ± 0.03	13.35 ± 0.08	35.53 ± 0.14
	30	1.42 ± 0.02	1.46 ± 0.03	6.70 ± 0.02	3.49 ± 0.02	13.36 ± 0.07	35.59 ± 0.27
	35	1.28 ± 0.05	1.68 ± 0.06	7.54 ± 0.06	3.85 ± 0.05	13.55 ± 0.09	35.39 ± 1.14
50 ± 1.0	25	1.30 ± 0.01	1.59 ± 0.03	7.69 ± 0.03	3.74 ± 0.05	17.46 ± 0.05	50.16 ± 0.65
	30	1.26 ± 0.04	1.68 ± 0.03	7.97 ± 0.05	3.80 ± 0.06	17.58 ± 0.06	50.41 ± 0.79
	35	1.10 ± 0.04	2.04 ± 0.04	9.08 ± 0.09	4.47 ± 0.06	17.90 ± 0.05	50.08 ± 0.48

Mean values ± standard deviations from triplicate analysis. [a] D(v,0.1), D(v,0.5), D[3,2], D[4,3] and D(v,0.9) respectively represent 10%, 50%, Sauter mean diameter, mean particle diameter and 90% of all particles finer than this size.

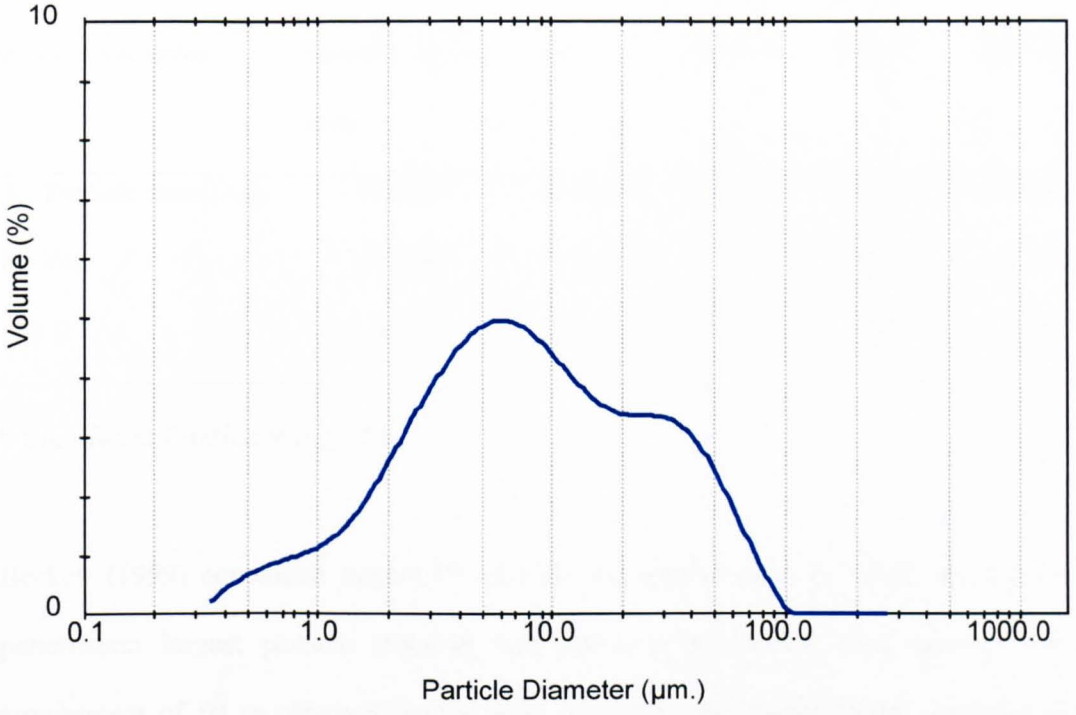
a.


10
Volume (%)
0
0.1
1.0
10.0
100.0
1000.0
Particle Diameter (µm.)

b.


10
Volume (%)
0
0.1
1.0
10.0
100.0
1000.0
Particle Diameter (µm.)

c.



d.

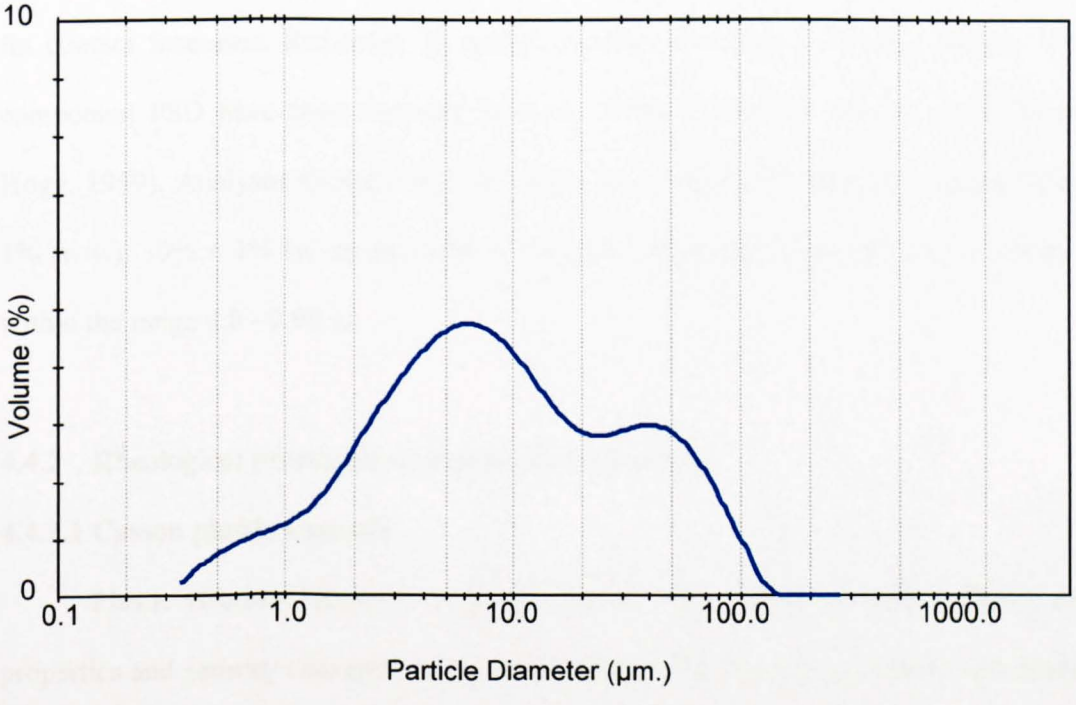


Figure 4.4. Particle size distribution of dark chocolate with D_{90} of (a) 18 µm, (b) 25 µm, (c) 35 µm, (d) 50 µm

Table 4.3. ANOVA Summary of F-ratios from particle size distribution

Process variables	Specific surface area	D(v,0.1)	D(v,0.5) [a]	D[3,2] [a]	D[4,3] [a]
A : Particle size (D_{90})	302.77*	455.54*	1007.84*	546.01*	8388.61*
B : Fat	115.88*	312.87*	311.17*	228.10*	23.21*
A x B	4.37*	6.63*	2.59*	3.52*	2.08

* Significant F-ratios at $p \leq 0.05$

Beckett (1999) concluded largest PS and specific surface area of solids are the two key parameters: largest particle diameter impacting on coarseness, and surface area with requirement of fat to obtain desirable flow properties. As size increases, particles become more spherical, leading to broadening of PSD with consequential reduction in solid loading as fat content increases. Reduction in specific surface area with increasing particle sizes of component PSD have been reported (Beckett, 1999; Sokmen & Gunes, 2006; Ziegler & Hogg, 1999). Analyses showed total fat values were within the stipulated ranges of 25% ± 1% (w/w), 30% ± 1% (w/w) and 35% ± 1% (w/w) respectively and moisture contents were within the range 0.8 - 0.98%.

4.4.2 Rheological properties of molten dark chocolate

4.4.2.1 Casson plastic viscosity

Plastic viscosity relates to pumping characteristics, filling of rough surfaces, coating properties and sensory character of body (Seguine, 1988). Increasing particle size drastically decreased plastic viscosity with 25% fat and 0.3% lecithin samples; while only slight decreases were noted with 30% and 35% fat at all lecithin levels (Fig. 4.5). Plastic viscosity with 25% fat and 0.3% lecithin was reduced from 20.42 Pas to 9.46 Pas respectively for 18

μm and 50 μm PS samples representing over 50% reduction but not with 0.5% lecithin at 25% fat. Likewise, changes in PS and lecithin content had no significant effect on plastic viscosity with 30% and 35% fat samples. Higher plastic viscosities in low fat chocolate can be explained as when distribution of particle sizes becomes wider with a large specific surface area, the smaller particles fill spaces between larger, reducing viscosity of suspension for any given solid concentration. Increasing fat content reduces specific surface area (Table 4.2), restricting solids packing ability with no apparent change in plastic viscosity. In addition, as the particles become finer, their number increases with parallel increase in points of contact between particles, thus increasing their plastic viscosities. Servais et al. (2002) reported viscosity can double with solid content increases of a few percent for high solid content suspensions.

Increasing fat content from 25% to 30% led to reduced plastic viscosities at all particle sizes and lecithin concentrations. At 18 μm, 5% increase in fat gave up to 10-fold reduction in plastic viscosity, indicating that fat had effects on plastic viscosity especially at lower PS (18 - 25 μm) and lower lecithin levels. However, at and above 30% fat, differences in plastic viscosity were small at all PS and lecithin levels. Beckett (1999) also attributed this to free-moving lubricating plastic flow related to forces between solid particles. Fat fills spaces or voids between particles in molten chocolate and reduces resistance to flow, with greatest effect noticeable at lower PS.

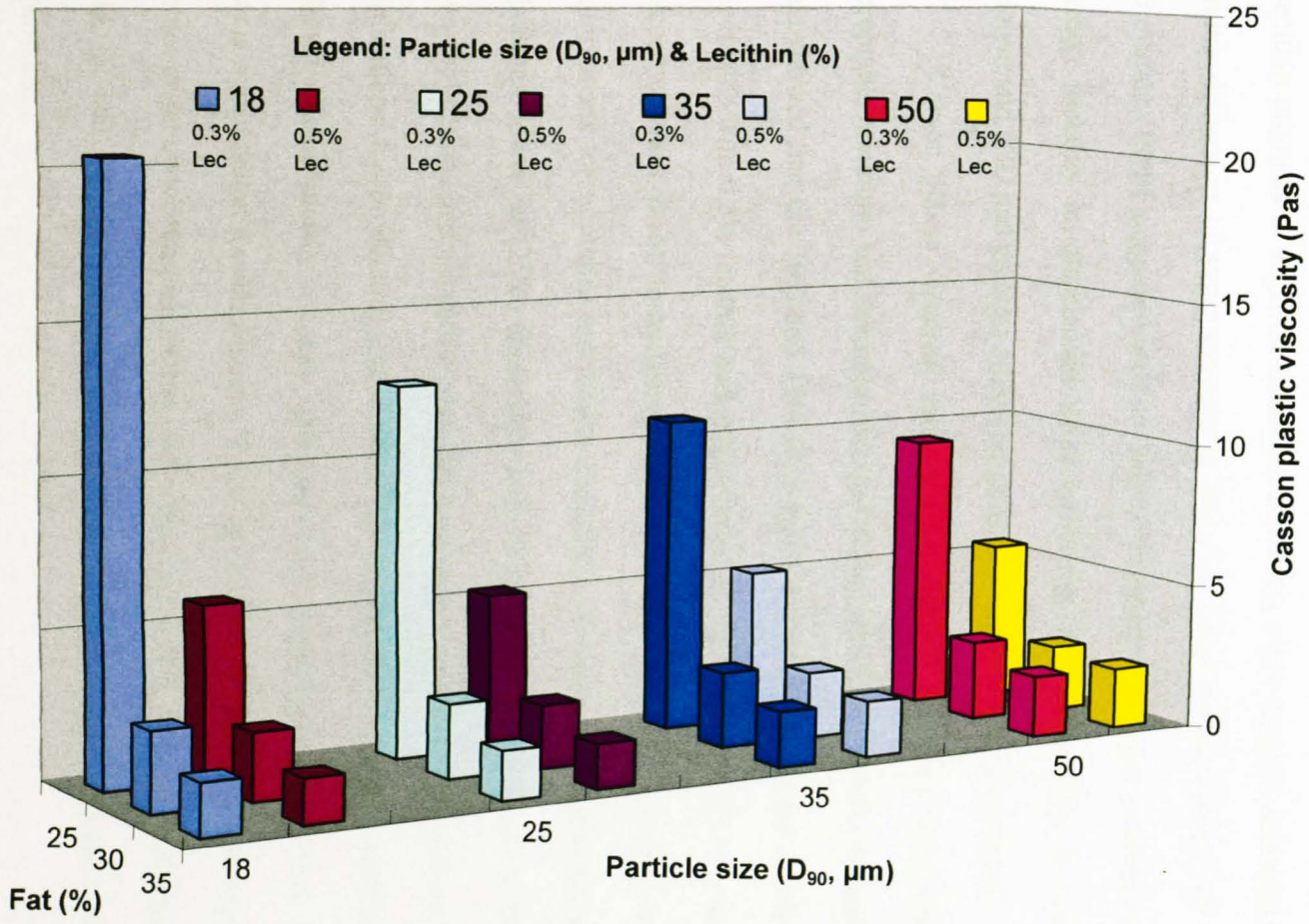


Figure 4.5. Effect of PSD, fat and lecithin content on Casson plastic viscosity of dark chocolate

Similar reductions in plastic viscosity were noted with increasing lecithin from 0.3% to 0.5%, especially at lower fat contents and PS where up to 4-fold reductions were noted. Plastic viscosity reductions from lecithin are attributed to association with sugar particles. Lecithin migrates to sugar/fat interfaces and coats sugar crystals, influencing rheology and aiding dispersion of sugar crystals in the continuous phases (Dhonsi & Stapley, 2006). Chevalley (1999) suggested lecithin forms a monolayer on sugar particle surfaces allowing greater mobility in suspensions while increasing fat spreadability. It was concluded fat content and PSD had greatest effects on plastic viscosity of dark chocolates.

Casson plastic viscosity values of 2.1 and 3.9 Pas have been reported to be the acceptable minimum and maximum for dark chocolates (Aeschlimann & Beckett, 2000). The data showed that the 30% and 35% fat samples fell within range but all the low fat (25%) chocolates with 0.5% lecithin had values between 5.81 Pas and 5.21 Pas. Such high plastic viscosity means these formulations cannot be employed for enrobing or coating with requirements for smoother and thinner chocolates. However, with application of mechanical vibrations, lower fat (25%) chocolates with PS between 25-35 μm and 0.5% lecithin could have applications in solid eating chocolates, panned products and chocolate chips/drops with implications for production cost. PSD, fat and lecithin contents significantly ($p \leq 0.05$) affected Casson plastic viscosity with significant interactions (Table 4.4). Multiple range tests revealed that at low fat contents, PSD significantly ($p \leq 0.05$) influenced plastic viscosity but not at fat concentrations of 30% and 35%. This means that the combined influences of PSD, fat and lecithin contents could be manipulated to control plastic viscosity in dark chocolates.

Table 4.4. ANOVA Summary of F-ratios showing the rheological properties

Process variables	Casson plastic viscosity	Casson yield value	Apparent viscosity	Yield stress	Thixotropy
Main Effects					
A : PSD (D_{90})	20.38*	363.58*	464.41*	1364.97*	8381.44*
B : Fat	1278.85*	1097.87*	2956.29*	6554.36*	53299.17*
C : Lecithin	413.47*	383.78*	688.96*	2054.07*	12149.36*
Interactions					
A x B	30.02*	104.72*	197.51*	472.35*	3518.89*
A x C	21.70*	95.86*	161.22*	475.16*	2928.37*
B x C	322.96*	275.64*	536.93*	1587.24*	9725.58*
A x B x C	22.43*	68.72*	123.82*	351.97*	2204.33*

* Significant F-ratios at $p \leq 0.05$

4.4.2.2 Casson yield value

Casson yield values showed an inverse relationship with particle size, fat and lecithin contents. Increasing particle sizes caused significant ($p \leq 0.05$) reductions in the yield values at all fat contents (Fig. 4.6). The greatest reductions were observed with low fat (25%) and 0.3% lecithin, from 408.8 Pa at 18 μm to 57.53 Pa at 50 μm, representing ~ 70-fold reduction (Fig. 4.6). Similar reductions were noted with 30% and 35% fat with 0.3% lecithin at all particle sizes with effects being less pronounced at higher particle sizes (Fig. 4.6). Higher values with the low (25%) fat and lower (18-25 μm) particle sizes can be attributed to high particle-particle interactions at lower PS, specific surface area and mean particle diameter, forming spanning stress bearing paths increasing yield values. Yield value is affected largely

by inter-particle contacts, and shows a linear dependency on mean particle size or, more accurately, specific surface area (Prasad et al., 2003). The yield stress or yield value relates to shape retention, pattern holding, feet and tails, inclined surface coating and presence of air bubbles (Seguine, 1988).

Increasing fat content gave significant ($p \leq 0.05$) decreases in yield values at all particle sizes and lecithin levels (Fig. 4.6). At 18 μm, yield values decreased from 408.80 Pa to 32.37 Pa representing ~120-fold reductions with fat increases from 25% to 35%. Similarly, with 50 μm, reductions of up to 90-fold were noted when fat was increased from 25% to 35%. This explains that combined effects of fat content and PSD having greatest influence on the yield values in dark chocolates. This effect is however less pronounced at higher fat and lecithin contents (Fig. 4.6). Fat coats the particle surfaces and reduces their inter-particle interaction to induce chocolate flow. Beckett (2000) explained that the effect of an extra 1% fat upon yield value depends on the amount already present. Above fat content of 32%, there is very little change in yield value with any further additions.

Similar significant ($p \leq 0.05$) reductions in Casson yield value were noted when lecithin was increased from 0.3% to 0.5% as reported previously (Beckett, 1999; Chevalley, 1999). The lecithin molecule has two long fatty acid chains capable of forming a non-polar tail that gives it a good stability in lipids and its amphiphilic nature promotes deagglomeration of clumps and wetting contributing to lowering of viscosity. Significant ($p \leq 0.05$) interactions were observed among all parameters (Table 4.4), indicating complex effects on yield value (Beckett, 1999, 2000; Chevalley, 1999), which remain a challenge.

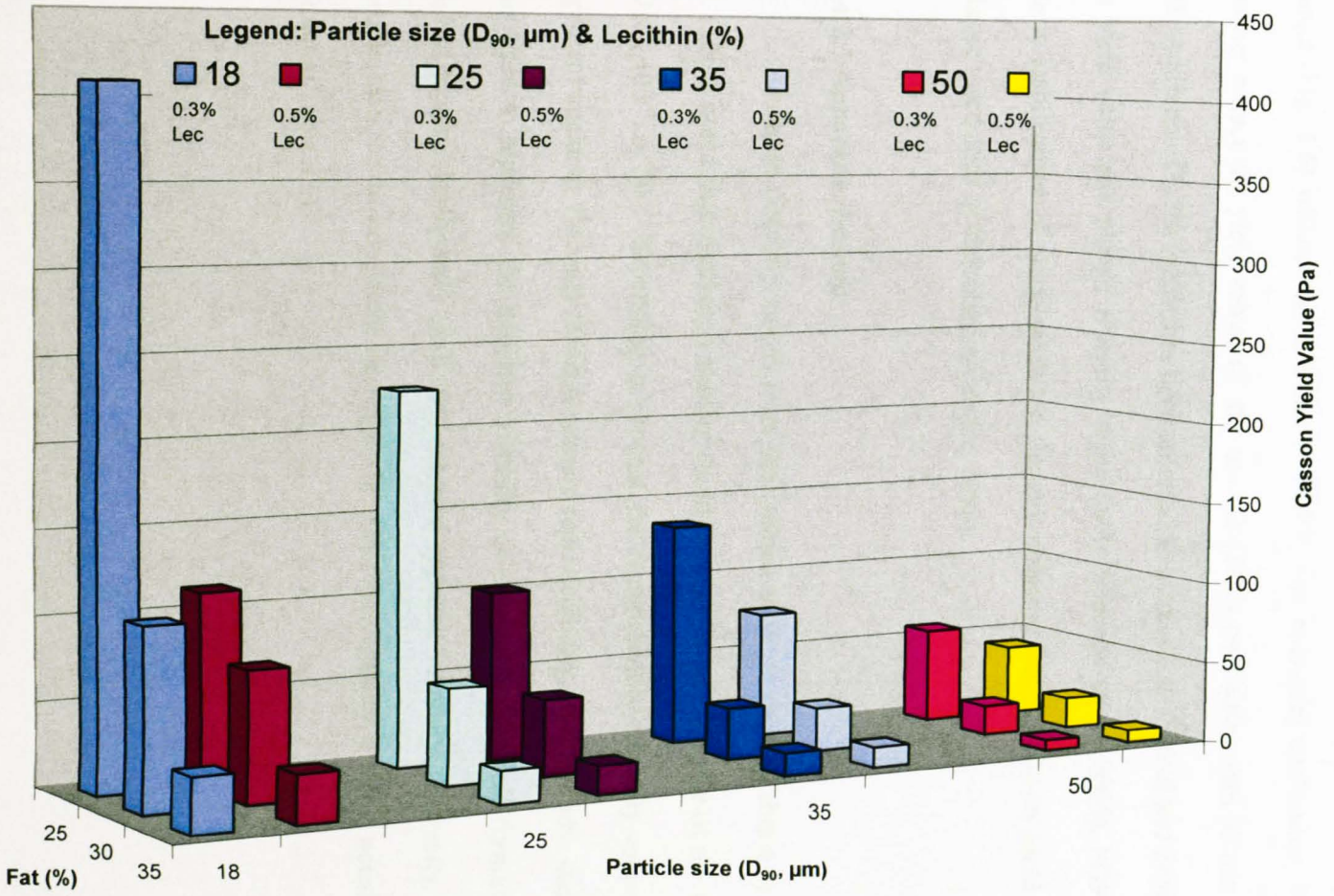


Figure 4.6. Effect of PSD fat and lecithin content on Casson yield value of dark chocolate

Casson yield values for dark chocolate have been reported as between 4 Pa and 32 Pa (Aeschlimann & Beckett, 2000). Most 30% and 35% fat formulations fell within this range (Fig. 4.6) without the addition of PGPR. For industrial application, PGPR could further reduce the yield values of the low fat (25%) chocolates with 35 μm and 50 μm PS. Addition of 0.5% PGPR has been reported to effect up to 12-fold and 24% reductions in yield value and plastic viscosity respectively (Haedelt et al., 2005). PGPR achieves steric stabilization of sugar particles, thereby reducing interactions on yield values and plastic viscosities in chocolates (Vernier, 1998).

4.4.2.3 Apparent viscosity

Apparent viscosity values were determined at 30 s^{-1} shear (Table 4.5). Servais et al. (2004) noted that apparent viscosity could be represented by value of the viscosity at 30 s^{-1}, 40 s^{-1}, or 50 s^{-1} depending on product, but recommended viscosity value at 40 s^{-1} to represent apparent viscosity through relative reproducibility. In this study, shear at 30 s^{-1} was used to represent the apparent viscosity as obtainable from all formulations. All studied factors significantly ($p<0.001$) affected apparent viscosity. Generally, increasing particle size led to consistent decreases in apparent viscosity a trend noted at all fat contents (Table 4.5).

Table 4.5. Effect of PSD, fat and lecithin contents on apparent viscosity and yield stress of dark chocolates

Particle size D_{90} (μm)	Fat Content (%)	Lecithin (%)	Apparent viscosity (Pas)	Yield stress (Pa)
18 ± 1.0	25	0.3	61.03 ± 1.60	920.77 ± 6.22
		0.5	20.26 ± 0.40	260.33 ± 4.98
	30	0.3	13.51 ± 0.08	211.63 ± 5.79
		0.5	10.55 ± 0.06	157.77 ± 4.76
	35	0.3	5.63 ± 0.05	79.47 ± 1.38
		0.5	4.93 ± 0.08	69.85 ± 1.32
25 ± 1.0	25	0.3	32.42 ± 1.24	441.50 ± 5.26
		0.5	18.37 ± 0.07	232.37 ± 3.21
	30	0.3	8.53 ± 0.04	123.63 ± 2.54
		0.5	7.88 ± 0.08	113.93 ± 3.10
	35	0.3	4.61 ± 0.03	56.43 ± 1.56
		0.5	4.05 ± 0.02	51.02 ± 1.42
35 ± 1.0	25	0.3	24.74 ± 0.07	346.10 ± 6.24
		0.5	15.78 ± 0.08	193.37 ± 3.16
	30	0.3	6.12 ± 0.05	84.89 ± 1.82
		0.5	6.27 ± 0.06	66.82 ± 1.72
	35	0.3	4.28 ± 0.03	49.98 ± 1.61
		0.5	3.84 ± 0.02	44.85 ± 1.26
50 ± 1.0	25	0.3	15.71 ± 0.17	225.57 ± 4.45
		0.5	11.50 ± 0.13	144.30 ± 3.24
	30	0.3	5.80 ± 0.23	62.60 ± 1.71
		0.5	5.27 ± 0.16	63.10 ± 1.68
	35	0.3	3.54 ± 0.02	35.49 ± 1.08
		0.5	3.45 ± 0.07	38.95 ± 0.82

Mean values ± standard deviations from triplicate analysis.

The trends with apparent viscosity were similar with Casson plastic viscosity; increasing particle sizes from 18 μm to 50 μm led to significant decreases in apparent viscosity, more pronounced at low fat (25%). Increasing fat content had similar inverse relationship with apparent viscosity but less effect at both 30% and 35% fat at all particle sizes; and finally, lecithin increase from 0.3% to 0.5% caused further reductions in apparent viscosity for all particle sizes and fat contents (Table 4.5). Influence on apparent viscosity of dark chocolates was more dependent on fat and lecithin content. ANOVA showed that PSD, fat and lecithin contents significantly ($p \leq 0.05$) affected dark chocolate apparent viscosity with significant interactions among factors (Table 4.4).

4.4.2.4 Yield stress

Yield stress relates to energy required to initiate chocolate flow and is important in keeping small solid particles in suspension and in coating of solid surfaces (Yoo et al., 1995). Values were significantly ($p \leq 0.05$) influenced by PSD, fat and lecithin contents as for Casson yield value. Increases in particle sizes from 18μm to 50 μm caused significant ($p \leq 0.05$) reductions at all fat contents with the greatest reductions with low fat (25%) chocolates containing 0.3% lecithin, from 920.77 Pa for 18 μm to 225.57 Pa in the 50 μm (Table 4.5). Trends were similar for 30% and 35% fat and 0.3% lecithin with increasing particle sizes but less pronounced with 30% and 35% fat contents. Higher yield stress with lower particle sizes and lower fat levels could result from higher specific surface area with lower PS, showing an inverse relationship. Servais et al. (2004) suggested yield stress depends on proportion of small particles (specific surface area) and on their interactions, originating in mechanical (friction) and chemical effects.

Fat and lecithin content effects on yield stress were comparable to trends noted with the Casson yield values. Increasing fat led to significant ($p \leq 0.05$) decreases in yield

stress at all PS and lecithin levels (Table 4.5) with higher yield stress values noted at 18 μm and lower fat content (25%), decreasing from 25% to 35% fat at all particle sizes, that could be attributed to coating of fat on particle surfaces, reducing inter-particle interaction and inducing flow in a direct relationship with fat content. Similarly, significant ($p \leq 0.05$) reductions in yield stress were noted when lecithin was increased from 0.3% to 0.5%, at all particle sizes and fat contents. Significant ($p \leq 0.05$) interactions were observed among all the processing parameters (Table 4.4). Fat content had the greatest influence in reducing yield stress in dark chocolates followed by lecithin content and then PSD.

4.4.2.5 Thixotropy

Thixotropy is when apparent viscosity or shear stress decreases with time of shear at a constant rate (Chhabra, 2007). During shearing, the continuous decrease in apparent viscosity and subsequent recovery of shear stress or apparent viscosity when flow is discontinued creates a hysteresis loop. Thixotropy is quantified from the area of loop or specific point on ramp curves of shear stress or apparent viscosity at a specific shear rate usually 5 s^{-1} or 40 s^{-1}. A certified method has still to be denoted (Servais et al., 2004; ICA, 2000; Cheng, 2003) but a well conched chocolate should not be thixotropic. Difference between yield stresses measured at a shear of 5 s^{-1} during ramp up and down in shear was used to represent thixotropy.

PSD, fat and lecithin content all had significant effects on thixotropy although, this observation was only made on the low fat (25%). The samples containing 30% and 35% fat contents exhibited little thixotropy, implying that irrespective of PSD, fat and lecithin content, chocolates of $\geq$ 30% fat were not thixotropic (Fig. 4.7).

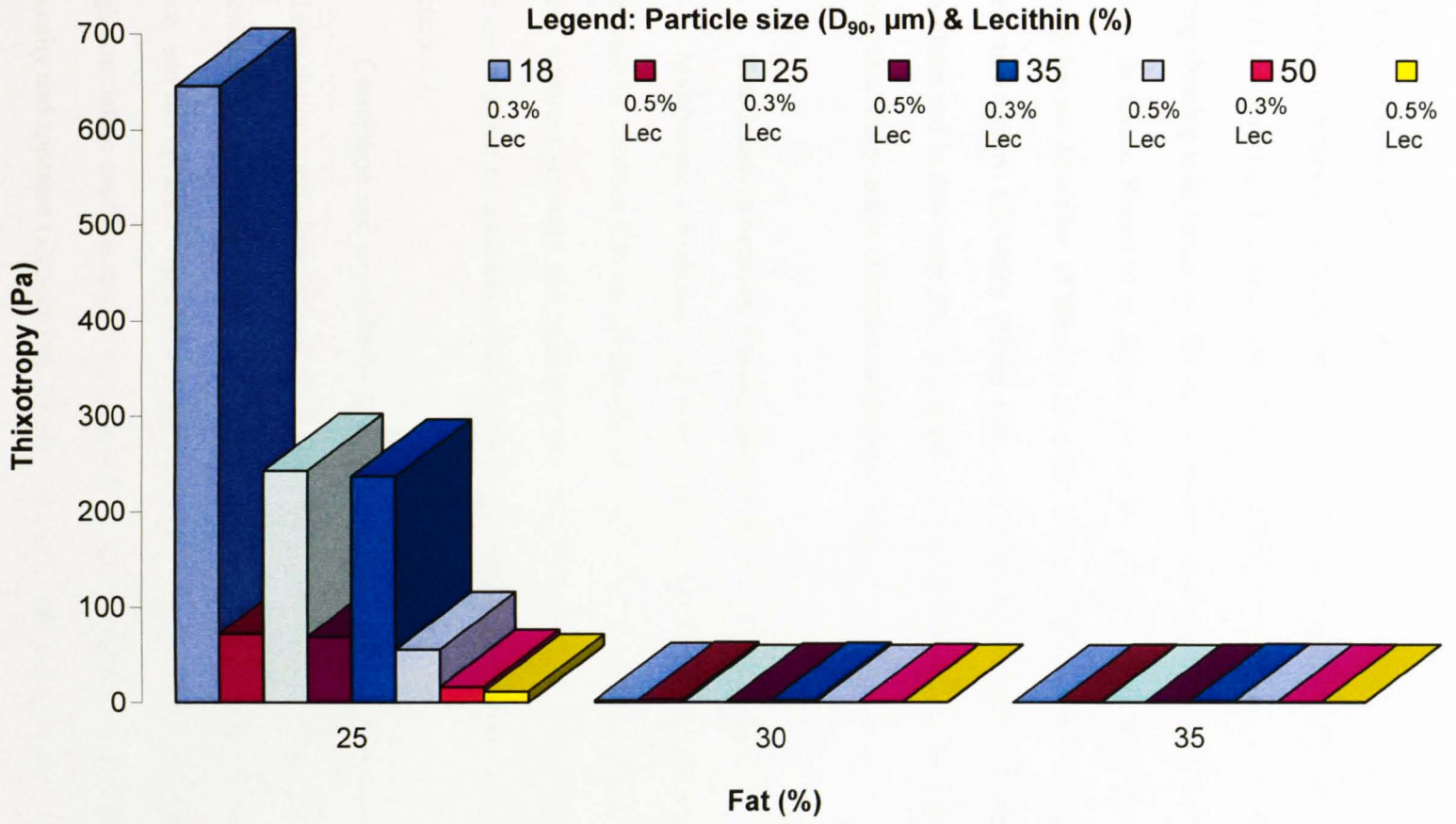


Figure 4.7. Effect of PSD, fat and lecithin content on thixotropy of dark chocolate

With the exception of 50 μm, which had reduced thixotropy values, all 25% fat samples exhibited high thixotropic behaviour, suggesting that thixotropy is dependent on particle size and fat content (Fig. 4.7) which could be attributed to the crowding of the particulate system during shearing with formation of sample spanning aggregates due to low interaction energy at low fat levels. Prasad et al. (2003) noted that the rates of formation and disruption of aggregates are functions of the flow induced shear stresses, particle volume fraction and interaction energy. Chevalley (1999) suggested thixotropy is especially important for thick chocolates and in this study PSD and lecithin were key factors that could be manipulated to reduce thixotropy in low fat and/or thick chocolates

4.5 Relationships between Casson model and ICA recommendations

Multivariate correlation, regression and principal component analyses evaluated relationships between Casson plastic viscosity and Casson yield value and the newer yield stress, apparent viscosity and thixotropy (ICA, 2000; Servais et al., 2004). Effects of PSD and composition on dark chocolate rheology using both models have been reported under Section 4.4.

Correlation and regression analyses conducted on the data revealed high regression and correlation coefficients among all rheological parameters (Table 4.6). Relationships were calculated using correlation analysis between Casson plastic viscosity and Casson yield value, and the apparent viscosity and (apparent) yield stress, the ICA recommended values. High correlation coefficients ($r = 0.95$, $p<0.001$) were observed between the Casson plastic viscosity and apparent viscosity and Casson yield value and yield stress ($r = 0.98$, $p<0.001$).

Table 4.6. Regression and correlation analyses between rheological parameters

Parameter	Analysis	Casson plastic viscosity	Casson yield value	Apparent viscosity	Yield stress	Thixotropy (YS)
Casson plastic viscosity	Regression	1.0000	0.8368*	0.9053*	0.8919*	0.9021*
	Correlation	1.0000	0.8903*	0.9467*	0.9349*	0.9447*
Casson yield value	Regression	-	1.0000	0.9582*	0.9694*	0.9665*
	Correlation	-	1.0000	0.9786*	0.9844*	0.9823*
Apparent viscosity	Regression	-	-	1.0000	0.9898*	0.9955*
	Correlation	-	-	1.0000	0.9941*	0.9977*
Yield stress	Regression	-	-	-	1.0000	0.9939*
	Correlation	-	-	-	1.0000	0.9957*
Thixotropy (AP)	Regression	-	-	-	-	0.9527
	Correlation	-	-	-	-	0.9761

* Significant at $P< 0.001$

Regression analyses (Figs. 4.8a and 4.8b) showed Casson plastic viscosity and apparent viscosity, and Casson yield value and yield stress were closely related. Contrary to the findings of Servais et al. (2004), Casson plastic viscosity and Casson yield value were highly correlated ($r = 0.89$, $p<0.001$), with a high and significant regression coefficient, $R^2 = 0.84$ (Table 4.6). The regression model is as shown on Figure 4.8c. Similarly, yield stress and apparent viscosity were highly correlated ($r = 0.99$, $p<0.001$), with regression coefficient, $R^2 = 0.99$ (Table 3). Figure 4.8d shows the regression model for yield stress and apparent viscosity.

Thixotropy is exhibited in chocolates if its apparent viscosity or shear stress decreases with time when sheared at a constant rate, and relates to degree of conching - well conched chocolate should not be thixotropic.

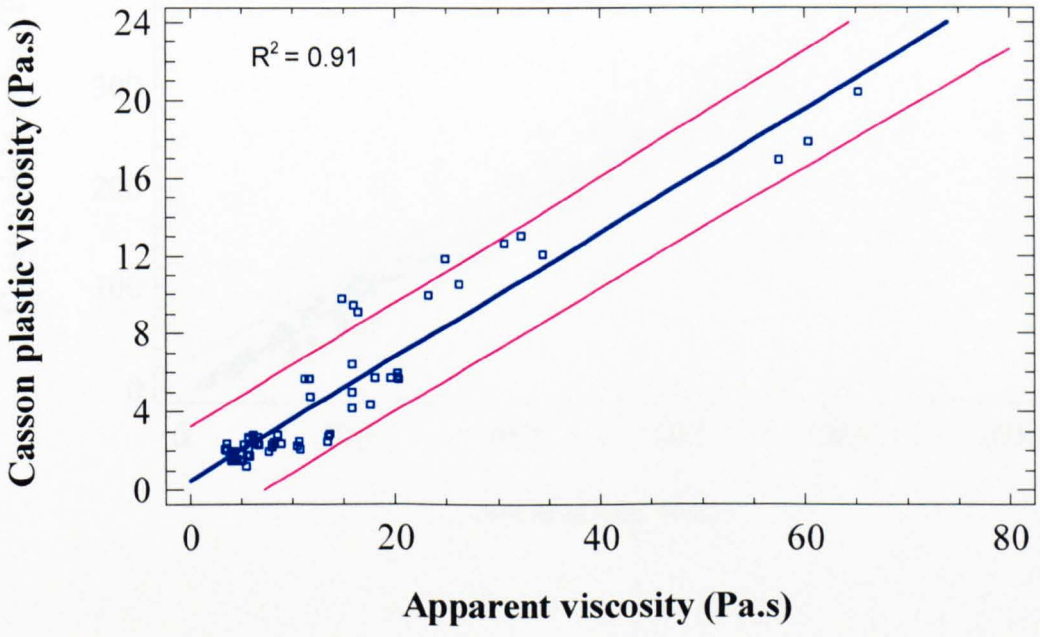


Figure 4.8a. Relationship between Casson plastic viscosity and apparent viscosity using bob and cup (Reference) geometry

Data points (Squares); Linear regression (Inner solid line); Minimum and maximum tolerance intervals (both outer lines); Casson plastic viscosity = 0.477564 + 0.31802* Apparent viscosity

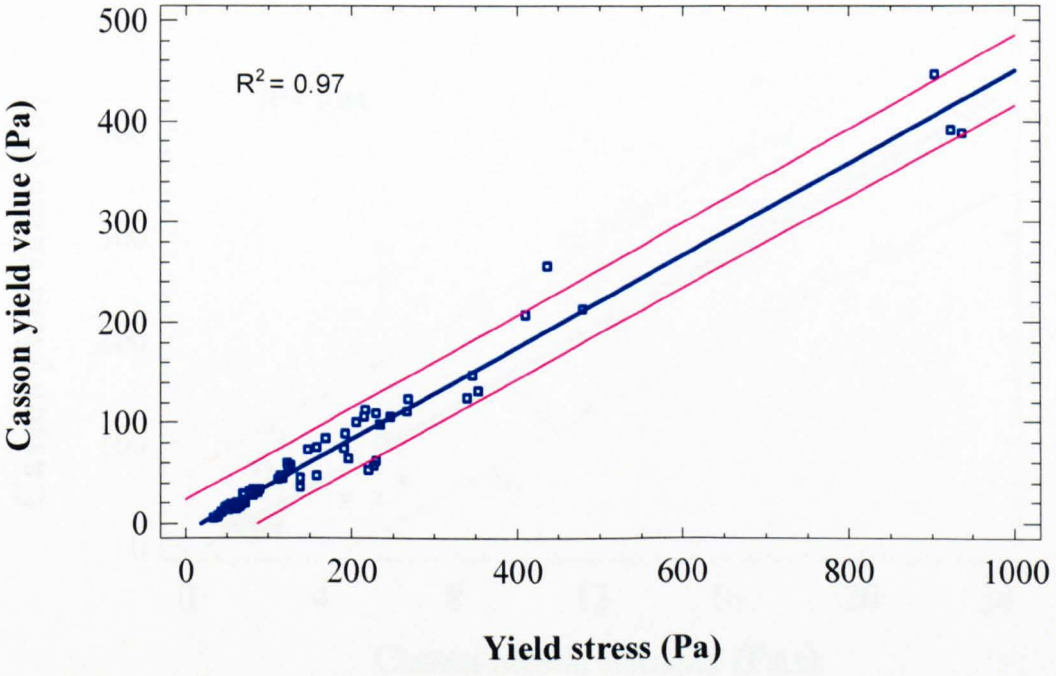


Figure 4.8b. Relationship between Casson yield value and yield stress using bob and cup (Reference) geometry

Data points (Squares); Linear regression (Inner solid line); Minimum and maximum tolerance intervals (both outer lines); Casson yield value = -8.29934 + 0.458911*Yield stress

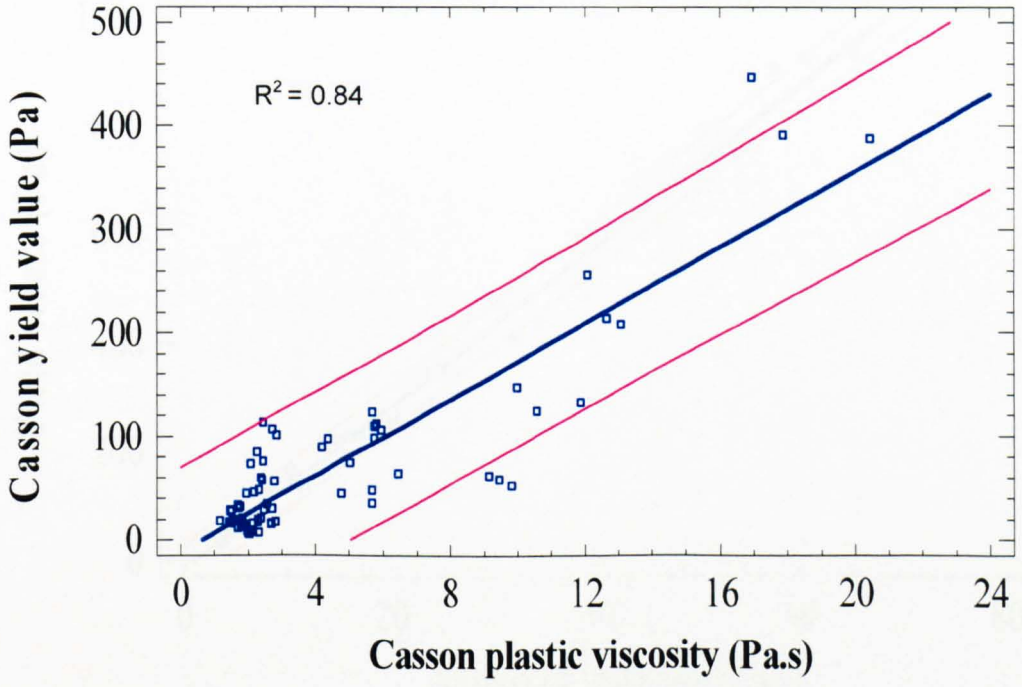


Figure 4.8c. Relationship between Casson yield value and Casson plastic viscosity using bob and cup (Reference) geometry

Data points (Squares); Linear regression (Inner solid line); Minimum and maximum tolerance intervals (both outer lines); Casson yield value = -11.9953 + 18.4325*Casson plastic viscosity

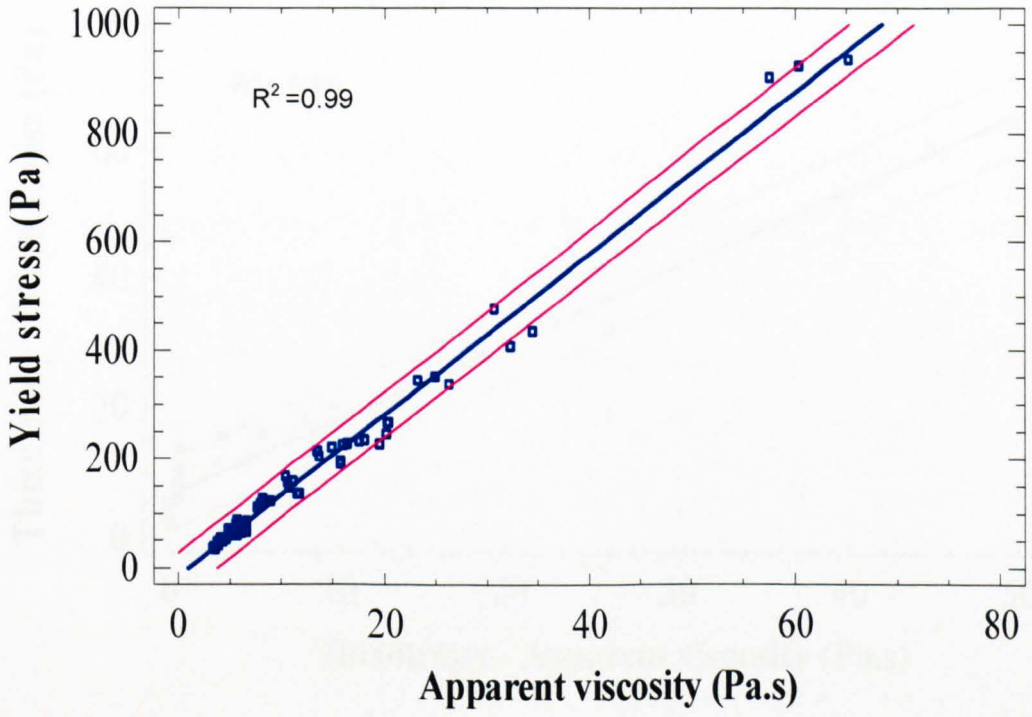


Figure 4.8d. Relationship between yield stress and apparent viscosity using bob and cup (Reference) geometry

Data points (Squares); Linear regression (Inner solid line Minimum and maximum tolerance intervals (both outer lines); Yield stress = -14.4174 + 14.8302*Apparent viscosity

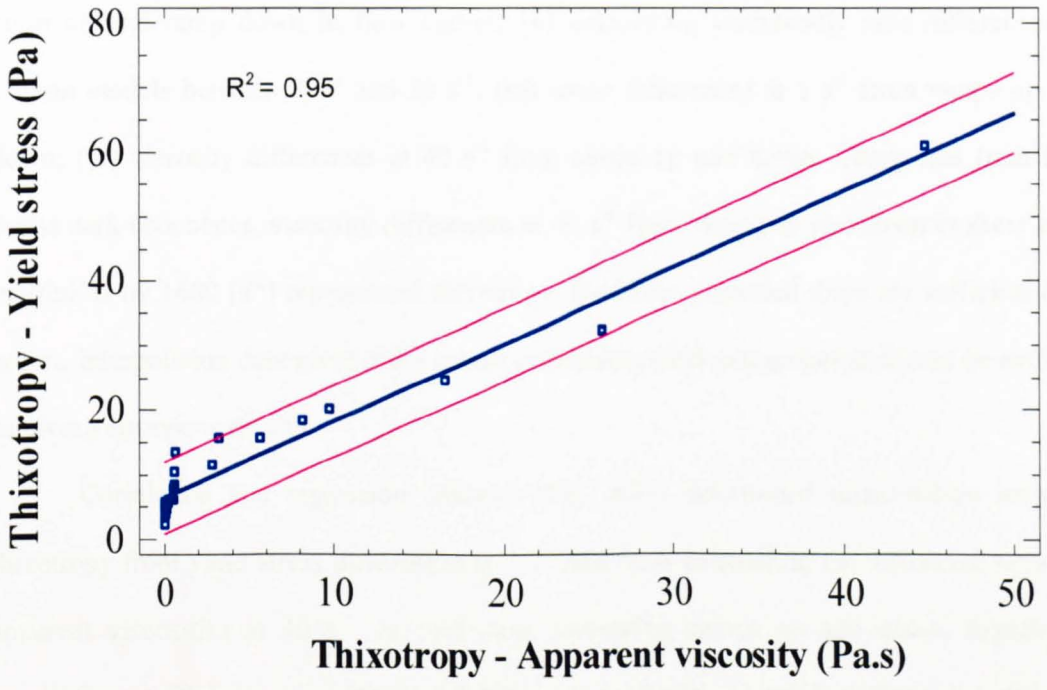


Figure 4.8e Relationship between thixotropy from yield stress and thixotropy from apparent viscosity

Data points (Squares); Linear regression (Inner solid line); Minimum and maximum tolerance intervals (both outer lines); Thixotropy (YS) = 6.42097 + 1.1907*Thixotropy (AP)

Interpolation and extrapolation data could be used to characterise thixotropy but no certified method has been denoted (ICA, 2000; Cheng, 2003; Chhabra, 2007). Servais et al. (2004) suggested that practically, thixotropy can be obtained by: (i) area differences between ramp up and ramp down in flow curves; (ii) calculating analytically area differences in Casson models between 2 s^{-1} and 50 s^{-1}; (iii) stress differences at 5 s^{-1} from ramps up and down; (iv) viscosity differences at 40 s^{-1} from ramps up and down. Using data from four Swiss dark chocolates, viscosity differences at 40 s^{-1} from ramps up and down in shear rates multiplied by 1600 [s^{-2}] represented thixotropy. However, provided there are sufficient data points, interpolation data gives more robust information and extrapolation should be avoided as giving erroneous results.

Correlation and regression analyses (Fig. 4.8e) determined relationships between thixotropy from yield stress differences at 5 s^{-1} and from calculating the difference between apparent viscosities at 40 s^{-1}, in each case comparing ramps up and down. Significant correlation coefficients of $r = 0.98$ ($p<0.001$); and regression coefficient of determination of $R^2 = 0.95$ ($p<0.001$), among the two methods (Table 4.6), suggested that both yield stress and apparent viscosity could be used as reliable interpolation data to measure thixotropy. In contrast the use of extrapolation data from Casson parameters should be avoided as this gave lower coefficients of determination (Table 4.6).

The principal component (PCA) product space (Fig. 4.9) explained 95.2 % variance (74.2, 13.7 and 7.3%) (eigenvalue > 1) and showed rheological parameters very closely related with PSD, fat and lecithin content as key influencing factors. This PCA (Fig. 4.9) product space for Casson parameters (plastic viscosity and yield value) and ICA

recommended parameters (apparent viscosity and yield stress) were closely related and could be used independently to evaluate rheological properties of dark chocolates.

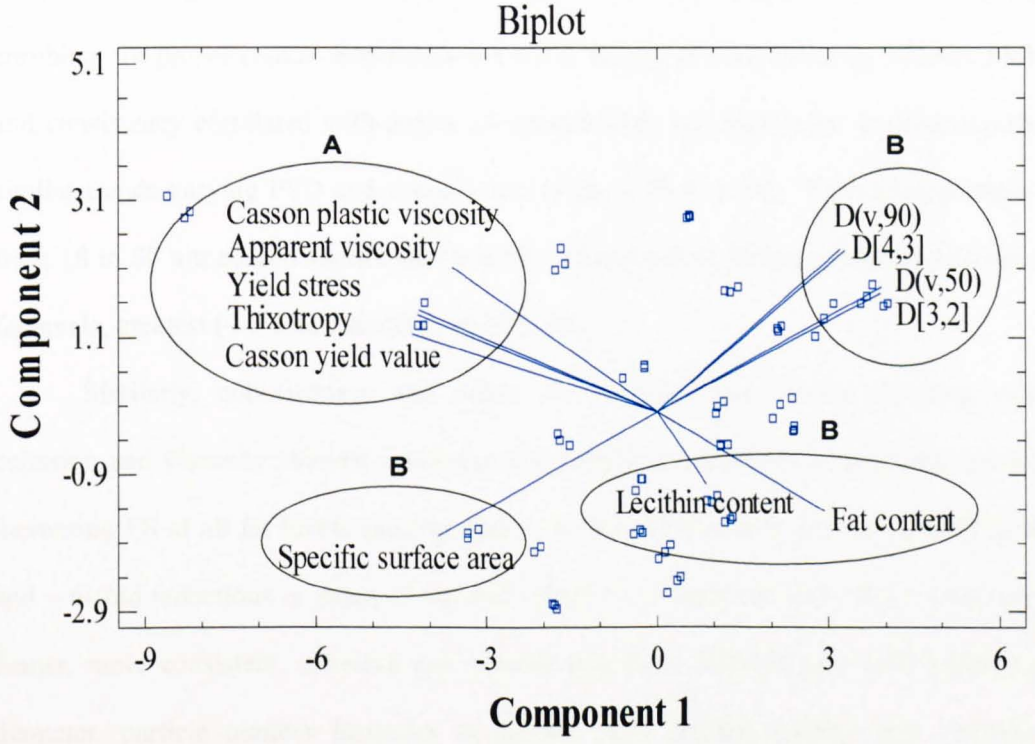


Figure 4.9. Principal component analysis showing relationship between parameters within two rheological models (A) and their influencing factors (B) PC1 (74.2 % variance) PC (13.7%)

4.6 Textural properties

4.6.1 Molten dark chocolate

Firmness, consistency, cohesiveness and index of viscosity were evaluated to ascertain degree of spreadability, consistency and resistance to flow behaviour (viscosity). Ziegler and Hogg (1999) concluded such flow behaviour is important for moulding and enrobing, for proper cookie drop formation and in design of bulk handling systems. Firmness and consistency correlated with degree of spreadability and particulate consistency showed similar trends varying PSD and composition (Figs. 4.10 & 4.11). Increasing particle sizes from 18 to 50 μm caused significant ($p<0.001$) reductions in firmness and consistency at all fat levels, greatest (~ 6-fold reduction) at 25% fat.

Similarly, cohesiveness and index of viscosity, respectively denoting work of cohesion and viscosity showed consistent and significant ($p<0.001$) decreasing trends with increasing PS at all fat levels causing upto ~ 8-fold reductions in cohesiveness (Fig. 4.12), and ~ 6-fold reductions in index of viscosity (Fig. 4.13). Samples with 18 μm particles were firmer, more consistent, cohesive and viscous that those with 50 μm: with reduced mean diameter, particle number increases in parallel with specific surface area (Table 4.2) enhancing particle surface-surface contacts yielding higher values for firmness, consistency and cohesiveness, restricting spreadability and viscosity for a specific solid concentration.

The high degree of reductions noticeable with low fat (25%) chocolate, with increasing PS might be due to the fact that, as the distribution of particle sizes becomes more spread out with a large specific surface area, the smaller particles fill the spaces between the larger particles, resulting in drastic reduction in the firmness, consistency, cohesiveness and viscosity. Likewise, increasing the fat content of the chocolates from 25% to 30% led to

drastic reductions in all textural parameters at all particle sizes and lecithin concentrations. At low PS (18 μm), 5% increase in fat content caused upto 5-fold reduction, indicating that fat has a marginally greater effect on firmness, consistency, cohesiveness and viscosity of dark chocolates especially at lower PS (18 - 25 μm) and lower lecithin levels. At 35% fat content, very little differences were observed at all PS and lecithin levels, attributable to fat inducing free-moving lubricating flow which is more connected with the forces between the solid particles. Beckett (2000) explained that fat fills the spaces or voids between the solid particles in molten chocolate and reduces their resistance to flow, with the greatest effect noticeable at lower PS.

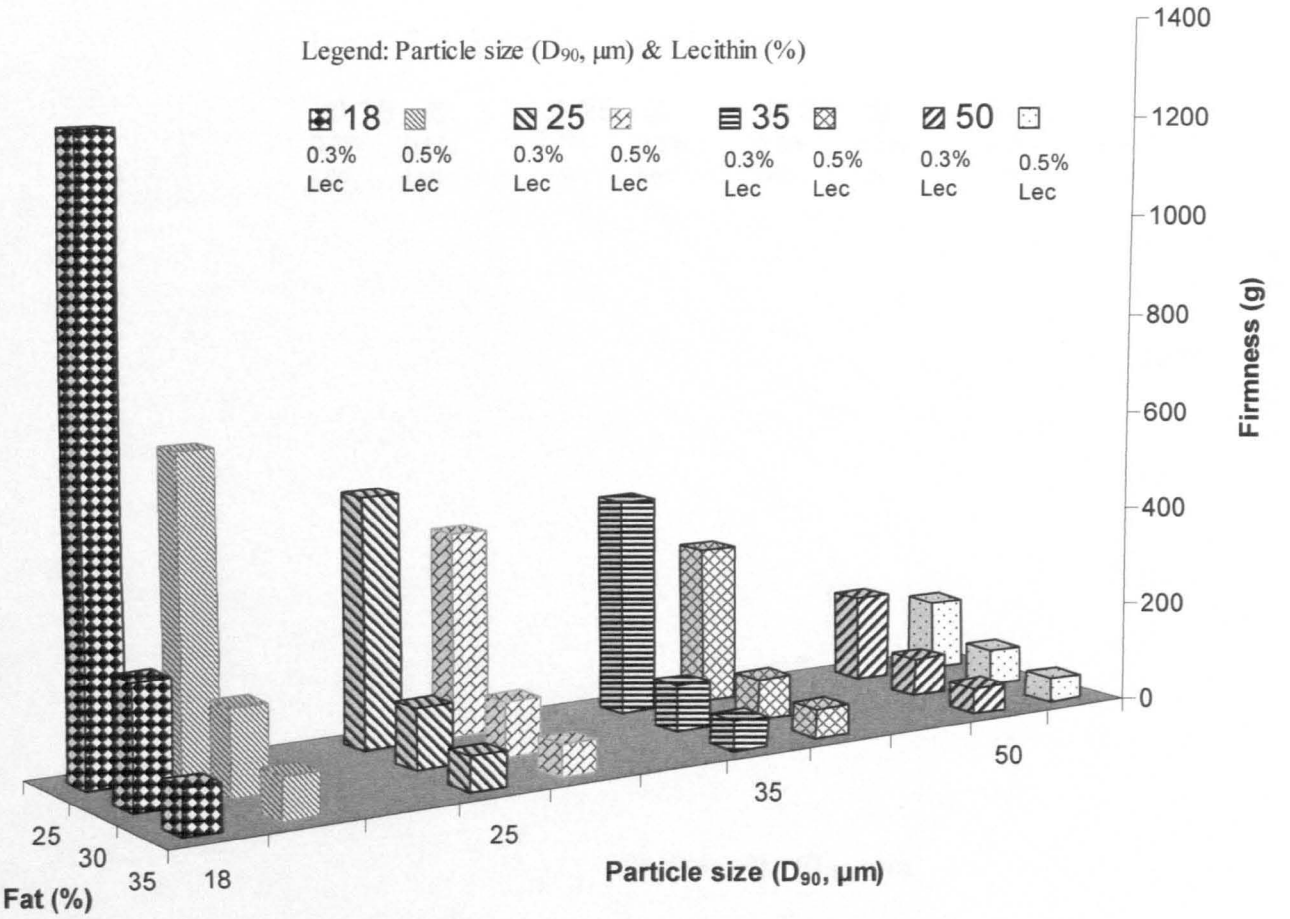


Figure 4.10. Effect of PSD and composition on firmness of molten dark chocolate

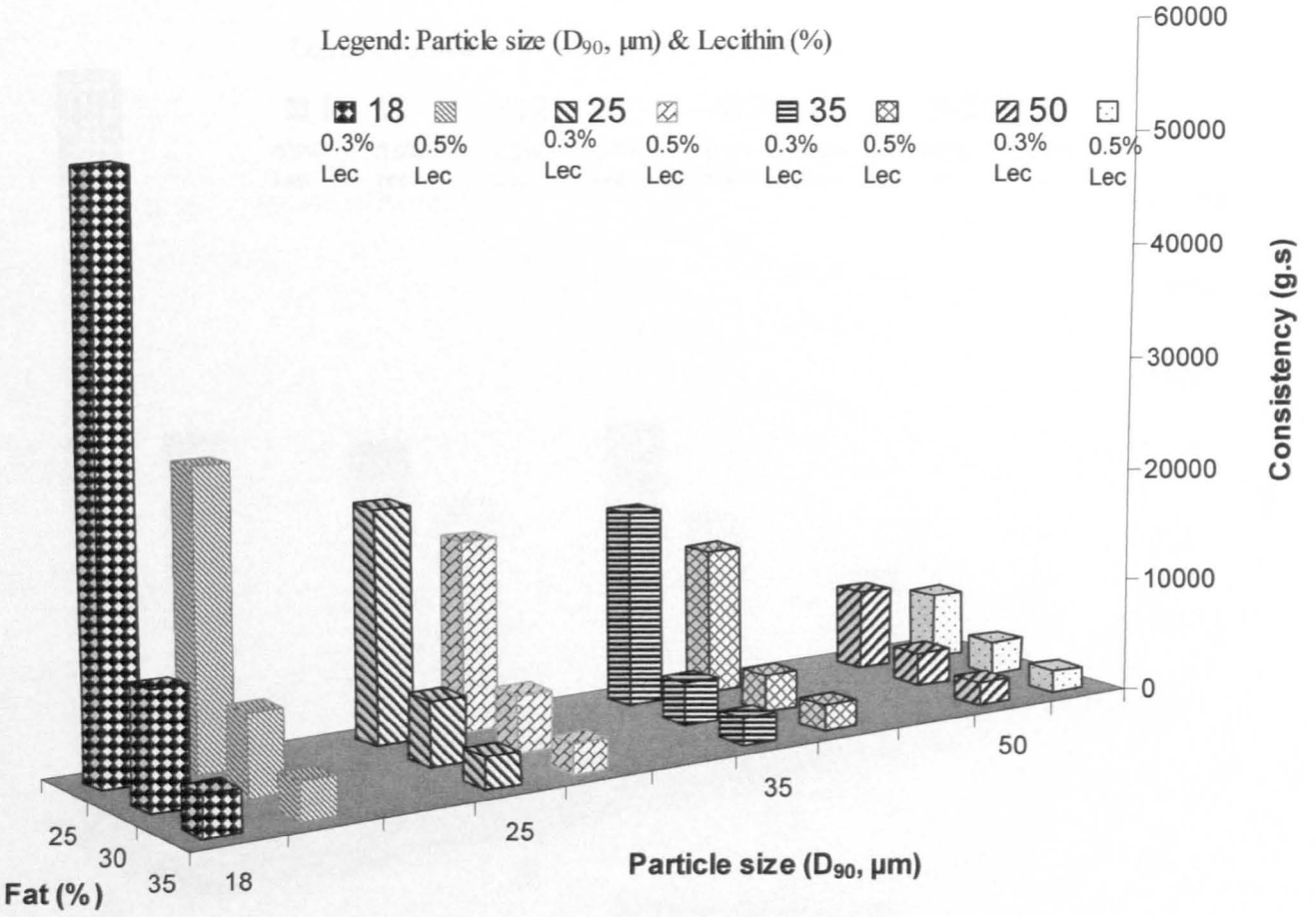


Figure 4.11. Effect of PSD and composition on consistency of molten dark chocolate

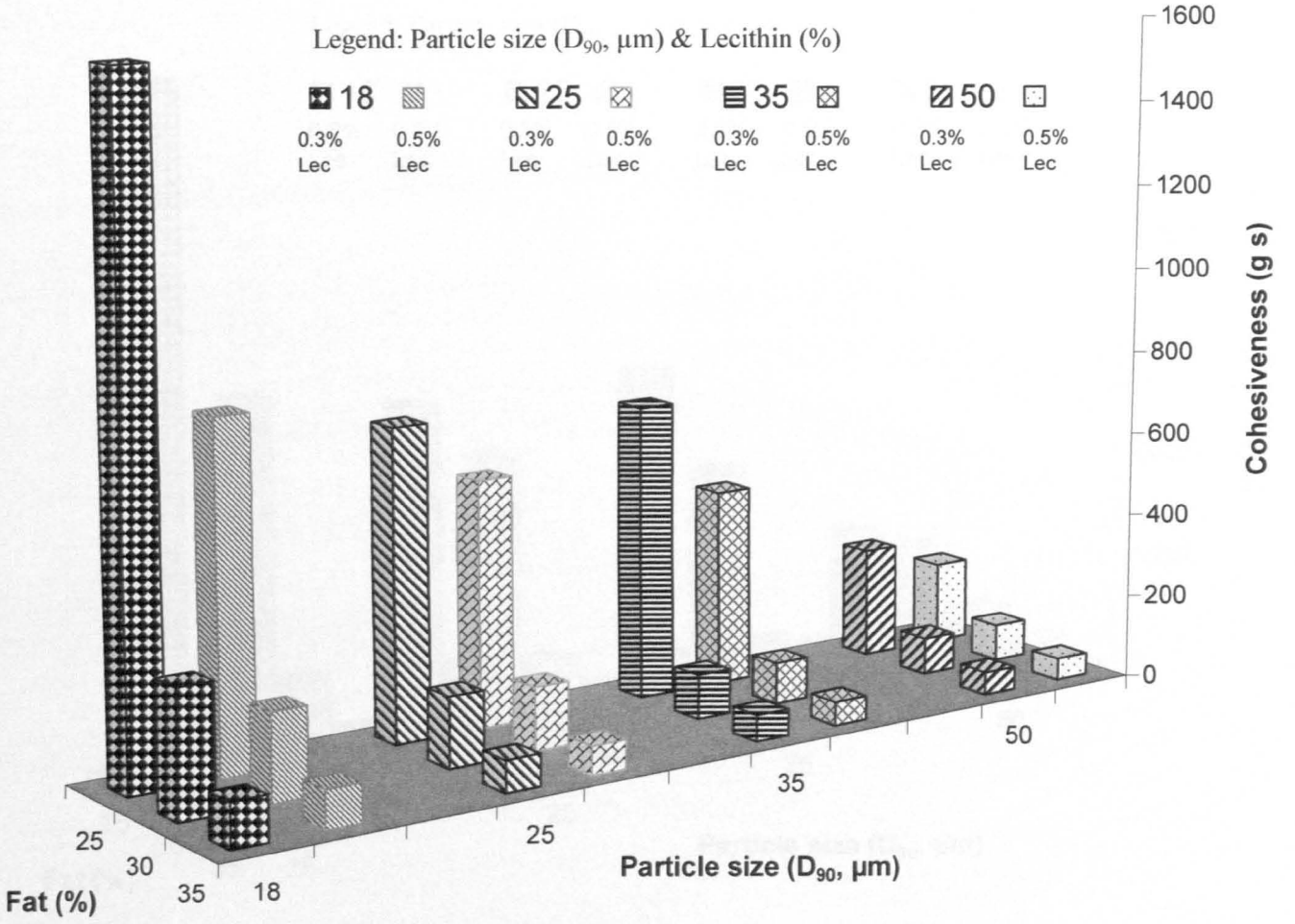


Figure 4.12. Effect of PSD and composition on cohesiveness of molten dark chocolate

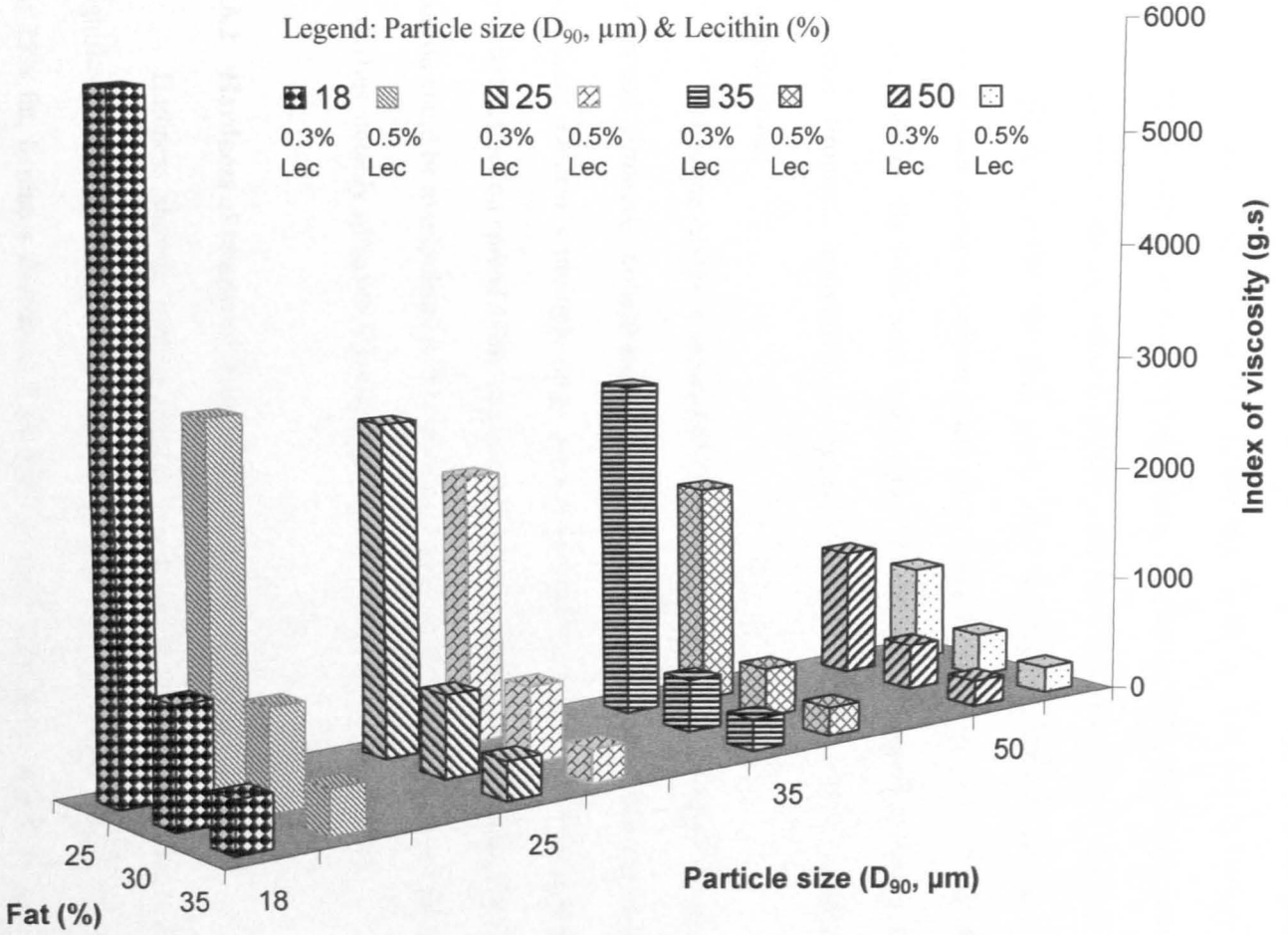


Figure 4.13. Effect of PSD and composition on index of viscosity of molten dark chocolate

Increasing lecithin from 0.3 to 0.5% significantly decreased firmness, consistency, cohesiveness and index of viscosity especially at lower fat content and lower PS where upto 2-fold reductions were noted, attributable to an association with sugar particles. Lecithin phospholipids migrate to sugar crystal surfaces making these lipophilic and thus acts as lubricant reducing internal friction and firmness, consistency, cohesiveness and viscosity (Beckett, 2000; Bueschelberger, 2004). Chevalley (1999) noted a monolayer of lecithin on sugar particle surfaces enhances suspension mobility with parallel increases in fat spreadability. Samples with 30% and 35% fat had comparable values for molten chocolate firmness, consistency, cohesiveness and viscosity with implications for manufacturing.

Univariate ANOVA showed PSD, fat and lecithin contents significantly ($p<0.001$) influenced firmness, cohesiveness and viscosity (Table 4.7) with interactions also significant. Duncan's multiple range tests revealed that at 25% fat, PSD had significant ($p<0.001$) effect on spreadability but less at 30 and 35%. Combined effect of PSD, fat and lecithin could be manipulated within stipulated legal regulations to achieve high fat textural properties, notably spreadability and viscosity, at reduced fat concentrations.

4.6.2 Hardness of tempered dark chocolate

Hardness showed inverse relationships with particle size, fat and lecithin with significant reductions at all fat contents, but greatest at 25% with 0.3% lecithin (Fig. 4.14). At 25% fat, hardness decreased from 7062 g with 18μm to 5546 g at 50μm. Trends in hardness were similar at 30% and 35% fat with 0.3% lecithin but less pronounced at higher particle sizes (Fig. 4.14). The greater hardness levels noted with 25% fat and 18 - 25 μm

particle sizes suggest more particle-particle interactions, and spanning of stress bearing paths. Hardness from particle contacts was a function of mean particle size and diameter and specific surface area.

Fat content was inversely related ($p<0.001$) to hardness at all particle sizes and lecithin levels (Fig. 4.14). Combined effects of fat content and PSD thus have greatest influences but are less pronounced at higher fat and lecithin contents (Fig. 4.14) where fat coating of particles reduces inter-particle interactions inducing product softening. Significant ($p<0.001$) reductions were noted when lecithin content was increased from 0.3% to 0.5%. Lecithin has amphiphilic (both hydrophilic and lipophilic) properties making the molecule an effective dispersant, promoting deagglomeration and wetting of clumps inducing chocolate softening. Significant ($p<0.001$) interactions (Table 4.7), showed that the combined effects of PSD, fat and lecithin could be manipulated to control softness and/or hardness of tempered dark chocolate, with implications for quality control and production cost.

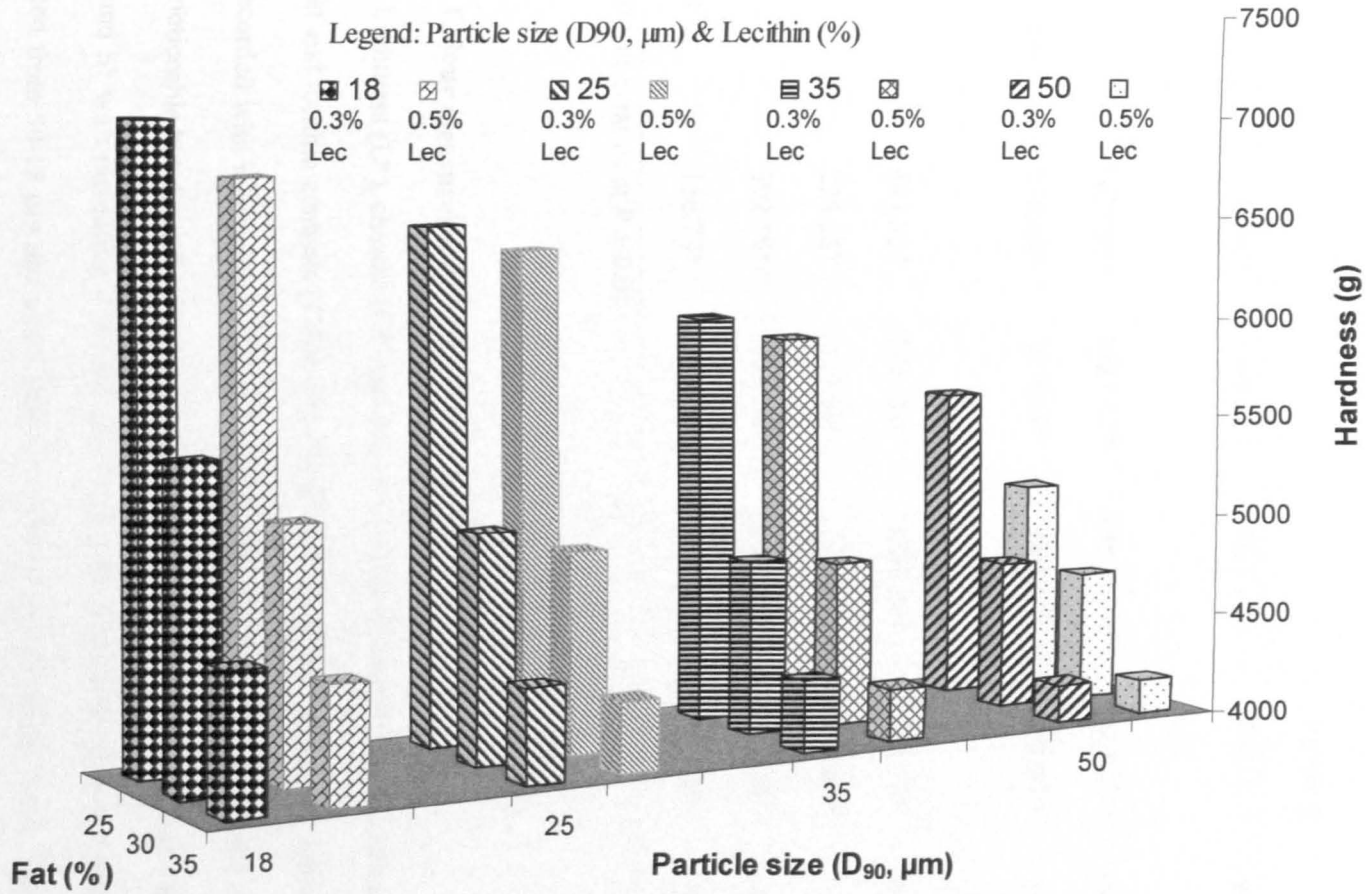


Figure 4.14. Effect of PSD and composition on hardness of tempered dark chocolate

Table 4.7. ANOVA Summary of F-values of the textural properties

Process variables	Firmness	Consistency	Cohesiveness	Index of viscosity	Hardness
A : Particle size (D_{90})	1595.40*	2340.25*	2027.61*	1018.71*	978.94 *
B : Fat	5971.02*	8583.65*	10547.21*	5919.75*	6921.18 *
C : Lecithin	578.48*	815.85*	988.35*	518.83*	166.74 *
A x B	841.85*	1221.89*	1083.29*	502.72*	160.94 *
A x C	224.58*	317.98*	279.52*	127.12*	7.48 *
B x C	392.59*	534.14*	698.08*	354.23*	17.74 *
A x B x C	156.79*	219.63*	186.26*	81.83*	32.48 *

* Significant F-ratios at $P \leq 0.05$

4.6.3 Colour measurements

Lightness (L*), chroma (C*) and hue (h°) followed similar trends with changes in PSD, fat and lecithin contents (Table 4.8). Significant ($p<0.001$) and linear effects on L* were recorded with increasing particles from 18 to 50 μm, with consequential decreases in L*, noticeable but dependent on fat contents (Table 4.8). Similar decreases were noted in C* and h° with increasing PSD and fat. Thus, dark chocolate became lighter as D_{90} decreased from 50-18 μm and as PS increased (18-50 μm) C* and h° were significantly decreased, pronounced at 25% fat. Increasing fat content reduced C* and h°, but the effects were less marked at 35% fat than at 30%. As lecithin had no noticeable effect on

L*, C* and h° (Table 4.9), appearance data was primarily dependent on PSD and fat content.

Hutchings (1994) stated that L*, C* and h° respectively represent food diffuse reflectance of light, degree of saturation and hue luminance, which are dependant on particulate distribution, absorptivity and scattering factors or coefficients. In a densely packed medium, scattering factor is inversely related to particle diameter (Saguy & Graf, 1991). Chocolates with varying particle sizes differ in structural and particulate arrangements (Table 4.2) influencing light scattering coefficients and thus appearance. Chocolates with finer particles (18-25 μm) have larger specific surface areas, lower particle diameters and more inter-particle interactions thus tend to be denser, scatter more light, appear lighter and more saturated than those with coarser (35-50 μm) particles. Such changes result in higher scattering coefficients, with subsequent paleness - higher L* values. Consequently, increases in saturation effects within suspensions yield higher C* and h° values. On the other hand, cocoa fat is an inherent crystalline network which scatters light reducing luminance and saturation indices in higher fat products.

From ANOVA, PSD and fat content significantly ($p<0.001$) influenced L*, C* and h° but lecithin had no significant effect on appearance (Table 4.9). No significant ($p\leq0.05$) interactions were observed among processing parameters (Table 4.8) with the exception of interaction between PSD and fat content. Fat content had greatest influence on dark chocolate appearance followed by PSD.

Table 4.8. Effects of particle size distribution and composition on colour measurements

Particle size (D_{90}) (μm)	Fat content (%)	Lecithin (%)	Colour measurements		
			L*	C*	h°
18 ± 1.0	25	0.3	43.49 ± 0.40	14.36 ± 0.40	43.9 ± 0.24
		0.5	43.52 ± 0.87	14.24 ± 0.46	43.7 ± 0.87
	30	0.3	40.39 ± 1.16	13.15 ± 0.08	42.6 ± 0.49
		0.5	37.43 ± 0.67	13.04 ± 0.52	42.4 ± 0.35
	35	0.3	35.19 ± 0.56	11.60 ± 0.07	40.4 ± 0.15
		0.5	34.73 ± 0.38	11.82 ± 0.34	41.4 ± 0.61
25 ± 1.0	25	0.3	42.16 ± 0.36	14.11 ± 0.48	42.9 ± 0.87
		0.5	41.72 ± 1.82	14.17 ± 0.57	42.8 ± 0.72
	30	0.3	35.96 ± 0.33	12.70 ± 0.28	42.5 ± 0.77
		0.5	36.43 ± 0.67	12.90 ± 0.75	42.5 ± 0.78
	35	0.3	34.01 ± 0.14	11.51 ± 0.19	40.5 ± 0.80
		0.5	34.61 ± 0.50	11.65 ± 0.33	40.6 ± 0.60
35 ± 1.0	25	0.3	40.94 ± 0.33	13.79 ± 0.38	42.9 ± 0.31
		0.5	40.38 ± 0.80	13.94 ± 0.15	42.6 ± 0.50
	30	0.3	34.85 ± 0.18	12.38 ± 0.22	42.5 ± 0.10
		0.5	35.27 ± 0.52	12.58 ± 0.27	42.0 ± 0.21
	35	0.3	33.50 ± 0.42	11.50 ± 0.07	39.7 ± 0.40
		0.5	33.86 ± 0.23	11.51 ± 0.06	40.1 ± 0.26
50 ± 1.0	25	0.3	38.64 ± 0.53	13.42 ± 0.28	42.5 ± 0.39
		0.5	36.93 ± 0.26	13.93 ± 0.16	42.7 ± 0.15
	30	0.3	34.22 ± 1.17	12.24 ± 0.46	41.6 ± 0.95
		0.5	34.90 ± 0.35	12.14 ± 0.26	41.2 ± 0.21
	35	0.3	33.25 ± 1.16	11.27 ± 0.42	39.5 ± 0.83
		0.5	33.42 ± 0.59	11.04 ± 0.15	38.9 ± 0.34

Means ± standard deviation from triplicate analysis

Table 4.9. ANOVA Summary of F-values of colour measurements

Process variables	L*	C*	h°
A : Particle size (D_{90})	88.19*	13.08*	13.41*
B : Fat	546.34*	317.49*	182.94*
C : Lecithin	2.01	1.05	10.97
A x B	12.87*	0.50	2.00
A x C	2.38	0.22	1.69
B x C	1.55	0.28	2.95
A x B x C	4.40	0.85	1.05

* Significant F-ratios at $P \leq 0.05$

4.6.4 Relationships between textural properties and appearance of dark chocolate

Correlation and principal component analyses established the extent that PSD, fat and lecithin contents influence textural properties and appearance with clear correlations. The correlation matrix (Table 4.10) for textural properties (firmness, consistency, cohesiveness, index of viscosity and hardness) and colour (L*, C* and h°) showed these were directly correlated with a highly significant correlation (r = 0.99-1.00; $p<0.001$) among textural properties, with high direct correlation (r = 0.71-0.96; $p<0.001$) between colour measurements. Thus, changes in textural properties in molten and solid tempered dark chocolates could predict finished product appearance although L* exhibited higher correlation (r = 0.87-0.96; $p<0.001$) than C* and h°, suggesting a better prediction.

The multivariate PCA product space (Fig. 4.15) explained >81% variance in the first two factors and showed texture and colour parameters were closely related with loadings for PSD, fat and lecithin content influencing factors. Fat content and PSD had polar influences on PC2 (16.4% variance) score. Further examination suggested PSD had multiple discrete components (specific surface area, largest PS (D_{90}), smallest PS (D_{10}), mean PS (D_{50}), and Sauter mean diameter (D[3,2])) together influencing texture and appearance.

Table 4.10. Correlation between textural properties and colour measurements of dark chocolate

Parameter	Firmness	Consistency	Cohesiveness	Index of viscosity	Hardness	L-value	Chroma	Hue
Firmness	1	0.9997*	0.9967*	0.9947*	0.9855*	0.9558*	0.9104*	0.7811*
Consistency	-	1	0.9928*	0.9845*	0.8814*	0.8691*	0.7069*	0.7287*
Cohesiveness	-		1	0.9982*	0.9865*	0.9584*	0.9099*	0.7834*
Index of viscosity	-		-	1	0.9861*	0.9563*	0.9086*	0.7841*
Hardness	-		-	-	1	0.9470*	0.9275*	0.8107*
L-value	-		-	-	-	1	0.8600*	0.7273*
Chroma	-		-	-	-	-	1	0.8760*
Hue	-		-	-	-	-	-	1

* Significant at $P < 0.05$

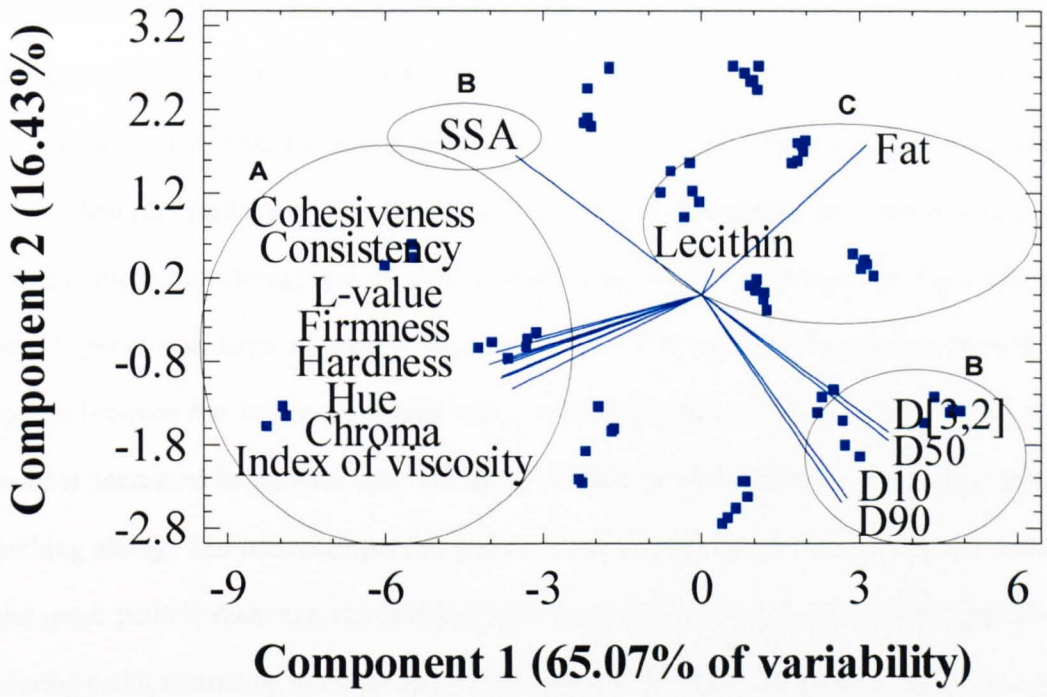


Figure 4.15. Principal component analysis of textural properties and appearance of dark chocolates (A) as affected by PSD (B) and composition (C)

4.7 Microstructural properties of molten dark chocolate

Light microscopy was used to characterise the variations in sugar crystalline network, particle-particle interaction strengths and particle-fat phase behaviour from molten dark chocolate with varying PSD and fat concentration. Micrographs (Figs. 4.16 – 4.18) showed clear variations in microstructure among samples from different PSD (Table 4.2) and fat contents.

Samples containing 25% fat showed high solids packing intensity with extensive particle-particle interaction strengths at all particle sizes (Figs. 4.16a – d) so that the crystalline network was disperse with large specific surface area (Table 4.2), and with smaller particles filling the spaces between the larger, the result was a high bed density. At lower PS (18 μm), particle number increased in parallel with points of contact, particle-particle interactions, and greater packing ability. The increased particle-particle interactions, amount of and specific surface area and mean particle diameter, resulted in flocculation and agglomeration, forming spanning stress bearing paths, restricting mobility and compartmentalization of the matrix (Figs. 4.16a – b).

With particle sizes ranges between 35-50 μm, particles were coarser, leading to PSD broadening into multimodal distributions (Fig. 4.4c-d), that reduced solid loading, and specific surface area (Table 4.2). As particle sizes were increased, the packing ability of solids became restricted, leading to fewer particle-particle interactions (Figs. 4.16c – d). Prasad et al. (2003) also noted rates of formation and disruption of aggregates were functions of flow induced shear stresses, particle volume fraction and interaction energy. The observed greater flocculation and agglomeration of sugar crystals network and high inter-particle interaction with 25% fat, explained higher rheological (Beckett 1999; Chevalley, 1999) and mechanical properties (firmness and hardness) observed with low fat chocolates. Servais et al. (2004) noted that yield stress depended on amount of small particles (specific surface area) and interactions, and

originated in mechanical (friction) and chemical interactions between particles. Prasad et al. (2003) concluded yield value was determined by inter-particle contacts, with a consequent linear dependence on mean particle size or, more accurately, on specific surface area.

With higher fat (Figs. 4.17 & 4.18) there were less dense sugar crystalline networks and reduced particle-particle interactions, with more open structures and void spaces between the crystals. This could be related to the higher fat content in the suspension, which tends to wet the matrix with fat thereby opening up the fat phase, as fat filled the voids within the crystal network. Beckett (1999) attributed this to the free-moving lubricating plastic flow, more connected with forces between solid particles. Fat fills spaces between solid particles in molten chocolate and reduces resistance to flow, with greatest effect at lower PS. However, the microstructure of D_{90} particle sizes >35 μm shows very large spherical and dispersed crystalline grains within the suspension (Figs. 4.16c-d, 4.17c-d, & 4.18c-d), that is suggested to be the cause of grittiness associated with chocolates processed with D_{90} >35 μm (Beckett, 2008).

The qualitative structural information illustrated by the micrographs thus provides a mechanistic explanation for quantitative differences in rheological, textural and sensory character in dark chocolates with varying PSD and fat content. This knowledge can improve the quality of models developed to optimise PSD influences on the flow, textural and sensory character in chocolate. Release of structural mobility and compartmentalization can be achieved by controlling microstructure during processing. Aguilera (2005) explained that structure has the largest effect on sound and food behaviour in biting. Structuring of particles within the multiphase chocolate systems during processing could be optimised to enhance resistance to flow, reduce grittiness perceived in chocolate with particle D_{90} > 35 μ, with consequential effects on the quality characteristics of finished chocolates.

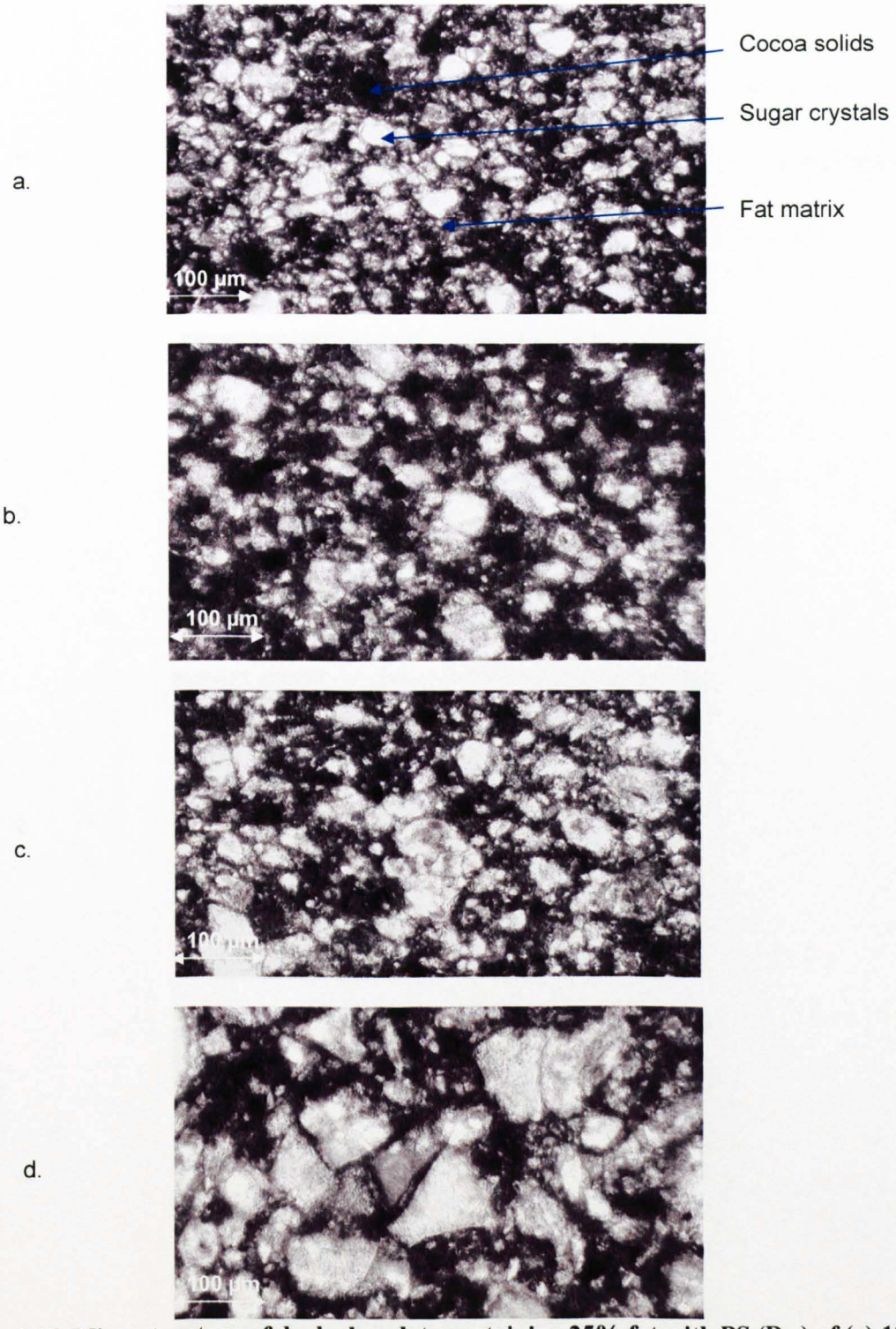


Figure 4.16. Microstructure of dark chocolate containing 25% fat with PS (D_{90}) of (a) 18 µm (b) 25 µm (c) 35 µm (d) 50 µm

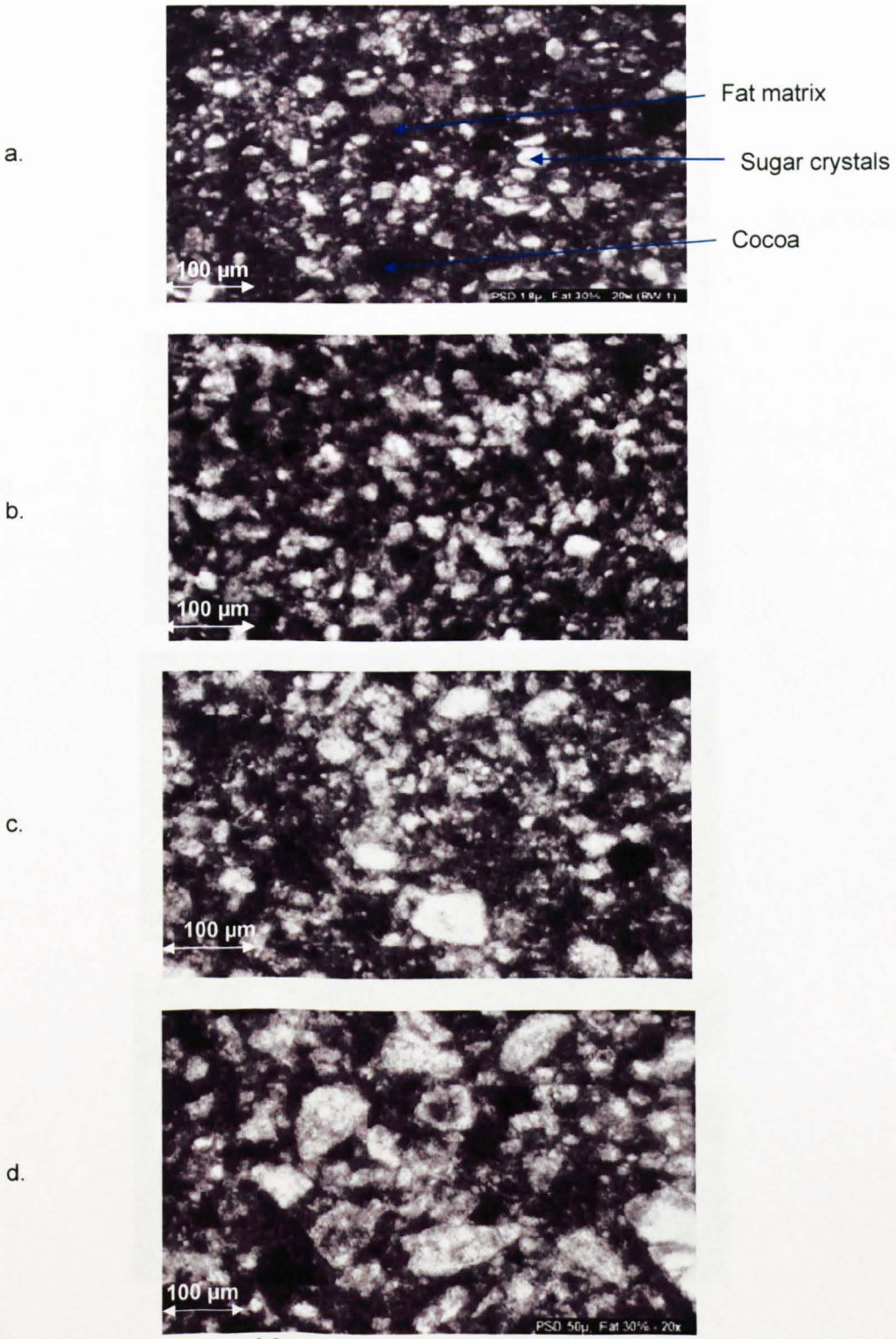


Figure 4.17. Microstructure of dark chocolate containing 30% fat with PS (D_{90}) of (a) 18 µm (b) 25 µm (c) 35 µm (d) 50 µm

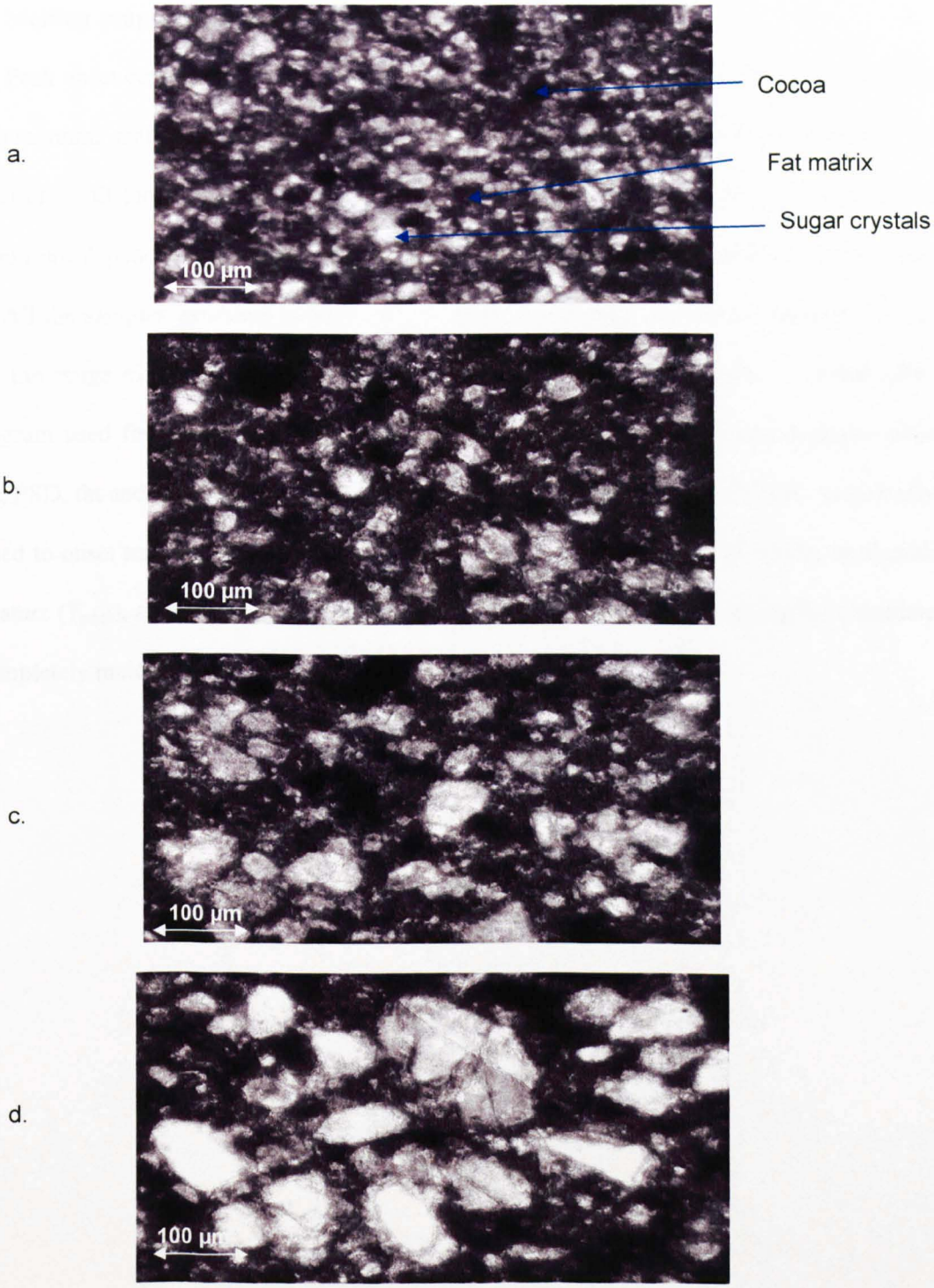


Figure 4.18. Microstructure of dark chocolate containing 35% fat with PS (D_{90}) of (a) 18 µm (b) 25 µm (c) 35 µm (d) 50 µm

4.8 Melting properties of dark chocolate

Peak onset corresponds to the temperature at which a specific crystal form starts to melt; peak maximum, that at which melting rate is greatest; and end of melting, completion of liquefaction – all these information are related to the crystal type. Peak height, position and resolution are dependent on sample composition and crystalline state distribution (McFarlane, 1999). All the samples exhibited similar distinct single endothermic transitions between 15 and 55 °C, the range expected for chocolate melting profiles. Figure 4.19 shows a typical DSC thermogram used for evaluating the melting properties of dark chocolates manufactured from varying PSD, fat and lecithin content. It recorded that heat capacity c_p gradually and consistently increased to onset temperature (T_{onset}), and then progressively increased more rapidly until peak temperature (T_{peak}), after which it decreased to the end temperature (T_{end}) indicating the chocolate was completely melted.

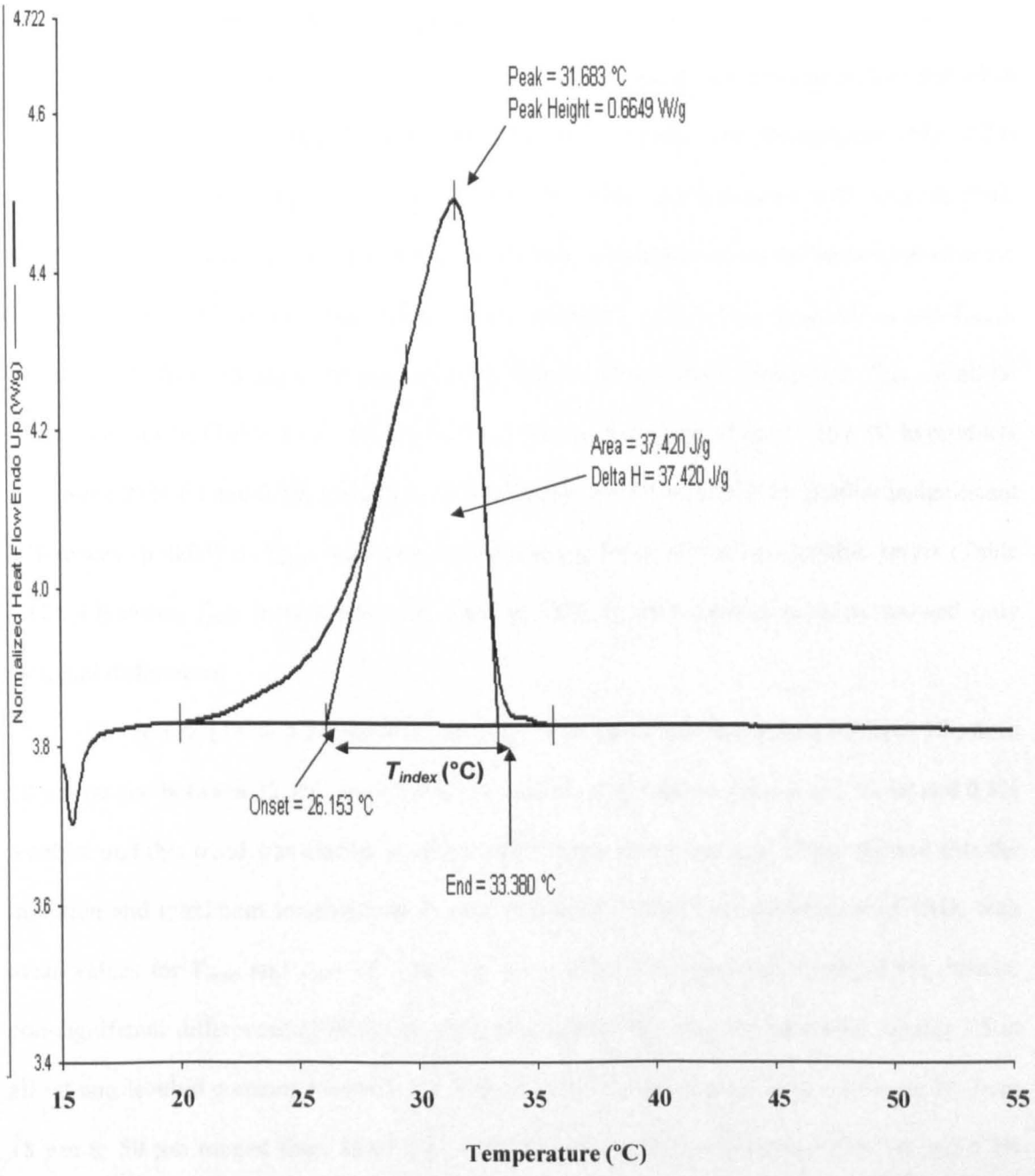


Figure 4.19. Illustration of DSC thermogram used to characterise the melting properties

4.8.1 Effects of particle size distribution

PSD influences chocolate rheological and microstructural properties as well as texture in derived molten and tempered products (Afoakwa et al., 2008). The thermogram (Fig. 4.20) showed similar peak shapes and sizes for dark chocolates manufactured with varying PSD, suggesting no characteristics differences in crystallinity and degree of crystallisation between the products. Table 4.11 shows values for key DSC parameters (T_{onset}, T_{end}, T_{peak}, ΔH_{melt} and T_{index}). Increasing PS from 18 μm to 50 μm caused no significant (p=0.675) changes in T_{onset}, at all fat and lecithin levels (Table 4.12). Values for T_{onset} were in the range of 26.5 – 26.6 °C in products containing 25% fat and 0.3% lecithin at 18 μm and 50 μm PS respectively. Similar insignificant differences (p>0.05) in T_{onset} were noted with varying PS at all fat and lecithin levels (Table 4.12). Likewise, T_{peak} in products with varying PSD, fat and lecithin contents showed only marginal differences.

The results (Table 4.11) showed that T_{peak} of products with increasing PS from 18 μm to 50 μm ranged between 32.3°C and 32.5°C respectively in products containing 25% fat and 0.3% lecithin, and this trend was similar at all fat and lecithin concentrations. These showed that the initiation and maximum temperatures in dark chocolate melting are independent of PSD, with mean values for T_{onset} and T_{peak} of ~ 26.5 °C and ~ 32.4 °C respectively (Table 4.11). Similar non-significant differences (p>0.05) in ΔH_{melt} were found between products with varying PS at all fat and lecithin contents (Table 4.12). Values of ΔH_{melt} in products with increasing PS from 18 μm to 50 μm ranged from 30.07 J/g to 30.62 J/g in products containing 25% fat and 0.3% lecithin, and this marginal and insignificant differences (p>0.05) in trends were similar at all fat and lecithin levels.

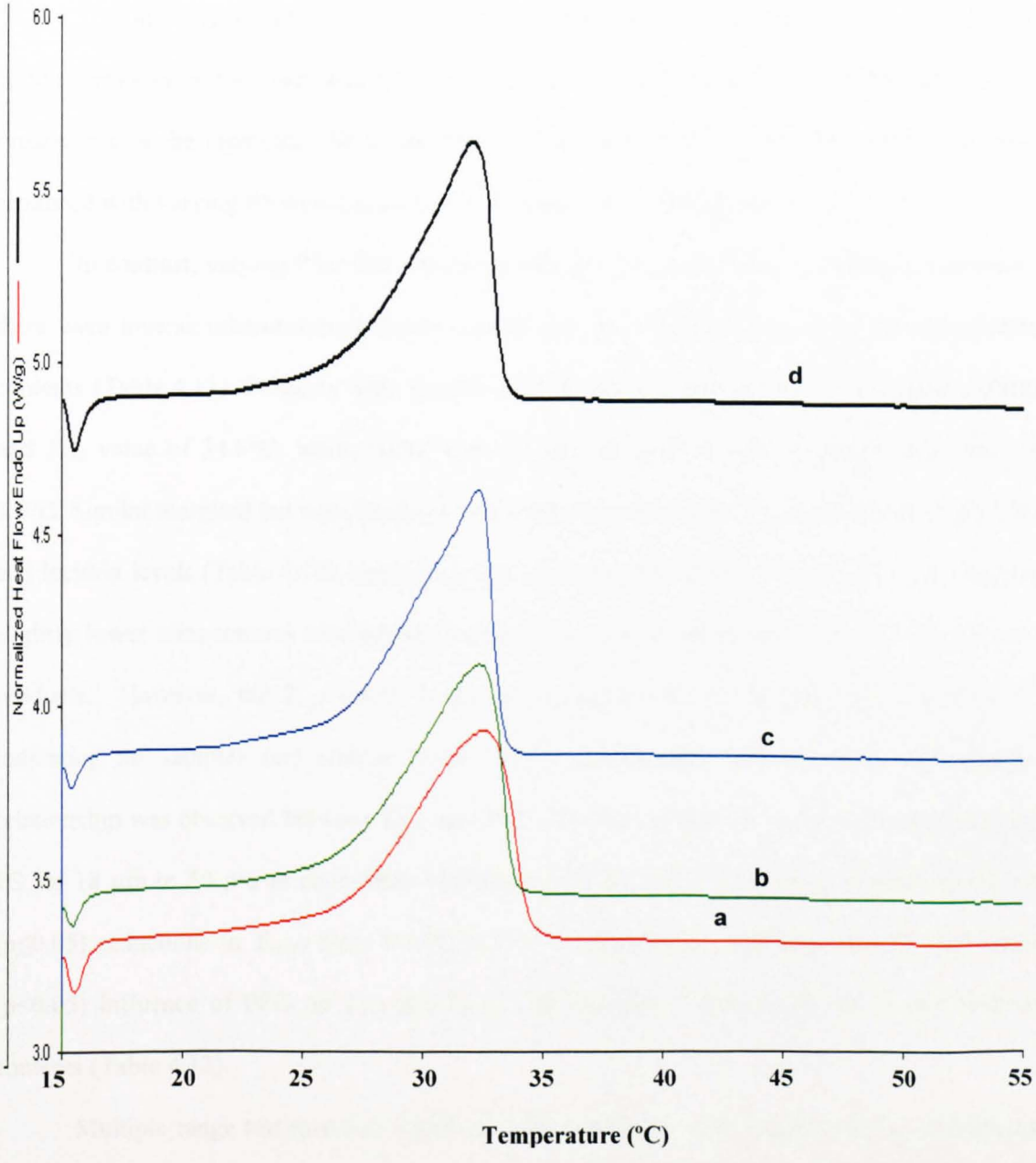


Figure 4.20. Typical DSC thermograms for dark chocolate at constant fat and lecithin content varying PSD: (a) 18 µm, (b) 25 µm, (c) 35 µm and (d) 50 µm

The non-significant relationship between PSD and ΔH_{melt}, implies that enthalpy of melting was similar for chocolates at all PS at specified fat and lecithin levels. This indicates that irrespective of the ingredient (fat or lecithin content) used for the formulation, dark chocolates produced with varying PS would require similar energy to complete melting.

In contrast, varying PSD had significant effects on T_{end} and T_{index} of products. Generally, there were inverse relationships between particle size and T_{end} and T_{index}, at all fat and lecithin contents (Table 4.11). Products with smaller PS (18 μm) at 25% fat and 0.3% lecithin content had T_{end} value of 34.6°C, whilst those with 50 μm had 34.0°C, representing a difference of 0.6°C. Similar marginal but significant ($p<0.05$) decreasing trends in T_{end} were observed at all fat and lecithin levels (Table 4.12), suggesting that dark chocolates with larger PS (50 μm) require slightly lower temperatures to complete melting than their corresponding smaller PS (18 μm) products. However, the T_{end} values in all the products were in the range 33.0 to 34.6 °C indicating all samples had similar Form V ($ß_2$) polymorphic stability. A similar inverse relationship was observed between T_{index} and PSD. The data (Table 4.11) showed that increasing PS for 18 μm to 50 μm in chocolates containing 25% fat and 0.3% lecithin caused significant ($p\leq0.05$) reductions in T_{index} from 8.4 °C to 7.4 °C respectively. ANOVA showed significant ($p<0.05$) influence of PSD on T_{end} and T_{index} with significant interactions for fat and lecithin contents (Table 4.12).

Multiple range test revealed significant differences ($p = 0.001$) between T_{end} of products containing 18 μm, 35 μm and 50 μm, indicating that chocolates with finer particles would take relatively longer time to melt than their corresponding products with larger particles, suggesting their possible relationships with the relative strengths of the inter-particle aggregations and flocculation in the different products.

Table 4.11. Melting properties of dark chocolate from varying PSD, fat and lecithin content

Particle size (D v, 0.9) (μm)	Fat (%)	Lecithin (%)	Melting properties				
			T_{onset} (°C)	T_{end} (°C)	T_{index} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)
18 ± 1.0	25	0.3	26.2 ± 0.2	34.6 ± 0.3	8.4 ± 0.2	32.5 ± 0.2	30.07 ± 0.38
		0.5	26.0 ± 0.1	34.2 ± 0.4	8.2 ± 0.4	32.0 ± 0.3	28.20 ± 0.12
	30	0.3	26.5 ± 0.3	34.4 ± 0.4	7.9 ± 0.2	32.5 ± 0.4	36.52 ± 1.05
		0.5	26.3 ± 0.2	33.9 ± 0.2	7.5 ± 0.3	32.4 ± 0.3	30.02 ± 0.42
	35	0.3	26.4 ± 0.1	33.8 ± 0.2	7.4 ± 0.1	32.4 ± 0.2	44.59 ± 0.62
		0.5	26.5 ± 0.2	33.7 ± 0.3	7.2 ± 0.3	32.5 ± 0.2	43.10 ± 1.23
25 ± 1.0	25	0.3	26.3 ± 0.1	34.4 ± 0.2	8.1 ± 0.2	32.4 ± 0.3	30.52 ± 0.73
		0.5	26.3 ± 0.2	34.1 ± 0.2	7.8 ± 0.3	32.4 ± 0.3	29.08 ± 0.46
	30	0.3	26.6 ± 0.2	33.9 ± 0.3	7.3 ± 0.4	32.2 ± 0.1	37.09 ± 1.24
		0.5	26.5 ± 0.1	33.5 ± 0.2	7.0 ± 0.3	32.5 ± 0.3	32.46 ± 0.66
	35	0.3	26.6 ± 0.3	33.7 ± 0.2	7.1 ± 0.3	32.2 ± 0.1	45.01 ± 1.42
		0.5	26.5 ± 0.2	33.5 ± 0.3	7.0 ± 0.1	32.2 ± 0.1	43.41 ± 1.28
35 ± 1.0	25	0.3	26.3 ± 0.1	34.2 ± 0.5	7.9 ± 0.2	32.6 ± 0.3	30.68 ± 0.28
		0.5	26.4 ± 0.1	34.0 ± 0.4	7.6 ± 0.3	32.4 ± 0.3	28.60 ± 0.34
	30	0.3	26.6 ± 0.3	33.8 ± 0.2	7.2 ± 0.2	32.4 ± 0.2	37.19 ± 0.94
		0.5	26.7 ± 0.3	33.7 ± 0.1	7.0 ± 0.1	32.6 ± 0.2	34.01 ± 0.63
	35	0.3	26.8 ± 0.4	33.6 ± 0.3	6.8 ± 0.3	32.8 ± 0.3	45.15 ± 1.05
		0.5	26.9 ± 0.4	33.4 ± 0.2	6.5 ± 0.1	32.2 ± 0.3	42.79 ± 0.84
50 ± 1.0	25	0.3	26.6 ± 0.2	34.0 ± 0.4	7.4 ± 0.5	32.3 ± 0.2	30.62 ± 0.53
		0.5	26.7 ± 0.1	33.9 ± 0.2	7.2 ± 0.3	32.4 ± 0.2	28.62 ± 0.23
	30	0.3	26.8 ± 0.3	33.5 ± 0.3	6.7 ± 0.3	32.9 ± 0.4	37.29 ± 0.15
		0.5	26.7 ± 0.3	33.3 ± 0.3	6.6 ± 0.2	32.2 ± 0.2	33.25 ± 1.05
	35	0.3	26.8 ± 0.4	33.2 ± 0.1	6.4 ± 0.1	32.7 ± 0.3	45.40 ± 0.87
		0.5	26.8 ± 0.3	33.0 ± 0.4	6.2 ± 0.4	32.4 ± 0.1	43.43 ± 0.46

Mean values from triplicate analysis ± standard deviation

Table 4.12. ANOVA Summary of F-values of the melting properties

Process variables	T_{onset} (°C)	T_{end} (°C)	T_{index} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)
A : Particle size	1.53	11.00*	199.84*	0.84	121.52
B : Fat	12.54	32.32*	2330.26*	0.23	3535.29*
C : Lecithin	2.43	18.18*	148.84*	3.13	376.74*
A x B	0.89	2.89*	99.22*	0.49	4.22
A x C	2.16	2.39	31.69*	0.91	1.46
B x C	2.45	0.53	198.58*	0.66	401.87*
A x B x C	1.73	1.01	19.76*	2.17	3.73

* Significant F-ratios at $P \leq 0.05$

Chocolates with smaller PSD (D_{90}, 18μm) have been found to contain higher particle-to-particle strengths with resultant increases in hardness (texture) than their corresponding larger PSD (D_{90}, 50μm) (Do et al., 2007; Afoakwa et al., 2008c). Do et al. (2007) also noted that decreases in the amount of particle aggregation and structure build up in flow affect chocolate melt down, suggesting that in its crystallised state, the particle skeleton of chocolates with larger PS is less interconnected, providing less resistance to breakage and melt down. This knowledge is important as it provides information on likely oral melting behaviour with an impact on temporal components of flavour release and also oral epithelial sensation. Beckett (1999) and Ziegler et al. (2001) noted that variations in PS might influence melt, flavour, colour and gloss of chocolates.

4.8.2 Effects of fat content

Data from the DSC (Fig. 4.21) indicated that varying fat content produced changes in crystallinity and melting properties observed in the differences in their peak widths. This suggests that the fat content in dark chocolates during manufacture influences the degree of crystallinity and crystal size distribution (CSD) of their corresponding tempered products. Lonchampt and Hartel (2004) also noted that amount and composition of fat in chocolate production had unpredictable effects on crystal size, and polymorphism and crystallisation rate in products. Hartel (2001) concluded distribution of crystal sizes in foods play key roles in final product quality, defined by total and specific characteristics of the crystalline material. Number of crystals and range of sizes, shapes, and polymorphic stability, as well as arrangements in network structures dictates mechanical and rheological properties. Knowledge and control of CSD can be important for optimising processing conditions.

Results from the DSC data on T_{onset}, T_{end}, T_{peak}, ΔH_{melt} and T_{index} with varying fat content are as shown on Table 4.11. ANOVA and multiple mean comparisons showed no significant difference ($p>0.05$) for T_{onset} and T_{peak} in chocolates with different fat contents (Table 4.12), implying limited influence on temperatures for onset and peak melting. There were significant differences ($p<0.05$) among melting end (T_{end}), index (T_{index}) and enthalpies (ΔH_{melt}) (Table 4.12). Increasing fat content from 25% to 35% caused consistent reductions in T_{end} from 34.6°C to 33.8°C in products containing 18 μm PS and 0.3% lecithin level.

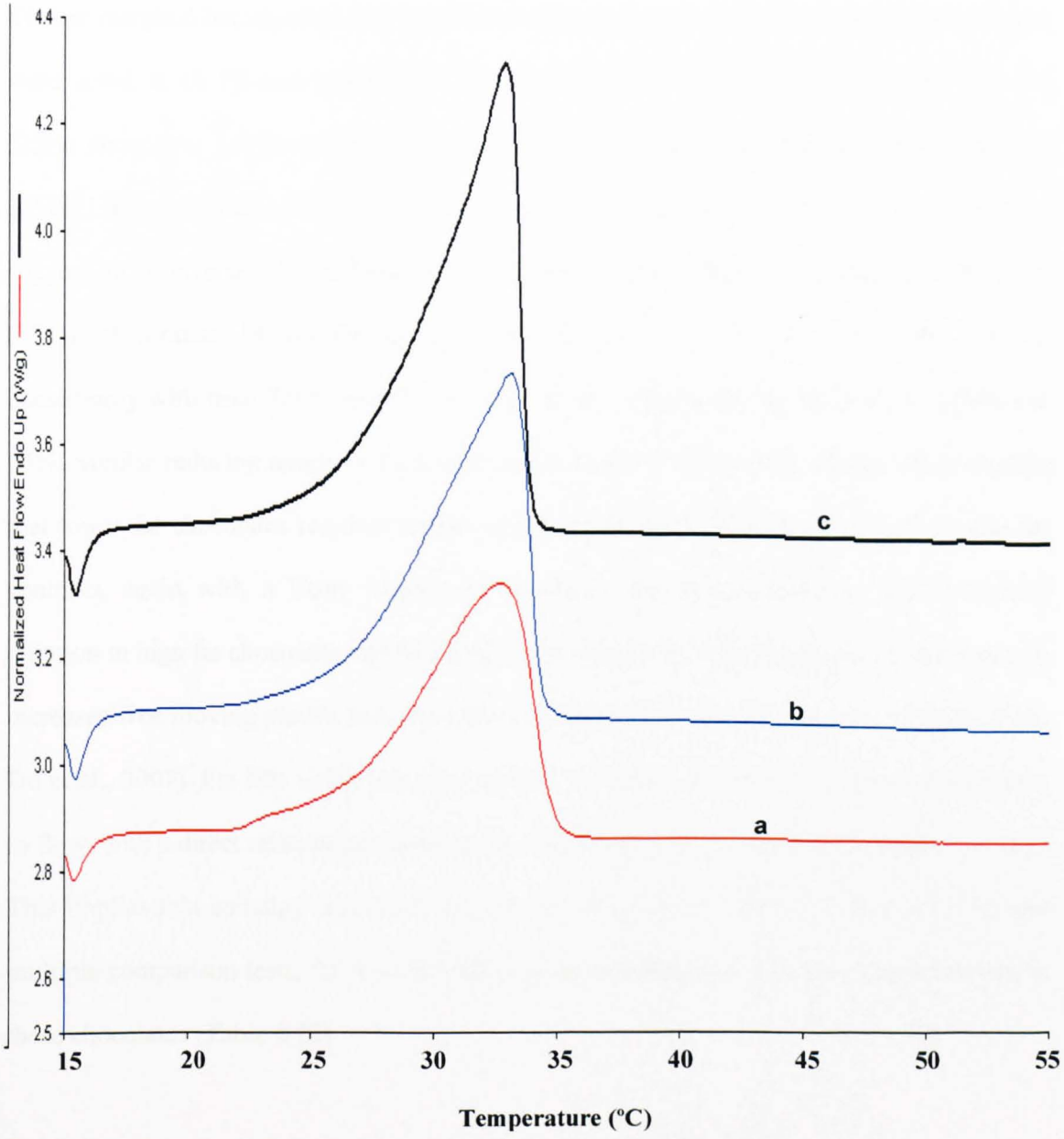


Figure 4.21. Typical DSC thermograms for dark chocolate at constant PS and lecithin content at constant PS and lecithin content with varying fat content: (a) 25%, (b) 30%, (c) 35%

Similar marginal but significant ($p<0.05$) decreasing trends in T_{end} with increasing fat content were noted at all PS and lecithin concentrations (Table 4.12). These suggest that low fat (25%) chocolates completed melting at higher temperatures than those with more fat (30-35%). Likewise, increasing fat content caused consistent decreases in T_{index} of products, suggesting an inverse relationship of T_{index} with fat content (Table 4.11). Products with lower (25%) fat content, 18 μm PS and 0.3% lecithin had T_{index} of 8.4 °C and this reduced consistently with from 7.9 °C and 7.4 °C respectively with increasing fat content to 30% and 35%. Similar reducing trends in T_{index} were noted at all PS and lecithin levels. These explain that lower fat chocolates required longer time to melt than similar products with higher fat contents, again with a likely impact on behaviour during consumption. Lower melting duration in high fat chocolates can be attributed to reductions in inter-particle interactions and increased free-moving plastic flow, possibly related to yield value of products (Beckett, 2000; Do et al., 2007). Fat fills voids between particles in molten chocolate and reduces resistance to flow, with a direct relationship between fat content and ΔH_{melt}, independent of particle size. This implies that enthalpy is reduced in products of lower fat contents. From ANOVA and multiple comparison tests, fat content had the greatest influence on melting characteristics in these chocolates (Table 4.12).

4.8.3 Effects of lecithin

The amphiphilic nature of lecithin promotes deagglomeration with effects on physical properties (Talbot, 1999; Beckett 2000; Lonchampt & Hartel, 2004; Dhonsi & Stapley, 2006). Figure 4.22 shows typical DSC thermograms for dark chocolate manufactured from varying lecithin content (0.3 and 0.5%) at 18 μm PS and 30% fat content. The thermograms (Fig. 4.22) revealed the effect of lecithin concentration on crystallinity of products.

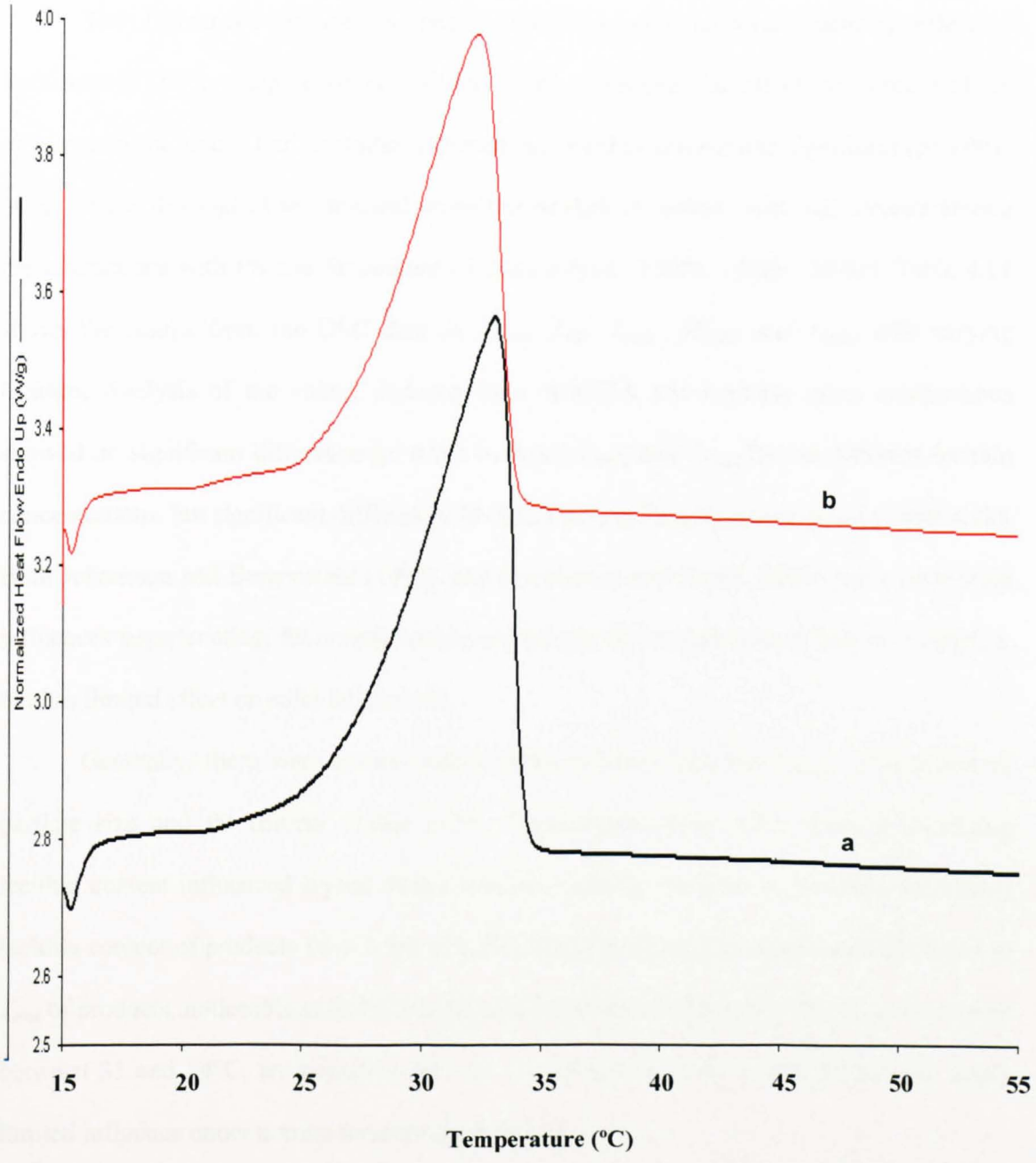


Figure 4.22. Typical DSC thermograms for dark chocolate with varying lecithin content showing (a) 0.3%, (b) 0.5% at constant PS and fat content

The differences observed in peak widths suggest a moderate reducing effect of lecithin addition on degree of crystallinity, with consequential effect on some melting properties of products. Earlier studies reported that lecithin content had significant ($p<0.001$) effect on the rheological and textural properties of dark chocolates with significance among the interactions with PS and fat content (Afoakwa et al., 2008a, 2008b, 2008c). Table 4.11 shows the results from the DSC data on T_{onset}, T_{end}, T_{peak}, ΔH_{melt} and T_{index} with varying lecithin. Analysis of the values deduced from ANOVA and multiple mean comparisons showed no significant difference ($p>0.05$) between T_{onset} and T_{peak} for the different lecithin concentrations, but significant differences ($p<0.05$) among T_{end}, T_{index} and ΔH_{melt} (Table 4.12). Both Johansson and Bergenstahl (1992), and Lonchampt and Hartel (2004) reported lecithin influences sugar coating, fat crystallisation, crystal growth, polymorphism and oil migration, but has limited effect on solid fat content.

Generally, there were inverse relationships between T_{end} and T_{index}, independent of particle size and fat content (Table 4.11). Thermograms (Fig. 4.22) showed increasing lecithin content influenced crystal dimensions and melting character in products. Increasing lecithin content of products from 0.3% to 0.5% caused marginal but significant differences in T_{end} of products, noticeable at all PS and fat concentrations (Table 4.11). The T_{end} values were between 33 and 34°C, an indication that the crystallisations were in ßV polymorph, imply limited influence under normal tempering conditions.

On the other hand, T_{index} decreased consistently with increasing lecithin content, suggesting that products containing lower lecithin levels (0.3%) might require relatively longer residence time to melt than those of their corresponding products with higher lecithin levels (0.5%), with likely impact on the melting residence time of products during consumption. The lower melting index (duration) observed with products containing higher lecithin levels might be attributed to sugar coating ability of lecithin during processing, and

thus reducing their inter-particle interaction to induce chocolate melting properties. Dhonsi and Stapley (2006) reported lecithin migrates to sugar/fat interfaces and coats sugar crystals, influencing rheology and aiding dispersion of sugar crystals in the continuous phases. Chevalley (1999) suggested lecithin forms a monolayer on sugar particle surfaces allowing greater mobility in suspensions while increasing fat spreadability. Increasing lecithin content caused significant and consistent decreases in ΔH_{melt}, trends noted at all PS and fat content (Table 4.11). This implies that products with relatively higher lecithin content would require lower enthalpies to melt than those of their corresponding products with lower lecithin levels. Significant ($p<0.05$) interactions were observed among all the processing parameters. Multiple comparison tests revealed that fat content had the greatest effect on T_{index} and ΔH_{melt} of dark chocolates followed by lecithin content and then PSD (Table 4.12).

4.9 Relationships between rheological, textural and melting properties of dark chocolate

The rheological properties (yield stress and apparent viscosity) of chocolate are of crucial importance to the properties of materials and their efficiency of manufacture (Afoakwa et al., 2007; Chevalley, 1999). On the other hand, textural properties (firmness and index of viscosity) determines the degree of consistency and spreadability, and resistance to flow (viscosity) of molten chocolate (Beckett, 2000). Ziegler and Hogg (1999) concluded knowledge of such flow behaviour is important for moulding and enrobing, for quality control of molten chocolate products, for proper cookie drop formation and in design of bulk handling systems. Hardness determines the physical rigidity (texture) of products and relates directly to sensory properties during consumption. Assessment of both molten and solid tempered chocolate using texture analyzer with various probes and procedures have been

reported previously (Beckett, 2000; Pereira et al., 2003; Full et al., 1996; Liang & Hartel, 2004).

Multivariate statistical techniques were employed to evaluate relationships between the rheological properties (apparent viscosity and yield stress), textural properties (firmness, index of viscosity and hardness) and melting index of dark chocolate systems. Correlation and regression analyses conducted on the data revealed very high and significant ($p<0.05$) regression and correlation coefficients among all rheological and textural parameters (Table 4.13). Relationships were calculated using correlation analysis between yield stress and apparent viscosity. As previously discussed, high correlation coefficients ($r = 0.99$, $p = 0.001$) were observed between the yield stress and apparent viscosity. The rheological measurements indicated that yield stress and apparent viscosity follow the same trend and have a strong linear correlation and regression model (Fig. 4.7d) with a highly significant ($p = 0.001$) regression coefficient, $R^2 = 0.99$, indicating the two rheological parameters were highly related and could individually be used to predict rheological behaviour of dark chocolate during manufacture. This confirms observations by Servais et al. (2004) that yield stress and apparent viscosity of chocolates are related, suggesting either of the two rheological parameters could be effectively used to predict chocolate viscosity during processing. Contrary to this observation, Mongia and Ziegler (2000) did not find any relationship between yield stress and apparent viscosity. They interpreted their data based on regression to the Casson model; thus, Casson yield stress and the Casson plastic viscosity. Servais et al. (2004) observed that for the same products a linear correlation could be found between yield stress and apparent viscosity using the new ICA (2000) recommendation, but not between Casson yield value and Casson plastic viscosity using the Casson model.

Table 4.13. Regression and correlation analyses between dark chocolate rheological, textural and melting parameters

Parameter	Analysis	Apparent viscosity	Yield stress	Firmness	Index of viscosity	Hardness	Melting index
Apparent viscosity	Regression	1.0000	0.9898**	0.9321**	0.9333**	0.7640*	60.38*
	Correlation	1.0000	0.9941**	0.9654**	0.9661**	0.8567**	77.70*
Yield stress	Regression	-	1.0000	0.9211**	0.9121**	0.7314*	58.80*
	Correlation	-	1.0000	0.9598**	0.9550**	0.8314*	76.68*
Firmness	Regression	-	-	1.0000	0.9896**	0.9764**	62.48*
	Correlation	-	-	1.0000	0.9947**	0.9855**	79.05*
Index of viscosity	Regression	-	-	-	1.0000	0.9742**	67.92*
	Correlation	-	-	-	1.0000	0.9861**	82.41**
Hardness	Regression	-	-	-	-	1.0000	82.74**
	Correlation	-	-	-	-	1.0000	90.96**
Melting index	Regression	-	-	-	-	-	1.0000
	Correlation	-	-	-	-	-	1.0000

* Significant at $P< 0.05$; ** Significant at $P< 0.001$

High correlation coefficients (r = 0.96, p = 0.001) were noted between both yield stress and index of viscosity, and yield stress and firmness (Table 4.13), suggesting their high inter-relationships. The regression models developed (Fig. 4.23 and 4.24) showed highly significant (p = 0.001) regression coefficient, R^2 = 0.92 and 0.91 respectively for yield stress and firmness, and yield stress and index of viscosity. Servais et al. (2004) noted that yield stress depends on proportion of small particles (specific surface area) and on interactions, originating in mechanical (friction) and chemical effects. As yield stress corresponds to the energy needed for chocolate to start moving and relates to the strength of inter-particle aggregates at rest, the observed high inter-relationship suggest that both firmness and index of viscosity could be related to strengths of the aggregated particle-to-particle network system of chocolate mass during manufacture (Servais et al., 2004; Beckett, 2000), with the distribution and arrangement of particle sizes, fat and lecithin contents as the main influential factors dictating their flow behaviour (Afoakwa et al., 2007, 2008a). This knowledge would be useful for engineering purposes such as pumping, mixing, storage and transportation of molten chocolate. Contrary to the high regression coefficients noted between yield stress and firmness and that of index of viscosity (Table 4.13), yield stress and hardness yielded high and significant, but relatively lower correlation (r = 0.83, p = 0.001) and regression coefficient, R^2 = 0.73 (Table 4.13), suggesting other processing factors play significant role in defining the texture (hardness) of solid tempered dark chocolate. Afoakwa et al. (2007) and Beckett (1999) noted that several factors including recipe, manufacturing techniques, temper, polymorphism (stability of fat crystals) and cooling temperature controls influence final texture (hardness) of solid tempered chocolate. Keogh et al. (2002) also concluded that hardness is a useful

indicator of good tempering, or degree to which a stable fat crystal network has been formed. The regression model forms Figure 4.25.

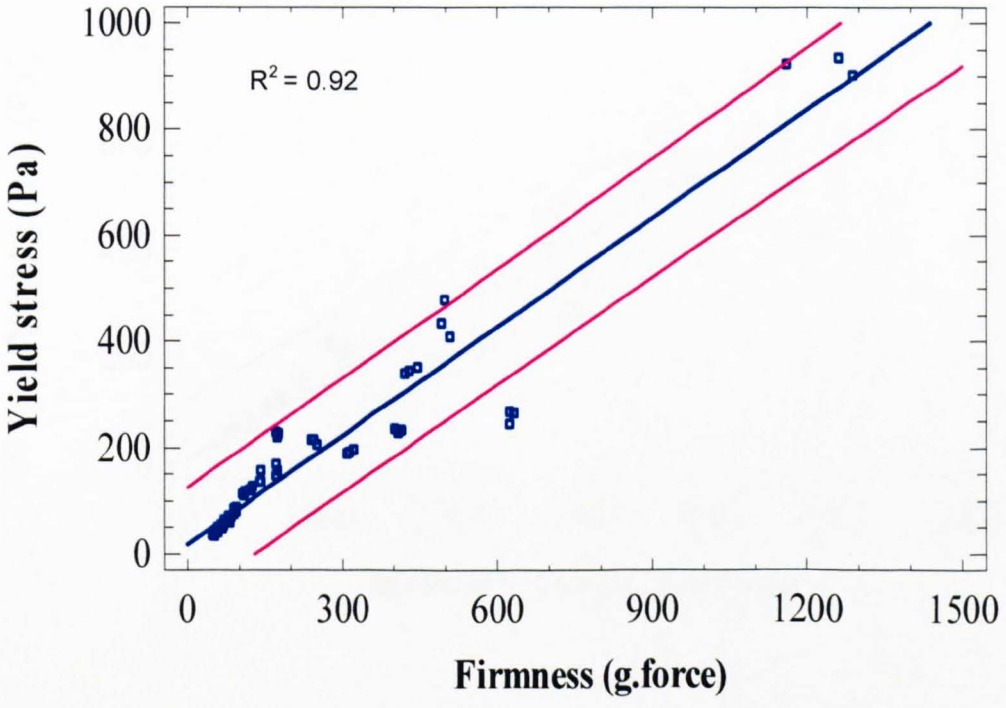


Figure 4.23. Relationship between yield stress and firmness in molten chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Yield stress = 18.2498 + 0.683086*Firmness

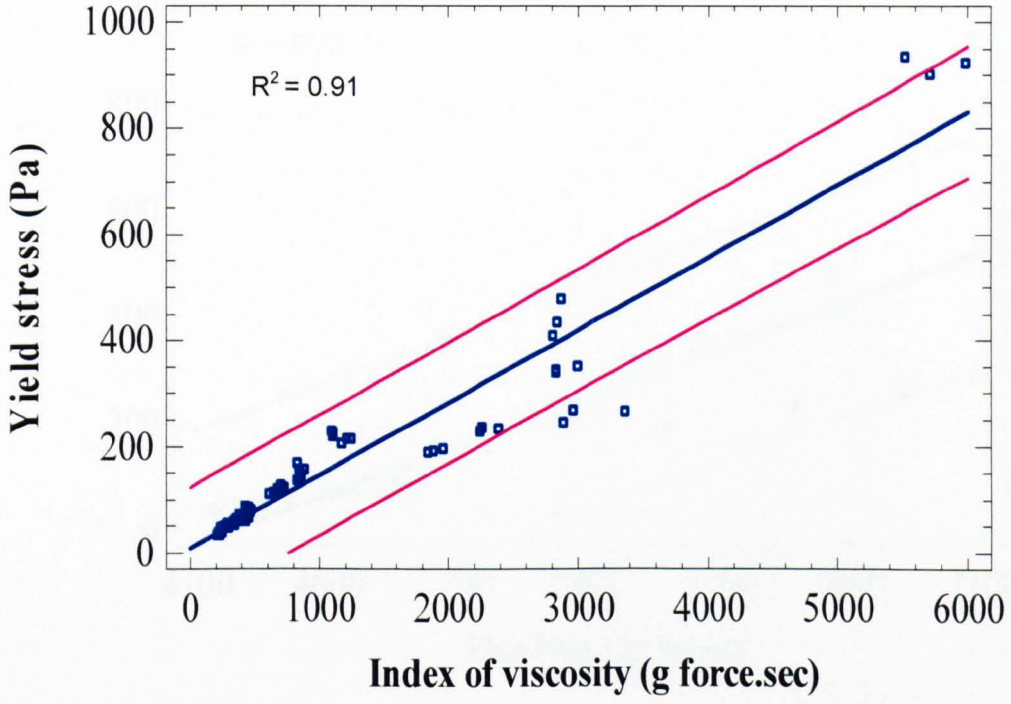


Figure 4.24. Relationship between yield stress and index of viscosity in molten chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Yield stress = 9.70711 + 0.13679*Index of viscosity

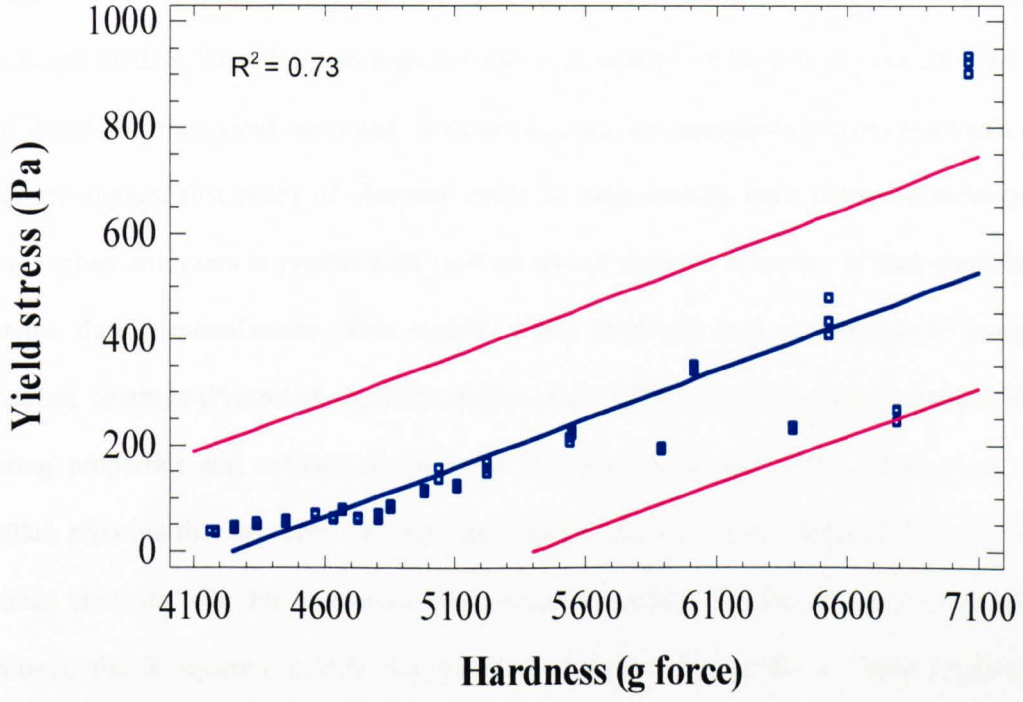


Figure 4.25. Relationship between yield stress and hardness in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Yield stress = -784.281 + 0.184538*Hardness

Apparent viscosity and firmness, and apparent viscosity and index of viscosity were highly positively correlated ($r = 0.97$, $p = 0.001$), with regression coefficient, $R^2 = 0.93$ (Table 4.13), indicating strong relationships. Figures 4.26 and 4.27 show the respective regression models. The observed high positive correlation (>95%) and regression (>94%) coefficients between yield stress and index of viscosity, and apparent viscosity and index of viscosity suggest that index of viscosity could be measured by back extrusion technique using texture analyzers to predict both yield stress and apparent viscosity of dark chocolate systems during manufacture. This would reflect thickness and uniformity of molten chocolate coatings (Prasad et al., 2003; Baker et al., 2006), and pumping characteristics, coating properties and sensory character of the mass (Seguine, 1988). Afoakwa et al. (2008a) reported that apparent viscosity and yield stress were more dependent on fat and lecithin contents with PSD showing only marginal effects. Similar to yield stress and hardness, the R-Squared statistic for hardness indicated the model as fitted explained 76.4% of the variability in apparent viscosity, with correlation coefficient, $r = 0.86$, $p = 0.001$, indicating a strong relationship between variables (Fig. 4.28). These suggest that although other factors contribute to final product hardness, both rheological parameters (yield stress and apparent viscosity) can predict ca. 75% variability in the final texture of tempered finished dark chocolate products.

The rheological and textural properties of the dark chocolate systems were related to the melting index (duration) of their respective tempered chocolate using regression and correlation analyses. The purpose was to establish the extent to which both rheological and textural properties of dark chocolates manufactured using varying PSD, fat and lecithin content could be used to predict the melting duration of their respective products during

consumption. This knowledge would be useful for new product development and process engineering purposes. The results showed moderately high correlation coefficients ($r = 0.77$ and $r = 0.78$, $p = 0.001$) respectively between both yield stress and melting index, and apparent viscosity and melting index (Table 4.13), indicating a moderately strong relationship between the variables. Similarly, moderately high regression coefficients were noted between the rheological properties (yield stress and apparent viscosity) of molten chocolate and melting index (Table 4.13). The regression models developed (Figs. 4.29 and 4.30) showed relatively lower but significant ($p = 0.001$) regression coefficient, $R^2 = 0.59$ and 0.60 respectively for yield stress and melting index, and apparent viscosity and melting index.

The relationships between the textural properties (firmness and index of viscosity) and melting index showed moderately higher positive correlations, $r = 0.79$, $p = 0.001$ and $r = 82$, $p = 0.001$, with regression coefficient, $R^2 = 0.62$ and $R^2 = 0.67$ respectively (Table 4.13), indicating their moderately strong relationships. Their regression models have been shown on Figures 4.31 and 4.32. These observations suggest that although other factors such as degree of fat crystal stability during tempering, tempering regime and cooling procedures might contribute to the melting behaviour of the products during consumption, both rheological parameters (yield stress and apparent viscosity) and textural properties of molten dark chocolate can be used to predict ca. 60 – 70% variability in the melting index or duration.

The relationship between hardness of the finished chocolate and melting index showed relatively higher coefficients of regression (Fig. 4.33) and correlation (Table 4.13). Multivariate analyses on the data showed fitting of a linear model to describe the

relationship between Hardness and Melting index. The R-Squared statistic indicated that the model as fitted explained 82.74% of the variability in Hardness, with correlation coefficient, r = 0.91, p = 0.001, indicating a relatively strong relationship between the variables. This explains that tempering, a fat crystallisation process which is used to convert molten chocolate into finished product plays a very significant role in defining the melting time or duration of the products. Beckett (1999) explained that melting of chocolate in the mouth is defined by the characteristics of the fat phase, and facilitates the perception of its characteristic taste, flavour and textural attributes. The intensity of perceived flavour changes dynamically over time as the chocolate is melted, manipulated and mixed with saliva for swallowing. Ziegler et al. (2001) also noted that particle size and rheology significantly influenced the melting time and sweetness intensity of milk chocolate using time intensity methodology.

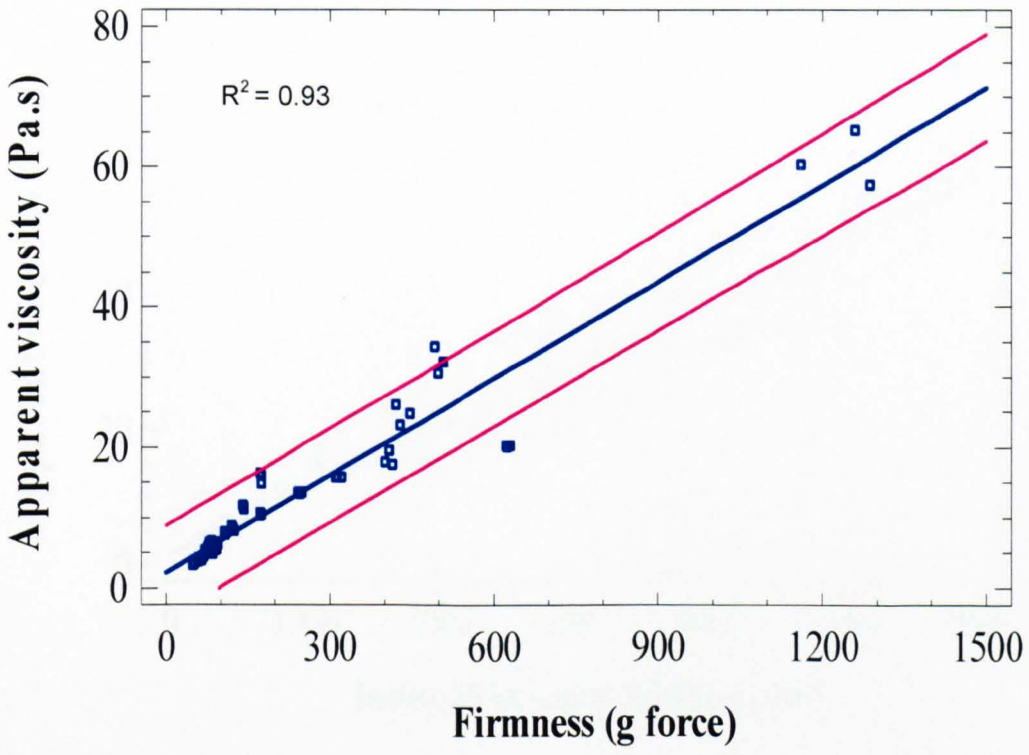


Figure 4.26. Relationship between apparent viscosity and firmness in molten chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Apparent viscosity = 2.20313 + 0.0460588*Firmness

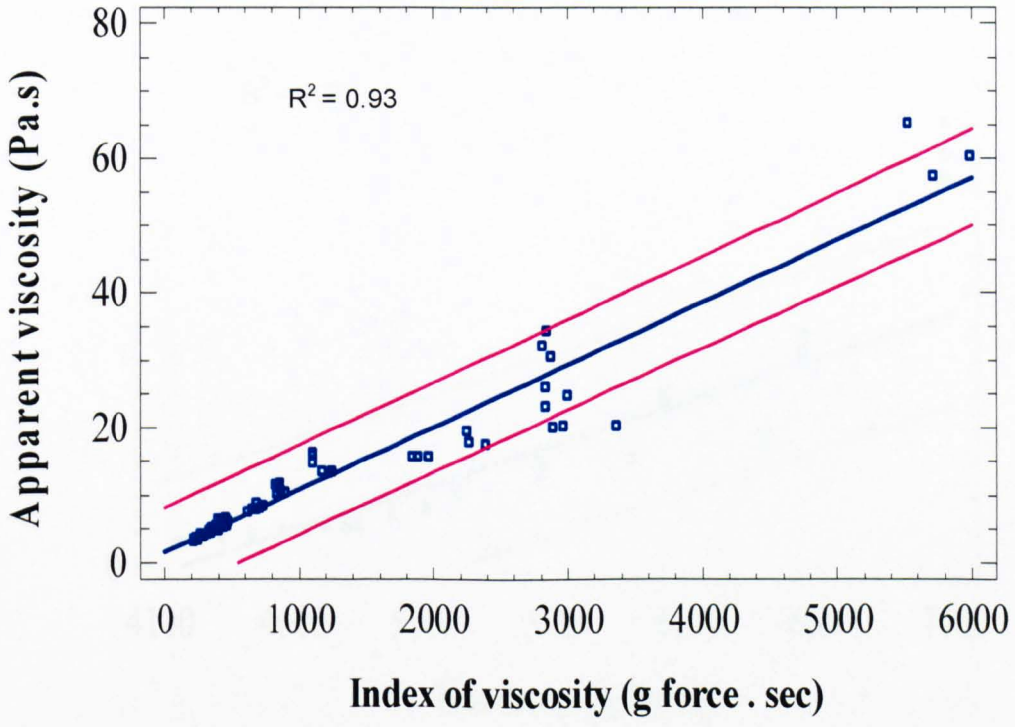


Figure 4.27. Relationship between apparent viscosity and index of viscosity in molten chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Apparent viscosity = 1.5664 + 0.00927531*Index of viscosity

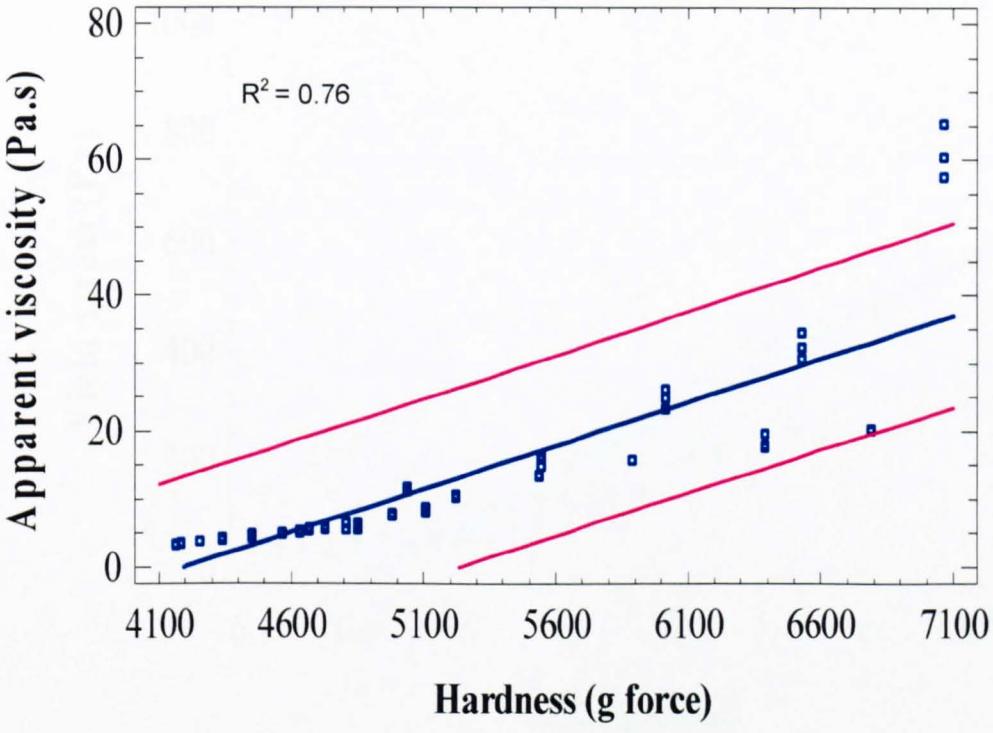


Figure 4.28. Relationship between apparent viscosity and hardness in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Apparent viscosity = -53.4812 + 0.0127469*Hardness

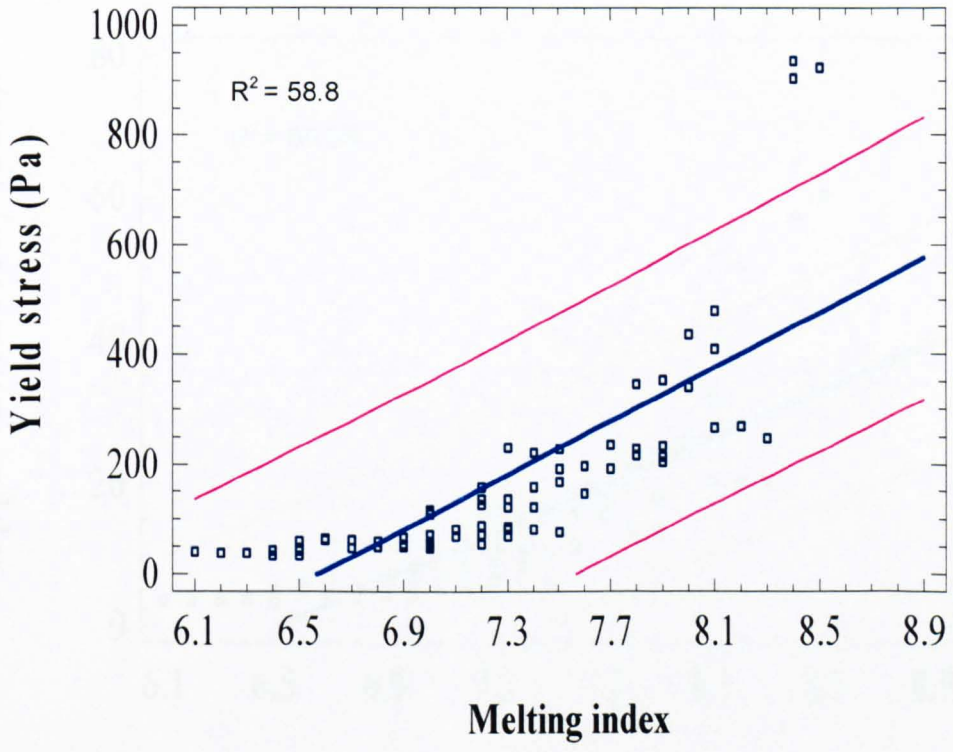


Figure 4.29. Relationship between yield stress and melting index in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Yield stress = -1620.41 + 246.64*Melting index

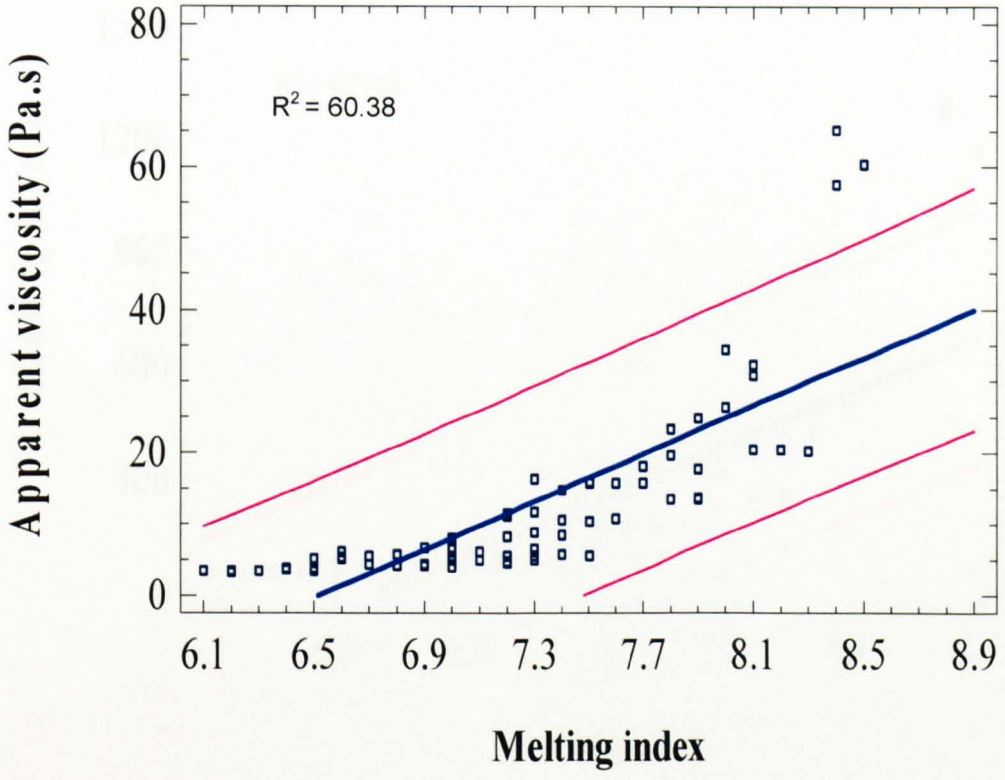


Figure 4.30. Relationship between apparent viscosity and melting index in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Apparent viscosity = -109.182 + 16.7535*Melting index

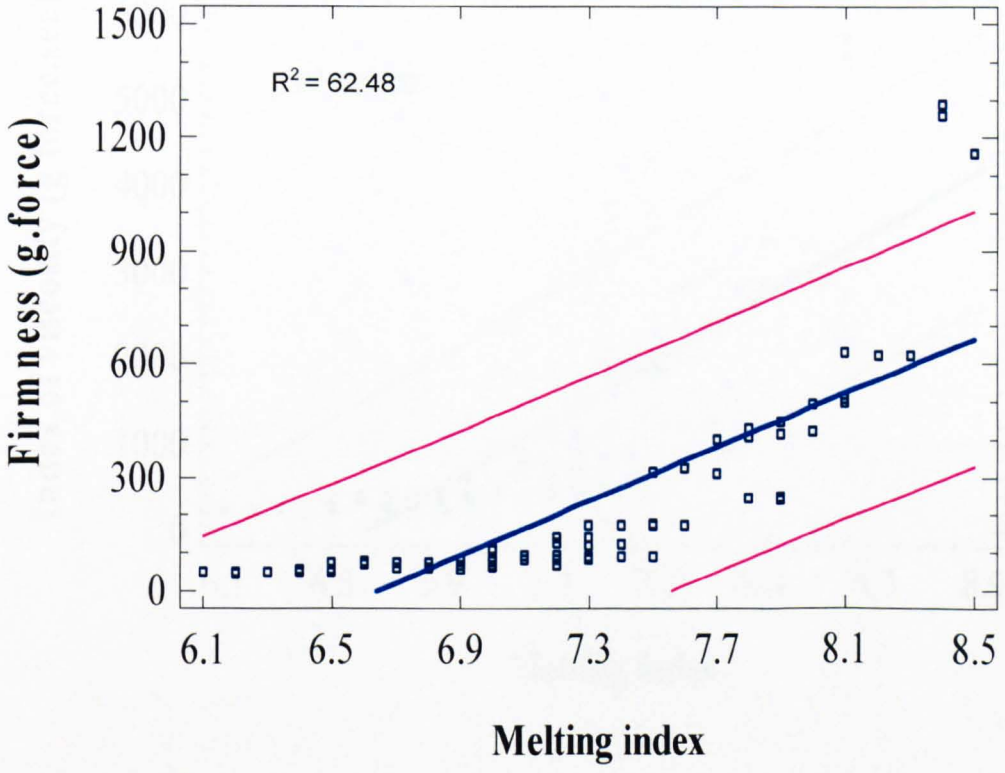


Figure 4.31. Relationship between firmness and melting index in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Firmness = -2371.11 + 357.237*Melting index

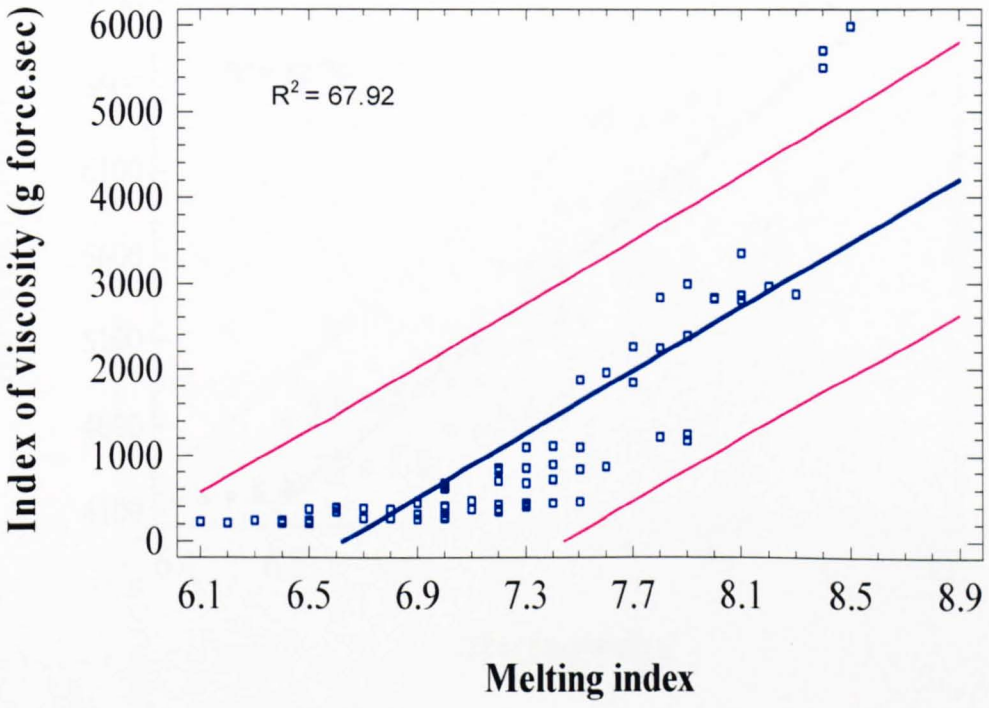


Figure 4.32. Relationship between index of viscosity and melting index in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Index of viscosity = -12263.5 + 1850.79*Melting index

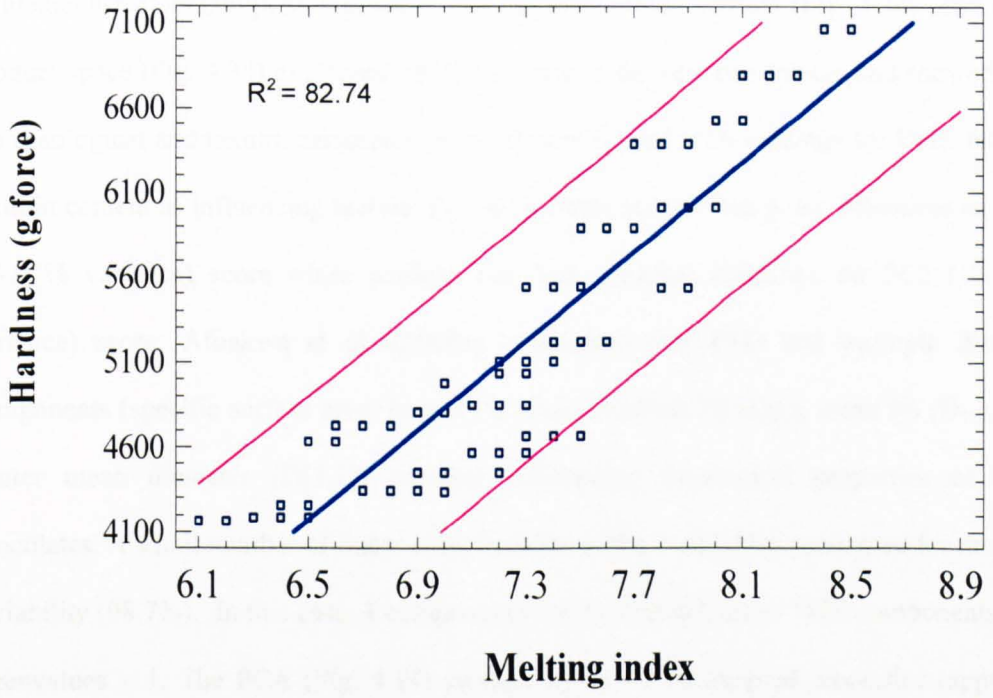


Figure 4.33. Relationship between hardness and melting index in chocolate

Data points (Squares); Linear regression (Inner solid line); 95% Minimum and maximum tolerance intervals (both outer lines); Hardness = -4396.97 + 1318.07*Melting index

Multivariate principal component analysis (PCA) evaluated the extent to which PSD, fat and lecithin contents influence rheological, textural and melting properties of dark chocolates. Group A is composed of rheological, textural and melting parameters and B influencing factors comprising particle size, fat and lecithin content (Fig. 4.34). The PCA product space (Fig. 4.34) explained >82% variance in the first two factors and showed that the rheological and texture parameters were closely related with loadings for PSD, fat and lecithin content as influencing factors. Fat and lecithin content had polar influences on PC1 (69.67% variance) score while particle size had marginal influence on PC2 (12.71% variance) score. Afoakwa et al. (2008a) established that PSD had multiple discrete components (specific surface area, largest PS (D_{90}), smallest PS (D_{10}), mean PS (D_{50}), and Sauter mean diameter (D[3,2]) together influencing rheological properties of dark chocolates. A small number of linear combinations of the 9 variables accounted for most of variability (98.7%). In this case, 3 components were extracted, since 1888 components had eigenvalues $\geq$ 1. The PCA (Fig. 4.34) product space for rheological properties (apparent viscosity and yield stress), textural properties (firmness, index of viscosity and hardness) and melting index were very closely related and could be used to predict the relative processing behaviours during dark chocolate manufacture.

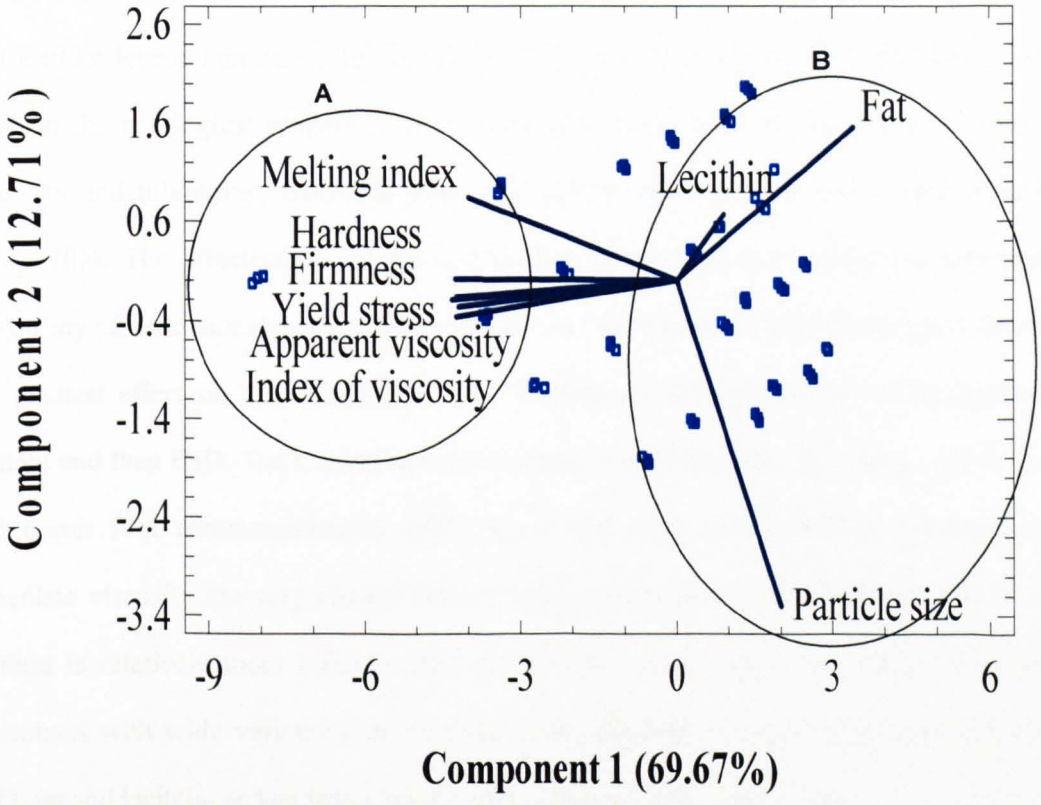


Figure 4.34. Principal component analysis of rheological, textural and melting properties of dark chocolates (A) as affected by PSD, fat and lecithin content (B)

4.10 CONCLUSION

Increasing particle sizes resulted in decreases in Casson plastic viscosity, Casson yield value, yield stress and apparent viscosity, which were more pronounced at lower fat and lecithin levels. Increasing the fat and lecithin levels enhanced the reducing effects of PSD on the rheological properties of the dark chocolates, with the exception of plastic viscosity and thixotropy, where no significant effects were noticed at fat levels at and above 30%. The effectiveness of fat and lecithin in reducing the plastic viscosity and thixotropy of dark chocolate depends on the level of fat already present. Fat content exerts the greatest effect on the variability in the rheological properties, followed by lecithin content and then PSD. The Casson reference parameters (yield value and plastic viscosity) and newer ICA recommendations (yield stress and apparent viscosity) for evaluating chocolate viscosity are very closely related, and could be used independently. The ICA method is relatively more efficient than the Casson model, which has limitations with chocolates with wide variations in viscosity. Both rheological models are dependent on PSD, fat and lecithin, as key factors under controlled processing conditions.

Increase in particle size resulted in linear decreases in textural properties of both molten and solid tempered dark chocolates, higher at lower fat and lecithin contents. At low (25%) fat contents, 5% and 2% increases in fat and lecithin levels respectively enhanced PSD effects on texture, with no significant effects at ≥30% fat. Effects on texture of changes in fat and lecithin depended on base fat content. Increasing PSD and fat inversely influenced appearance parameters (L*, C* and h°). Fat content exerted greatest effect on texture and appearance, followed by PSD and then lecithin content with the last having no significant effect on appearance.

Particle size distribution and ingredient content were significant factors determining microstructural properties of dark chocolates. Microstructural analysis revealed that the smaller particles (D_{10}, D_{50}), largest particles (D_{90}) and specific surface area had direct influence on packing ability and inter-particle interactions. At low (25%) fat concentrations, inter-particle interaction of crystals led to flocculation, with an impact on microstructure and behaviour of molten and tempered products. Increasing fat reduced the crystalline network density, created more open and void spaces which fill with fat, reducing resistance to flow, and enhancing spreadability and softening.

Variations in PSD, fat and lecithin content during dark chocolate manufacture influence to varying levels, the degree of crystallinity and melting properties (T_{end}, T_{index} and ΔH_{melt}) of their derived products. Changes in PSD had no effect on the crystallinity of products. Increasing fat content resulted in consistent increases in crystallinity of products formed during tempering. Products containing 25% fat had the smallest crystal size, followed by those with 30%, with the 35% fat having the largest crystal size, causing significant changes in T_{end}, T_{index} and ΔH_{melt} of products. Similarly, increasing lecithin content from 0.3% to 0.5% moderately reduced the crystallinity of products with significant variations in T_{end}, T_{index} and ΔH_{melt} of products. Neither PSD, fat nor lecithin content influenced initiation (T_{onset}) and maximum (T_{peak}) melting temperatures. Chocolates with finer particles, higher fat and lower lecithin contents, took longer and higher temperatures to complete melting than their corresponding products with larger PS, lower fat and higher lecithin content. This suggest that for chocolate of the same composition, processed under identical conditions, the PSD of the suspended non-fat solid, fat and lecithin contents play important roles in determining their melting behaviour. These

findings would have application in defining chocolate quality as the nature of crystalline material, dimensions of crystals and polymorphic stability dictate the mechanical and rheological properties of chocolate products.

Rheological parameters (apparent viscosity and yield stress), textural parameters (firmness, index of viscosity and hardness) and melting index (duration or time) were highly positively correlated suggesting effective prediction. Except for hardness which showed relatively lower correlation and regression coefficients with both apparent viscosity and yield stress, all other rheological and textural parameters had high correlation and regression coefficients >90%, suggesting that the rheological parameters and textural properties of molten dark chocolate were very highly related and predictive of character. Similarly, the rheological and textural properties of the molten dark chocolate showed relatively lower correlation and regression coefficients with melting index, while relatively higher correlation and regression coefficients were noted with hardness. Principal component analysis revealed that with the exception of melting index which showed a moderate shift in space, the rheological properties (apparent viscosity and yield stress) and textural properties (firmness, index of viscosity and hardness) were closely related. PSD, fat and lecithin contents all interact to determine rheological and textural properties, and melting index (duration) of dark chocolates, with significance for manufacturing improvements and quality control.

CHAPTER 5

TEMPERING BEHAVIOUR OF DARK CHOCOLATES FROM VARYING PARTICLE SIZE DISTRIBUTION AND FAT CONTENT USING RESPONSE SURFACE METHODOLOGY

This chapter has been published in *Innovative Food Science and Emerging Technologies,* Volume 9, Issue 4, Pages 527 – 533. doi:10.1016/j.ifset.2008.02.002. (Afoakwa et al., 2008)

5.1 ABSTRACT

Central Composite Rotatable Design (CCRD) for K = 2 was used to study the combined effects of multistage heat exchangers for Stages 1 (14-30 °C) and 2 (12-28 °C) coolant temperatures at constant Stage 3 coolant and holding temperatures during tempering of dark chocolates using laboratory-scale mini-temperer. Quantitative data on chocolate temper index (slope) were obtained for products with varying particle size distribution (PSD) (D_{90} of 18, 25, 35 and 50 μm) and fat (30% and 35%) content. Regression models generated using stepwise regression analyses were used to plot response surface curves, to study the tempering behaviour of products. The results showed that both Stage 1 and Stage 2 coolant temperatures had significant linear and quadratic effects on the crystallisation behaviour causing wide variations in chocolate temper slope during tempering of products with variable PSD and fat content. Differences in fat content exerted the greatest variability in temperature settings of the different zones for attaining well-tempered products. At 35% fat content, changes in PSD caused only slight and insignificant effect on tempering behaviour. No unique set of conditions was found to achieve good temper in dark chocolate with a specified tempering unit. Thus, different combinations of temperatures could be employed between the multi-stage heat exchangers to induce nucleation and growth of stable fat crystal polymorphs during tempering. Variations in tempering outcomes of the dark chocolates were dependent more on the fat content than PSD.

5.2 INDUSTRIAL RELEVANCE

Tempering consists of shearing chocolate mass at controlled temperatures to promote cocoa butter crystallisation in a stable polymorphic form. During industrial processing, multistage heat exchangers are used to control temperature adjustments to promote formation of appropriate stable polymorphic crystals to obtain products with good snap, colour, contraction, gloss and shelf life characteristics. The process employs varying time-temperature throughputs of the multistage units making it difficult to obtain standard tempering conditions for products with variable particle sizes and fat content, thus prolonging equipment standardisation periods with consequential effects on processing times and product quality characteristics. Modelling the tempering behaviour of dark chocolates from varying PSD and fat content would enhance our knowledge and understanding on the optimal temperature conditions for obtaining good tempered products during industrial manufacture, with significance for reducing processing (tempering) times and assurances in quality and shelf characteristics.

5.3 INTRODUCTION

Tempering is a directed pre-crystallisation that consists of shearing chocolate mass at controlled temperatures to promote cocoa butter crystallisation in a thermodynamically stable polymorphic form. During chocolate manufacture, tempering is used to obtain the stable form V (or $ß_2$) of cocoa butter having a melting temperature of 32-34 °C, which gives the desired glossy appearance, good snap, contraction and enhanced shelf life characteristics (Seguine, 1991; Talbot, 1999; Beckett, 1999; Timms, 2003; Lonchampt & Hartel, 2006; Afoakwa, et al., 2007). The process involves pre-crystallisation of a small

proportion of triglycerides (TAGs), with crystals forming nuclei (1 – 3% total) for the remaining lipid to set in the correct form. The final crystal form depends critically on the shear-temperature-time process which the material has undergone. The tempered chocolate is then deposited in moulds and cooled so that subsequent crystal growth occurs upon the existing seed crystals (Stapley et al., 1999; Hartel, 2001). Tempering has four key steps: melting to completion (at 50°C), cooling to the point of crystallisation (at 32°C), crystallisation (at 27°C), and conversion of any unstable crystals (at 29-31°C) (Talbot, 1999). Thereby, the tempering sequence is a function of recipe, equipment, and the final purpose.

Current industrial tempering machines consist of multistage heat exchangers (Fig. 5.1a) through which chocolate passes at widely differing rates and are used to control temperature adjustments to promote formation of appropriate stable crystals. Time-temperature combinations are of paramount importance in process design and in continuous tempering (Beckett, 1999; Nelson, 1999; Tewkesbury et al., 2000; Hartel, 2001). The varying time-temperature throughputs of these units make it difficult to obtain standard tempering conditions for products with variable particle sizes and fat composition, thus prolonging equipment standardisation periods with consequential effects on process times and product quality characteristics. Poorly tempered chocolates result in unstable crystal growth and poor setting characteristics, making products more susceptible to fat bloom, a physical imperfection that often manifests itself as a white or greyish-white layer on the surface of the chocolate product during storage. The occurrence of fat bloom is associated with the polymorphic transformation from a lower and unstable crystal Form IV to a higher and more stable Form VI (Bricknell & Hartel, 1998; Beckett, 2000; Lonchampt & Hartel,

2004; Lonchampt & Hartel, 2006). Particle size distribution and fat composition during dark chocolate manufacture affect their rheological (Afoakwa et al., 2008a) as well as microstructural and mechanical properties (Afoakwa et al., 2008b), establishing relationships between the rheology and structural character of products, however, their influence on pre-crystallisation and nucleation still remains unclear.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimising processes in which a response of interest is influenced by several variables and the objective is to optimise the response. Baş and Boyacı (2007) noted that RSM has important applications in the design, development and formulation of new products, as well as in the improvement of existing product designs. It defines the effect of the independent variables, alone or in combination, on the processes. In addition to analysing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical, biochemical or physical processes involved (Anjum et al., 1997; Myers & Montgomery, 1995; Senanayake & Shahidi, 2002; Vohra & Satyanarayana, 2002; Afoakwa et al., 2007). The objective of this work was to study tempering behaviour of dark chocolates varying in PSD and fat content using response surface methodology.

5.4. MATERIALS AND METHODS

5.4.1 Materials

Cocoa liquor of Central West African Origin was obtained from Cargill Cocoa Processing Company (York, UK); sucrose (pure extra fine granulated) from British Sugar Company (Peterborough, UK); pure prime pressed cocoa butter and soy lecithin from

ADM Cocoa Limited (Koog aan de Zaan, Netherlands) and Unitechem Company Ltd. (Tianjin, China) respectively.

The recipe, formulation and production of samples have been given in Table 4.1, but with only 0.5% lecithin content. Chocolates were formulated with total fat of 25 - 35% (w/w) from cocoa liquor and cocoa butter with >34% total cocoa: composition as specified for dark chocolate by relevant directives (European Commission Directive, 2000; Codex Revised Standard, 2003). Experimental samples (5 kg batch for each formulation) were produced by mixing sucrose and cocoa liquor in a Crypto Peerless Mixer (Model K175, Crypto Peerless Ltd, Birmingham, UK) at low speed for 2 min and then at high for 3 min, then using a 3-roll refiner (Model SDX 600, Buhler Ltd., CH-9240 Uzwil, Switzerland) to a specified particle size (D_{90} :18 ± 1 μm, 25 ± 1 μm, 35 ± 1 μm & 50 ± 1 μm) conducting particle size analysis, during refining, to ensure D_{90} values. Refined chocolates were placed in plastic containers and conditioned at 50 - 55 °C for 24 h to ensure melting of fat within chocolate mass prior to conching in a Lipp Conche (Model IMC-E10, Boveristr 40-42, D-68309, Mannhein, Germany) at low speed for 3.5 h at 60 °C. Lecithin and cocoa butter were added and mixtures then conched at high speed for 30 min to effect adequate mixing and liquefaction. Samples were kept in sealed plastic containers at ambient temperature (20 - 22 °C). Moisture and fat contents determined using Karl Fischer and Soxhlet methods (ICA, 1988; 1990).

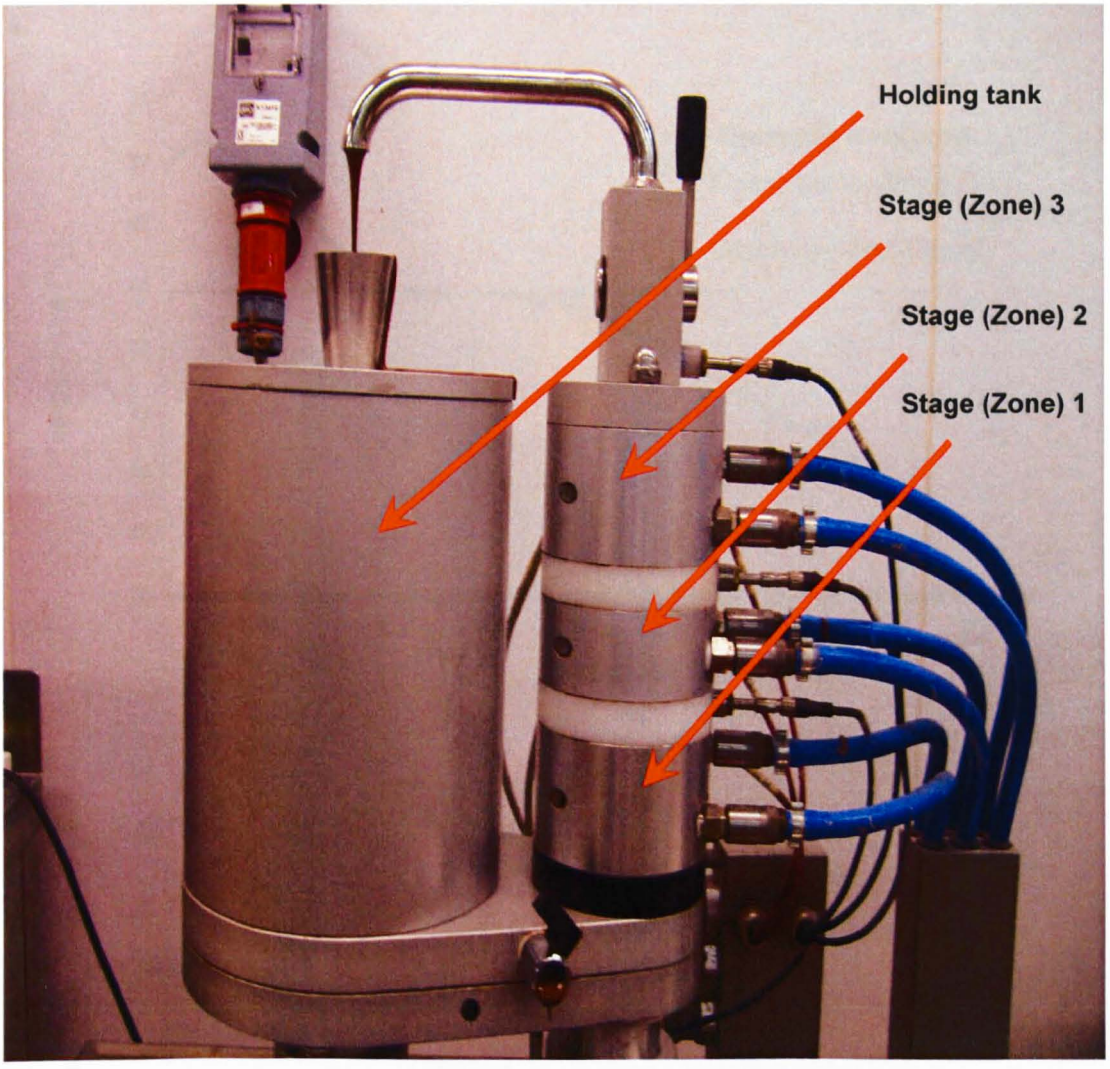
5.4.2 Tempering procedure

Samples were allowed to melt at 50 °C for 4 h for melting and tempered using an Aasted Mikrovert laboratory continuous three-stage tempering unit (Model AMK 10,

Aasted Mikroverk A/S, Farum, Denmark) as shown in Figure 5.1b. Chocolate is pumped through the multi-stage units and a worm screw drives the product through the heat exchangers. Sensors are located at specific points in the equipment to measure the temperature of both the chocolate and the coolant fluid at each stage. The temperatures of each of the coolant in each of the three stages were thus set and controlled independently of each other to obtain the temper status of the chocolate.

Pre-crystallisation status of the chocolate was measured as temper slope on cooling curves generated using a computerised tempermeter (Exotherm 7400, Systech Analytics, Neuchâtel, Switzerland) on a temperature-time graphs using Software version 5.0 (Exotherm 7400, Systech Analytics, Neuchâtel, Switzerland) and the readings recorded. The cooling curve measures the amount of heat released during solidification of chocolate with time. The tempering process generates two inflection points on the cooling curve – first inflection point being the temperature-time at which seed crystals begin to nucleate, and the second inflection point, where the actual nucleation takes place. The slope that is used to evaluate the temper status of the chocolate mass is developed at the second inflection as illustrated on Figure 5.2. This is directly related to the amount of seed crystals formed during the pre-crystallisation or tempering process. Figure 5.2 shows typical pre-crystallisation (cooling) curves and how the temper slopes generated by the computerised tempermeter to evaluate the temper status - optimally-tempered, under-tempered and over-tempered of the chocolate mass is processed. Triplicate measurements were taken for each product composition and the mean values recorded.

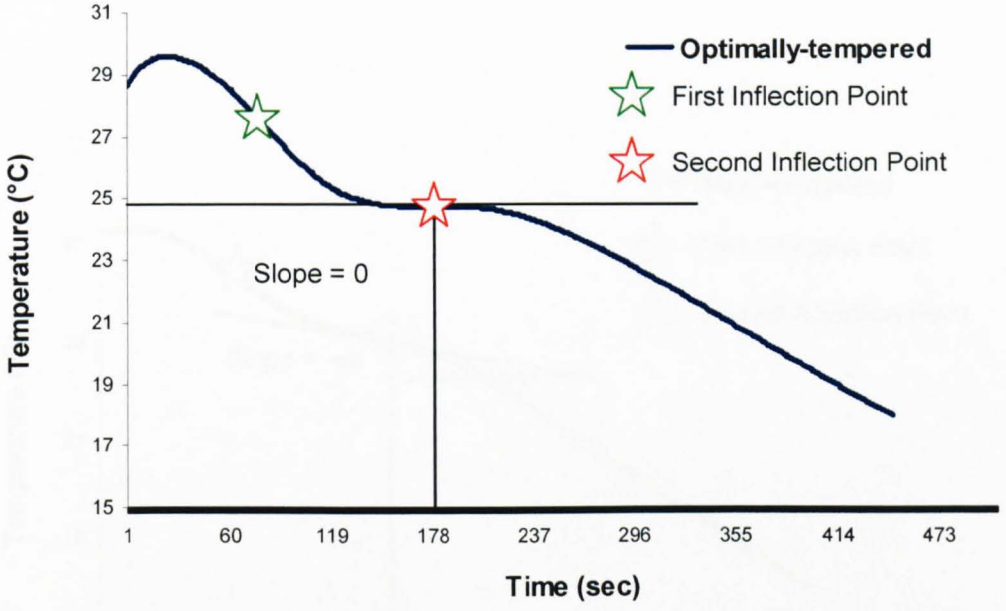
a.



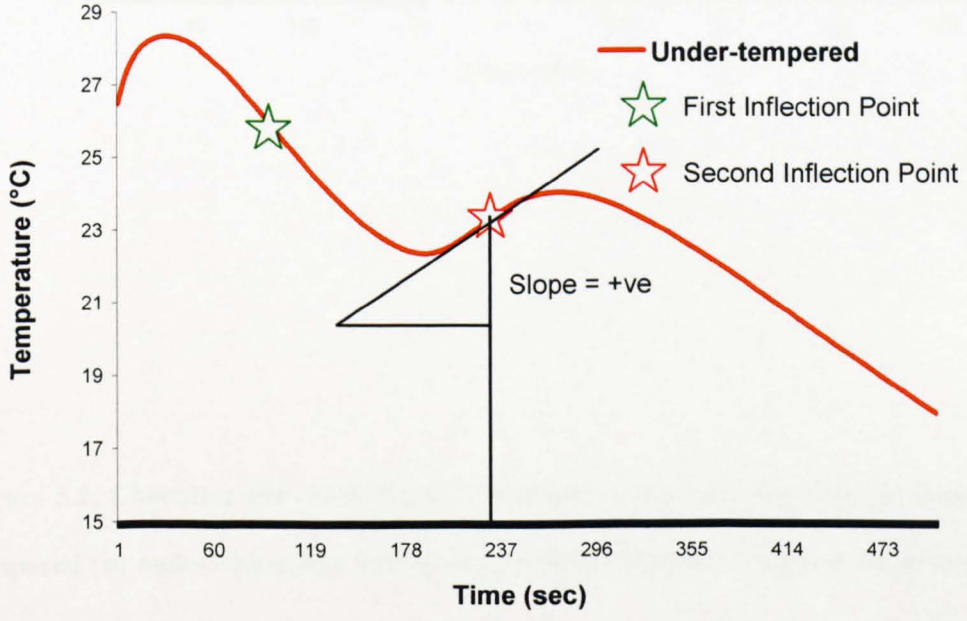
b.

Figure 5.1. Typical Aasted Mikrovert multistage tempering unit (temperers) (a) used in chocolate manufacture, (b) used for this experiment

a.


Optimally-tempered
First Inflection Point
Second Inflection Point
Slope = 0
Temperature (°C)
Time (sec)
31
29
27
25
23
21
19
17
15
1
60
119
178
237
296
355
414
473

b.


Under-tempered
First Inflection Point
Second Inflection Point
Slope = +ve
Temperature (°C)
Time (sec)
29
27
25
23
21
19
17
15
1
60
119
178
237
296
355
414
473

c.

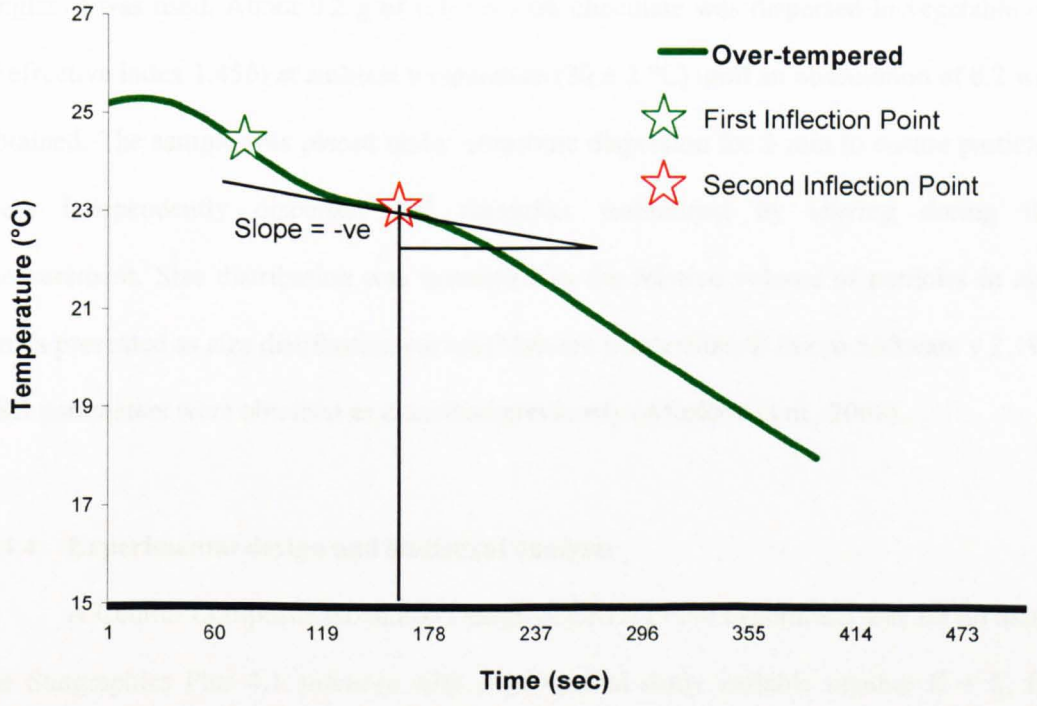


Figure 5.2. Chocolate pre-crystallisation (cooling) curves showing how (a) optimally-tempered (b) under-tempered and (c) over-tempered temper slopes were determined by the tempermeter

5.4.3 Determination of particle size distribution

A MasterSizer® Laser Diffraction Particle Size Analyzer equipped with MS 15 Sample Presentation Unit (Refractive index 1.590) (Malvern Instrument Ltd., Malvern, England) was used. About 0.2 g of refined dark chocolate was dispersed in vegetable oil (Refractive index 1.450) at ambient temperature (20 ± 2 °C) until an obscuration of 0.2 was obtained. The sample was placed under ultrasonic dispersion for 2 min to ensure particles were independently dispersed and thereafter maintained by stirring during the measurement. Size distribution was quantified as the relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Micro Software v 2.19). PSD parameters were obtained as described previously (Afoakwa et al., 2008).

5.4.4 Experimental design and statistical analysis

A Central Composite Rotatable Design (CCRD) of the experiment was set up using the Statgraphics Plus 4.1 software with experimental study variable number K = 2, for independent variables including Stage 1 coolant temperature (X_1) and Stage 2 coolant temperature (X_2). Stage 3 coolant temperature and the temperature of the holding tank (Fig. 5.2) were kept constant at 32°C and 45°C respectively. The variables used in the CCRD for K = 2 were processed using the software, and provided the dependent variable limits and their values (Table 5.1). The experiments were carried out in two separate sets to optimise these parameters. According to this design, the total number of treatment combinations is $2k + 2k + no$, where 'k' is the number of independent variables and no is the number of repetitions of the experiments at the center point. For statistical calculation, the variables X_i have been coded as x_i according to the following transformation:

$$x_i = \frac{X_i - X_0}{\delta X} \quad (5.1)$$

where xi is the dimensionless coded value of the variable X_i, X_0 is the value of the Xi at the center point and δX is step change. A $2k$-factorial design with four axial points (α = 1.414) and six replicates at the center point with a total number of 14 experiments was employed for the studied parameters. The number of center point replications was chosen to verify any change in the estimation procedure, which was also a measure of precision described by the following equation:

$$no = \lambda 4(\sqrt{F} + 2)^2 - F - 2k \quad (5.2)$$

where F is the number of points in factorial portion, i.e., the first four experiments in experimental design (run numbers 1–4 in Table 5.2) and $\lambda 4$ is the mixed fourth order moment. The total number of center point replications obtained after substituting the values in Eq. (5.2) is five, but six replications were performed to reduce error. The behaviour of the system was explained by the following quadratic model:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_{ii}^{\ 2} + \Sigma\beta_{ij} X_{ij} \quad (5.3)$$

where Y is the predicted response, β_0 the offset term, β_i the linear effect, β_{ii} the quadratic effect and β_{ij} is the interaction effect. The dependent variables studied were the chocolate temper index (slope) as measured by the Tempermeter, for samples processed from 18 μm, 25 μm, 35 μm and 50 μm particle sizes, D_{90} at 35% fat to study the tempering behaviour of samples from varying PSD. The tempering behaviour of samples processed from 25 μm and 35 μm PSD at 30% fat content were also studied and compared to their respective samples with 35% fat, to determine the effect of varying fat content. These samples were

selected following trends in rheological properties observed from earlier studies (Afoakwa et al., 2008a). The design matrix and variable combinations in experimental runs are as shown on Tables 5.1 and 5.2.

Table 5.1. Process variables and their levels used in the Central Composite Rotatable Design for K = 2

Independent variables	Code	Variable levels				
		-1.414	-1	0	1	1.414
Stage 1 coolant temp. (°C)	X_1	13.9	16.0	21.0	26.0	28.1
Stage 2 coolant temp. (°C)	X_2	11.9	14.0	19.0	24.0	26.1

The experiments conducted on the various combinations and the results (temper slopes) obtained are as shown on Tables 5.3 and 5.4. These were analysed using stepwise regression analysis. Analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms determined. The significance of all terms in the polynomial was judged statistically by computing the F-value at a probability (P) of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculation to generate response plots from the regression models. Table 5.5 shows the coefficients of the variables in the models and their contribution to the model's variation. A test for the lack of fit and the R^2 values were used to judge the adequacy of the models. The R^2 of a model refers to the proportion of variation in the response attributed to the model rather than random error. For a good fit of a model, an R^2

of 0.80% was used. Malcolmson et al. (1993), commented that R of 0.80% is perfect for a good model study and but recommended that an R^2 of 0.60% can be used for a preliminary study.

Table 5.2. Design matrix and variable combinations in experimental runs

Runs	Block	Variable codes		Levels	
		X_1	X_2	Stage 1 coolant temp. (°C)	Stage 2 coolant temp. (°C)
1	1	1.0	1.0	26.1	24.0
2	1	-1.0	-1.0	16.0	14.0
3	1	1.0	-1.0	26.0	14.0
4	1	-1.0	1.0	16.0	24.0
5	1	0.0	0.0	21.0	19.0
6	1	0.0	0.0	21.0	19.0
7	1	0.0	0.0	21.0	19.0
8	2	-1.414	0.0	13.9	19.0
9	2	1.414	0.0	28.1	19.0
10	2	0.0	1.414	21.0	26.1
11	2	0.0	-1.414	21.0	11.9
12	2	0.0	0.0	21.0	19.0
13	2	0.0	0.0	21.0	19.0
14	2	0.0	0.0	21.0	19.0

X_1 – Stage 1 coolant temperature (°C); X_2 - Stage 2 coolant temperature (°C)

5.5 RESULTS AND DISCUSSION

5.5.1 Particle size distribution of dark chocolates

Variations in PSD were observed for 18, 25, 35 and 50 μm (Fig. 5.3) using D_{90} values (>90% finer) that relate to chocolate character (Beckett, 2000). Data from the PSD showed variations in specific surface area, mean particle volume D(v,50), Sauter mean (D[3,2]) and mean particle diameter (D[4,3]) with increasing D_{90} particle sizes. These findings have been previously reported (Afoakwa et al., 2008a). Increasing fat from 25 to 35% led to significant ($P<0.001$) reductions in specific surface area and to an increase in all other PSD parameters, suggesting fat content, inversely correlated with specific surface area, during refining has a direct influence on PSD. Beckett (1999) concluded that the largest particle size and solids specific surface area are the two key parameters for chocolate manufacture. The largest particle size determines chocolate coarseness and textural character, the solids specific surface area with desirable flow properties. Specific surface area is inversely correlated with the different components of PSD (Beckett, 1999; Ziegler & Hogg, 1999; Sokmen & Gunes, 2006). Fat contents were 25 ± 1, 30 ± 1 and 35 ± 1%; moisture within the range 0.80 - 0.98.

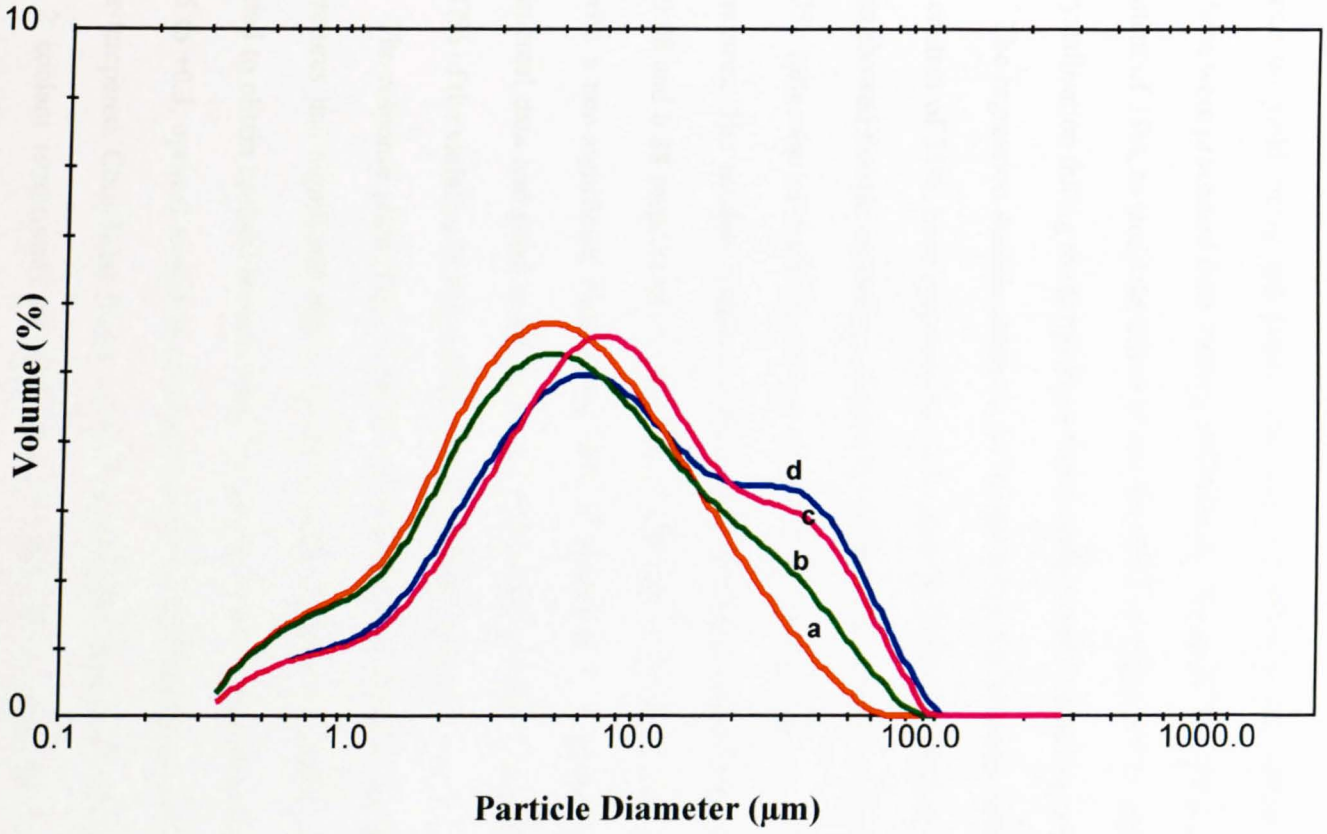


Figure 5.3. Particle size distribution of dark chocolate with D_{90} of (a) 18 µm (b) 25 µm (c) 35 µm (d) 50 µm

5.5.2 Effect of particle size distribution on tempering behaviour

Particle size distribution (PSD) has been reported as a key determinant of the microstructure, rheological and mechanical properties in dark chocolates with a direct influence on yield stress and plastic viscosity (Afoakwa et al., 2008a, 2008b). Dark chocolates were processed from varying PSD mainly 18 μm, 25 μm, 35 μm and 50 μm, at fat content of 35%, to study the effect of varying PSD on degree of fat crystal nucleation and crystallisation during the tempering process using a multi-stage temperer.

The regression models obtained for temper slope for products with varying PSD at fat content of 35%, have respectively been given in Table 5.5. Statistical analyses on the data showed that the models developed for all products showed strong and significant ($p<0.05$) influence of both linear and quadratic factors of Stages 1 and 2 coolant temperatures. The models obtained showed coefficient of determination (R^2) of 0.91, 0.88, 0.88 and 0.88 respectively for products with PSD of 18 μm, 25 μm, 35 μm and 50 μm, with a non-significant *F*-ratios for lack of fit (Table 5.5), explaining that all the experimental data had good fit of the model and could be used to explain in each case over 87% of the variation in tempering behaviour of the products.

The response plots (Figs. 5.4-5.7) showed that both Stage 1 and Stage 2 coolant temperatures had significant effects on the temper slope of the products and could be regulated to obtain optimal temper. From the study, it was observed that at temper slope of -0.5 to +0.3, optimal temper is achieved, above 0.3 is under-tempered and below -0.5 is over-tempered. Combining Stage 1 coolant temperature between 14 °C and 20 °C and Stage 2 coolant temperature of between 12°C and 18°C, produced too low cooling temperatures, resulting in unsatisfactory tempered (over-tempered) products with temper

slopes well below -0.4. Tewkesbury et al. (2000) noted that chocolate melts over a temperature range, and the presence of lower polymorphs will mean that a greater fraction of the cocoa butter will be liquid at room temperature, thus affecting texture and consumer acceptability. Increasing Stage 1 coolant temperature to between 20°C and 26 °C, and Stage 2 coolant temperature between 18°C and 24°C led to optimally tempered products. This explains that there is no unique set of conditions needed to achieve optimal temper with a given dark chocolate in a given tempering unit, but a wide range of conditions exist, all of which could result in tempered product.

Similarly, at all Stage 1 coolant temperatures (12-32°C), any Stage 2 coolant temperature combination above 24°C (Figs. 5.4-5.7) resulted in drastic heat generation within the chocolate system, and these causes complete re-melting of all the nucleated stable fat crystals initially formed within the chocolate, thus leaving the product unsatisfactory tempered (under-tempered). This temperature combination led to under-tempering of the products, and would effect formation of fat bloom in storage. Likewise, at higher Stage 1 coolant temperatures above 30°C, all Stage 2 coolant temperature points resulted in complete re-melting of the stable fat crystals formed, thus causing under-tempering of products. This observation is particularly true with products processed from lower (18 and 25μm) PS (Figs. 5.4 & 5.7) and at 35% fat content, as products with larger (35 and 50 μm) PS showed quite different tempering behaviour.

Table 5.3. Design matrix, variable combinations temper slopes obtained from experimental runs for dark chocolates containing 35% fat with varying PSD

Runs	Variable codes		Levels		Dependent variables			
	X_1	X_2	Stage 1 temp. (°C)	Stage 2 temp. (°C)	Temper slope for 18 μm	Temper slope for 25 μm	Temper slope for 35 μm	Temper slope for 50 μm
1	1.0	1.0	26.1	24.0	0.62	-0.03	0.75	0.41
2	-1.0	-1.0	16.0	14.0	-1.72	-1.71	-1.74	-1.73
3	1.0	-1.0	26.0	14.0	0.20	0.01	-0.04	0.00
4	-1.0	1.0	16.0	24.0	1.22	0.48	0.59	0.54
5	0.0	0.0	21.0	19.0	-0.01	0.01	0.06	0.53
6	0.0	0.0	21.0	19.0	-0.01	0.01	0.06	0.53
7	0.0	0.0	21.0	19.0	-0.01	0.01	0.06	0.53
8	-1.414	0.0	13.9	19.0	-0.13	-1.76	-0.09	-0.01
9	1.414	0.0	28.1	19.0	1.17	1.36	1.73	2.40
10	0.0	1.414	21.0	26.1	1.35	2.15	2.06	1.97
11	0.0	-1.414	21.0	11.9	-1.85	-1.73	-1.80	-1.70
12	0.0	0.0	21.0	19.0	-0.01	0.01	0.06	0.53
13	0.0	0.0	21.0	19.0	-0.01	0.01	0.06	0.53
14	0.0	0.0	21.0	19.0	-0.01	0.01	0.06	0.53

X_1 – Stage 1 coolant temperature (°C); X_2 - Stage 2 coolant temperature (°C)

Table 5.4. Design matrix, variable combinations temper slopes obtained from experimental runs for dark chocolates varying in fat content (30% and 35%) and PSD (25 μm and 35 μm)

Runs	Variable codes		Levels		Dependent variables			
	X_1	X_2	Stage 1 temp. (°C)	Stage 2 temp. (°C)	Temper slope for PS 35 μm, fat 30%	Temper slope for PS 50 μm, fat 30%	Temper slope for PS 35 μm, fat 35%	Temper slope for for PS 50 μm, fat 35%
1	1.0	1.0	26.1	24.0	0.05	0.57	0.75	0.41
2	-1.0	-1.0	16.0	14.0	-1.89	-2.00	-1.74	-1.73
3	1.0	-1.0	26.0	14.0	-0.26	-0.20	-0.04	0.00
4	-1.0	1.0	16.0	24.0	-0.06	0.15	0.59	0.54
5	0.0	0.0	21.0	19.0	-0.27	-0.02	0.06	0.53
6	0.0	0.0	21.0	19.0	-0.27	-0.02	0.06	0.53
7	0.0	0.0	21.0	19.0	-0.27	-0.02	0.06	0.53
8	-1.414	0.0	13.9	19.0	-0.35	-0.33	-0.09	-0.01
9	1.414	0.0	28.1	19.0	0.56	1.30	1.73	2.40
10	0.0	1.414	21.0	26.1	1.67	1.82	2.06	1.97
11	0.0	-1.414	21.0	11.9	-0.31	-0.35	-1.80	-1.70
12	0.0	0.0	21.0	19.0	-0.27	-0.02	0.06	0.53
13	0.0	0.0	21.0	19.0	-0.27	-0.02	0.06	0.53
14	0.0	0.0	21.0	19.0	-0.27	-0.02	0.06	0.53

X_1 – Stage 1 coolant temperature (°C); X_2 - Stage 2 coolant temperature (°C)

Table 5.5. Regression coefficients from second order polynomials used for the response plots

Coefficients	Slope (18μm PS, 35% fat)	Slope (25μm PS, 35% fat)	Slope (35μm PS, 35% fat)	Slope (50μm PS, 35% fat)	Slope (25μm PS, 30% fat)	Slope (35μm PS, 30% fat)
Constant	-5.6339	-10.7366	-6.3643	-9.7863	-4.8394	-4.0873
X_1	0.186044**	0.474608**	0.028105*	0.169025**	0.027762*	0.181715**
X_2	0.228753***	0.359311**	0.40447***	0.659722***	0.25184**	0.0415585*
X_1^2	0.002729*	-0.003763*	0.004770*	0.0023979*	0.002806*	-0.000179*
X_1X_2	-0.012857*	-0.011378*	-0.007857*	-0.0094898*	-0.005306*	-0.006480*
X_2^2	0.003647*	0.0004209*	-0.002270*	-0.0084183*	-0.003571*	0.004975*
R^2	0.9073	0.8759	0.8992	0.8811	0.9280	0.8636
F [a]	0.4220	0.2028	0.5140	0.3189	0.8658	0.3265
Probability of F	$p \leq 0.001$	$p \leq 0.01$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.01$

*$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

[a] Models have non-significant lack of fit ($p > 0.05$)

A close examination of the response plots (Figs. 5.5 & 5.7) showed that at all Stage 1 coolant temperatures, it was possible to find an alternative stage 2 coolant temperature condition to yield an optimally tempered product. Contrary to the observations made with the 18 μm and 25 μm (Figs. 5.4 & 5.5), setting Stage 1 coolant temperature above 30°C yielded optimal tempered products when a lower corresponding Stage 2 coolant temperature (12-14°C) was used (Figs. 5.6 & 5.7), suggesting that with larger (35 and 50 μm) PS, lower coolant temperatures at Stage 2 are required to induce nucleation of the required stable polymorphs in the fat. These findings were probably due to the different apparent viscosities and yield stress values of 4.93 Pas, 4.05 Pas, 3.84 Pas and 3.45 Pas, and 69.85 Pa, 51.02 Pa, 44.85 Pa and 38.95 Pa respectively noted with the 18 μm, 25 μm, 35 μm and 50 μm samples containing 35% fat and 0.5% lecithin (Afoakwa et al., 2008a), which is suspected to be influencing the pumping and cooling rates of products through the multi-stage heat exchangers.

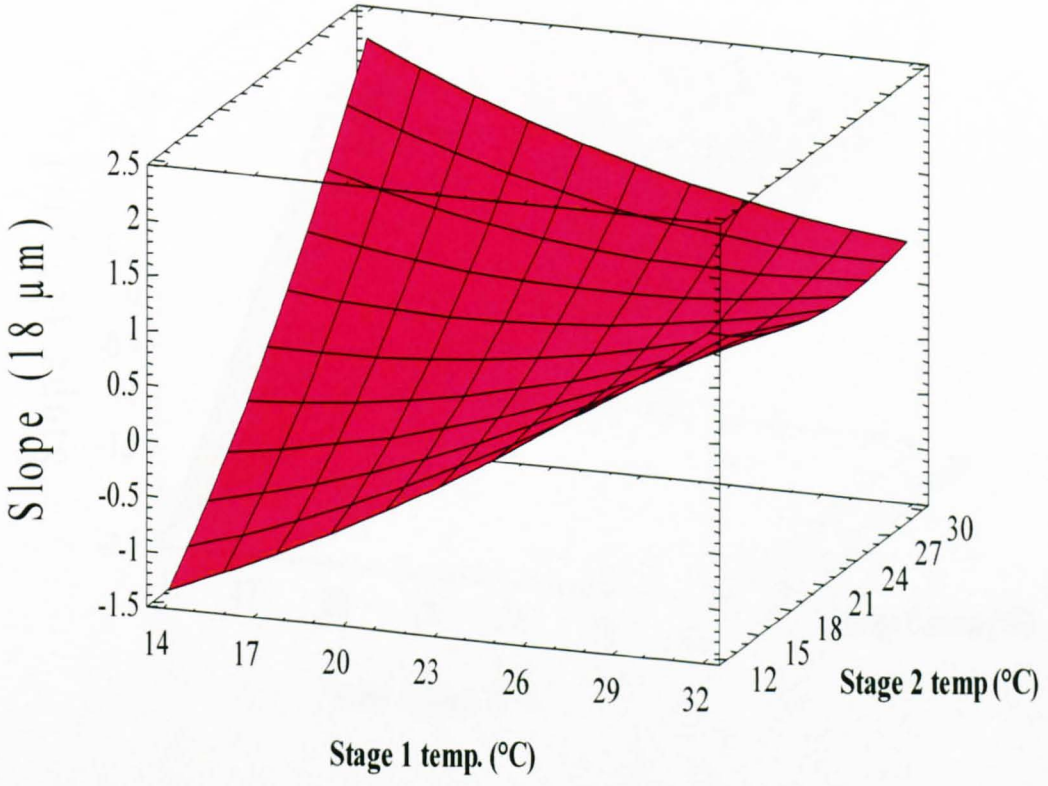


Figure 5.4. Response plot showing chocolate temper slope for sample containing 18 µm PS at 35% fat content

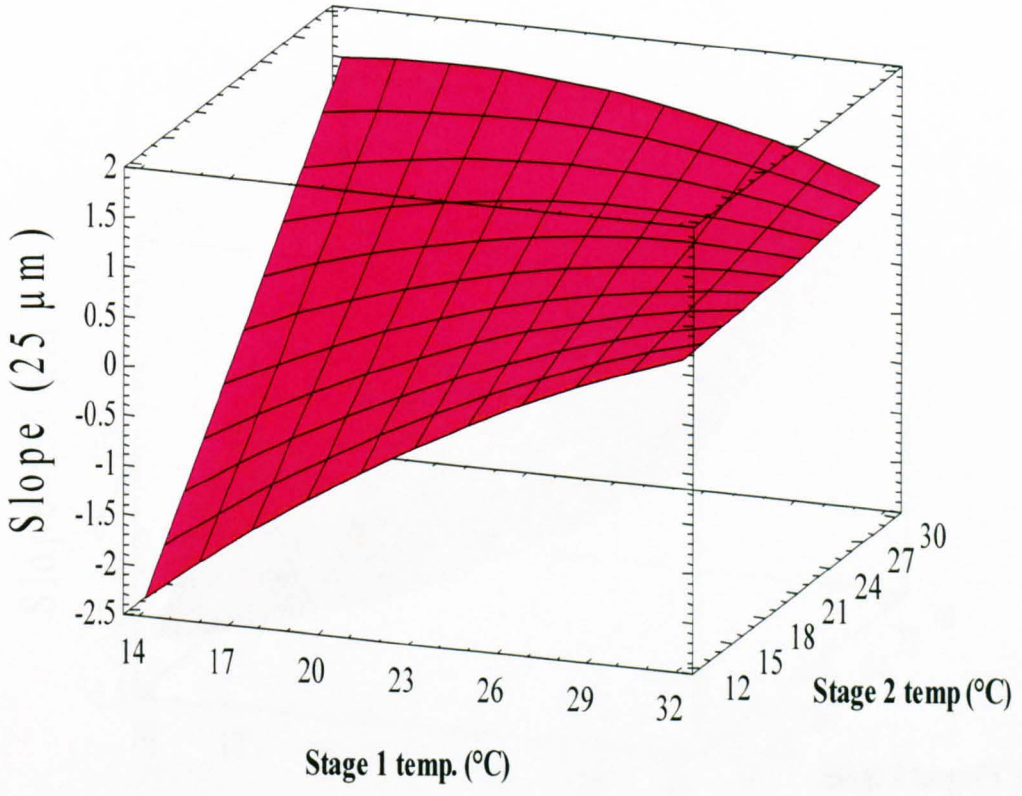


Figure 5.5. Response plot showing chocolate temper slope for sample containing 25 µm PS at 35% fat content

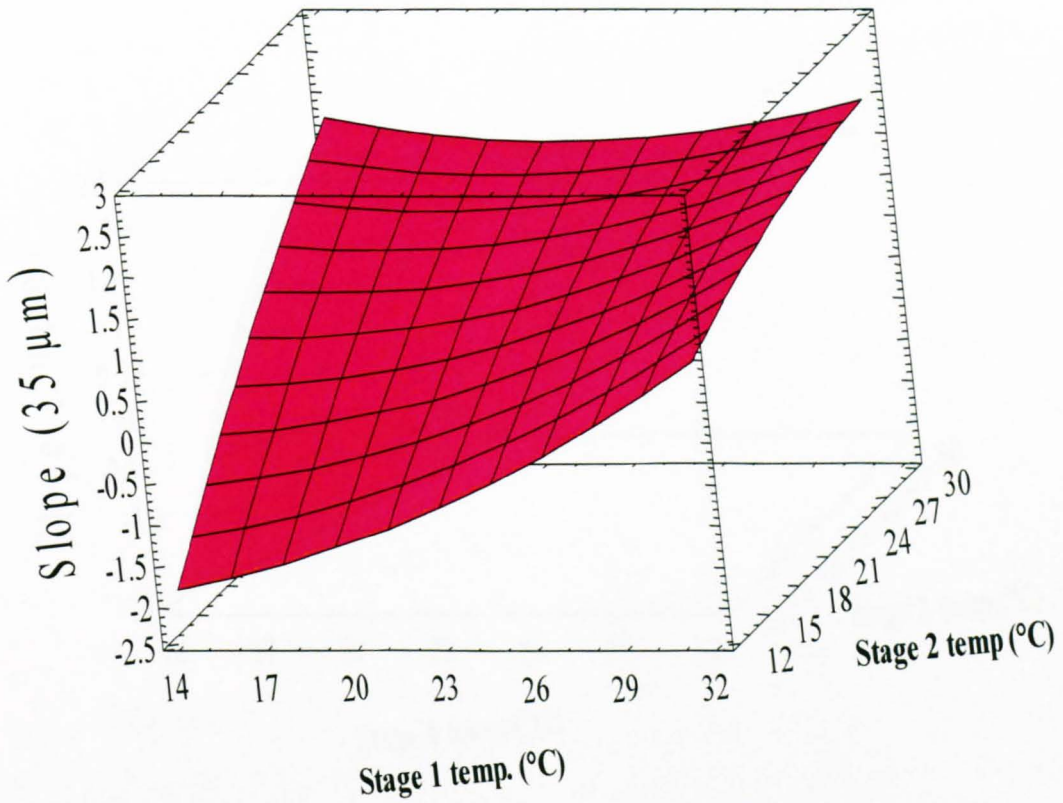


Figure 5.6. Response plot showing chocolate temper slope for sample containing 35 µm PS at 35% fat content

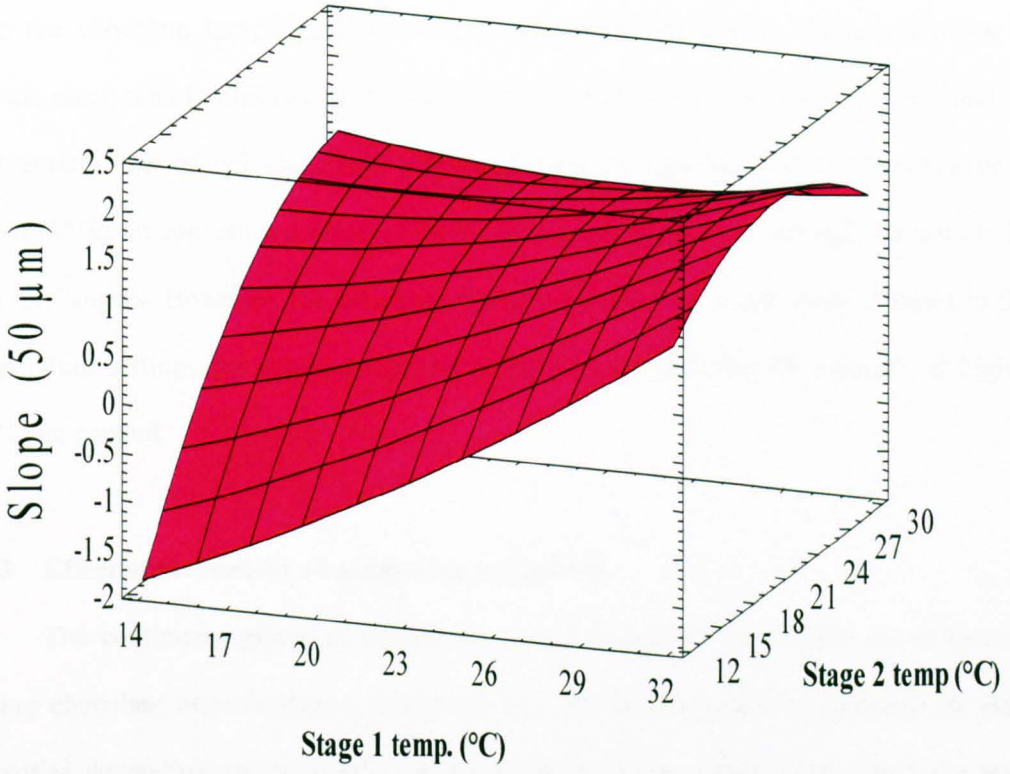


Figure 5.7. Response plot showing chocolate temper slope for sample containing 50 µm PS at 35% fat content

Nelson (1999) noted that the stage 1 coolant temperature in a multi-stage heat exchangers serves to gently cool the warm chocolate through the tempering machine, gradually reducing the temperature to 'strike seed' and initiate the first stages of crystal growth. At this phase the crystals grow very fast, and as the viscosity increases there is the need to raise the chocolate temperature to prevent runaway solidification. Thus, variations in particle sizes, mainly differences in smaller (18 μm and 25 μm) and larger (35 μm and 50 μm) particle sizes of dark chocolates influence the tempering behaviour of products even at higher (35%) fat content as a result of their varying cooling rates or throughputs within the heat exchangers. However, the effect is minimal and does not cause wide changes in the temperature settings for attaining optimal temper in the different PS products at higher (35%) fat content.

5.5.3 Effect of fat content on tempering behaviour

The continuous phase of molten chocolate consists of fat (mainly cocoa butter). During chocolate manufacture, a minimum level of fat is required to maintain its flow properties depending on the solids component particle size distribution, being the void fractions between the packed bed and the specific surface area of the particles (Chevalley, 1999). To study the tempering behaviour of products with varying fat content, dark chocolate containing 30% fat at 25 μm and 35 μm PS were further tempered using the multi-stage temperer and the models developed compared with those processed with 35% fat at 25 μm and 35 μm PS.

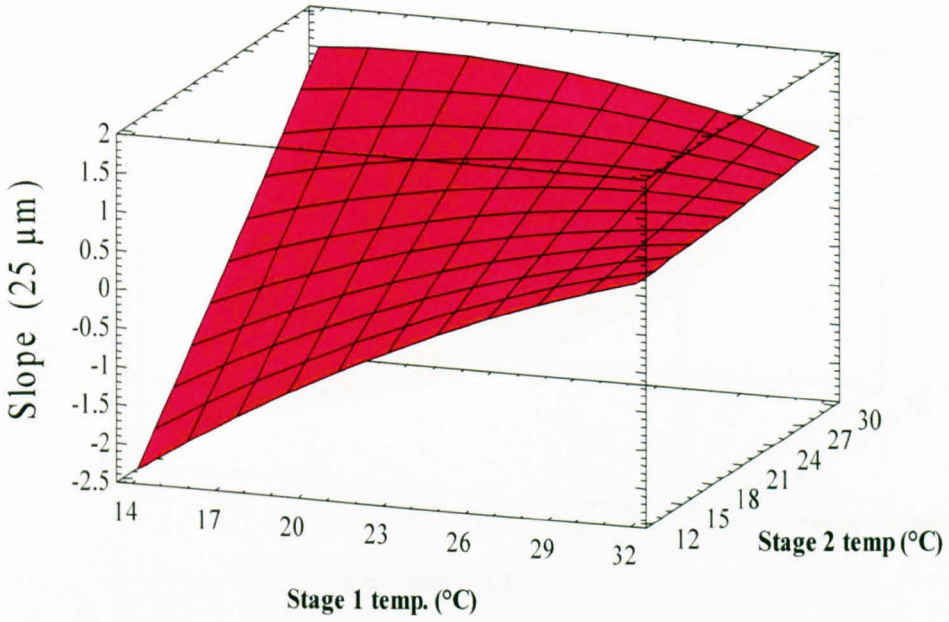
The multiple regression models developed for products containing 30% fat at 25 μm and 35 μm PS have respectively been given in Table 5.5. Statistical analyses revealed that

the models developed for products with different fat content (30% and 35%) showed strong and significant ($p<0.05$) influence of both linear and quadratic factors of Stages 1 and 2 coolant temperatures. The models obtained for products containing 30% fat content at 25 μm and 35 μm showed coefficient of determination (R^2) of 0.93 and 0.86 respectively, with a non-significant *F*-ratios for lack of fit (Table 5.5), explaining that the experimental data had good fit of the model and could be used to explain in each case over 86% of the variation in tempering behaviour of the products at 30% fat content. This was very similar to coefficient of determinations of 0.88% and 0.90% noted for products containing 35% fat at 25 μm and 35 μm PS (Table 5.5), meaning the four models all have good fit for effective comparative study of their tempering behaviours.

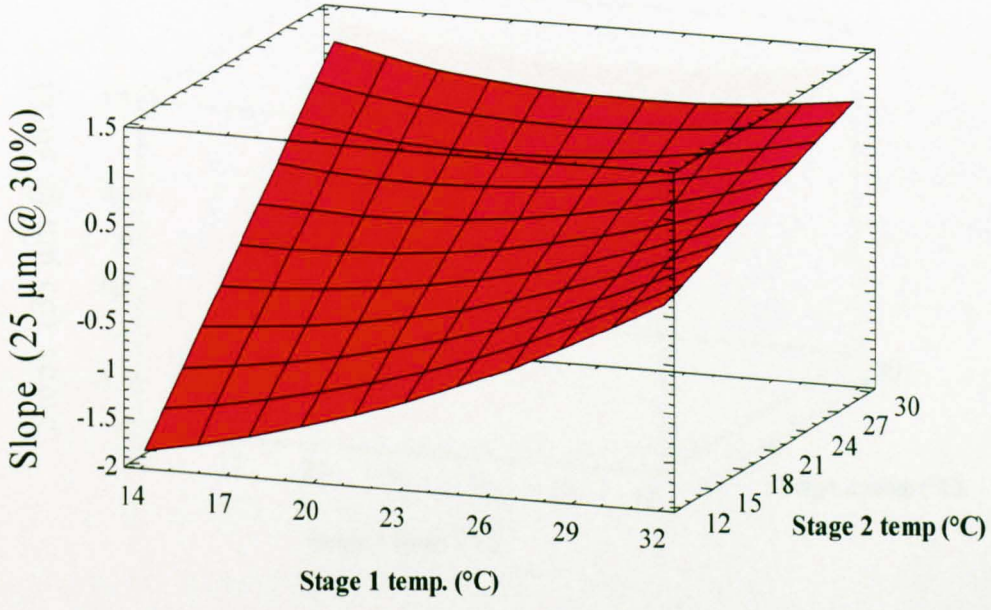
Variations in fat content had the greatest influence in dictating the tempering behaviour of products. The response plots (Figs. 5.8 – 5.9) showed that both Stages 1 and 2 coolant temperatures had significant effects on the Temper slope. Contrary to observations made with models developed for products containing 35% fat at 25 μm PS where combined coolant temperatures for Stage 1 (14°C and 20°C) and Stage 2 (12°C and 18°C) resulted in too low cooling temperatures effecting over-tempering of products, reducing the fat content of products to 30% showed a different tempering behaviour. The response plots developed for products containing 30% fat at 25 μm PS (Fig. 5.8), showed that at all Stage 1 coolant temperatures, there was a corresponding Stage 2 coolant temperature that gave an optimal temper. The model showed that at lower Stage 1 coolant temperature between 17 °C and 23 °C, a corresponding Stage 2 coolant temperature between 18°C and 21°C yielded an optimal temper, whereas reducing Stage 2 temperature (12-18°C) caused too low chocolate temperature resisting nucleation of fat crystals within the system with resultant over-

tempered products. Similarly, increasing Stage 2 temperature to between 24-30°C caused too high chocolate temperature resulting in re-melting of all the initial nucleated fat crystals thereby rendering the product under-tempered. This explains that at all Stage 1 coolant temperatures a corresponding Stage 2 temperature could be found to achieve successful nucleation and growth of fat crystals yielding an optimal temper (Fig. 5.8). It could be observed that at low Stage 1 coolant temperature (14-20°C), a corresponding intermediate Stage 2 coolant temperature between 18-21°C was ideal to effect nucleation of the fat crystals and growth; extending the temperature beyond this range would raised the nucleated fat crystal growth temperature beyond the required limit, effecting re-melting of the nucleated crystals to unstable polymorphic state causing under-tempering of products. Alternatively, at higher Stage 1 coolant temperature between 21-32°C, a corresponding lower Stage 2 coolant temperature between 12-21°C was required for an optimal temper, beyond which the products were under-tempering.

Similar tempering behaviours were noted between products containing 35% and 30% fat at 35 μm PS respectively (Figs. 5.6 & 5.9). The models explained that for samples containing 30% fat at 35 μm PS, all Stage 1 coolant temperatures could successful effect nucleation of stable fat crystals and growth independently, when a correct corresponding Stage 2 coolant temperature was identified and used, at constant Stage 3 coolant temperature. Nelson (1999) noted that Stage 3 coolant temperature zone is known as the retention stage, being the time period at which crystal maturity is promoted within the equipment.

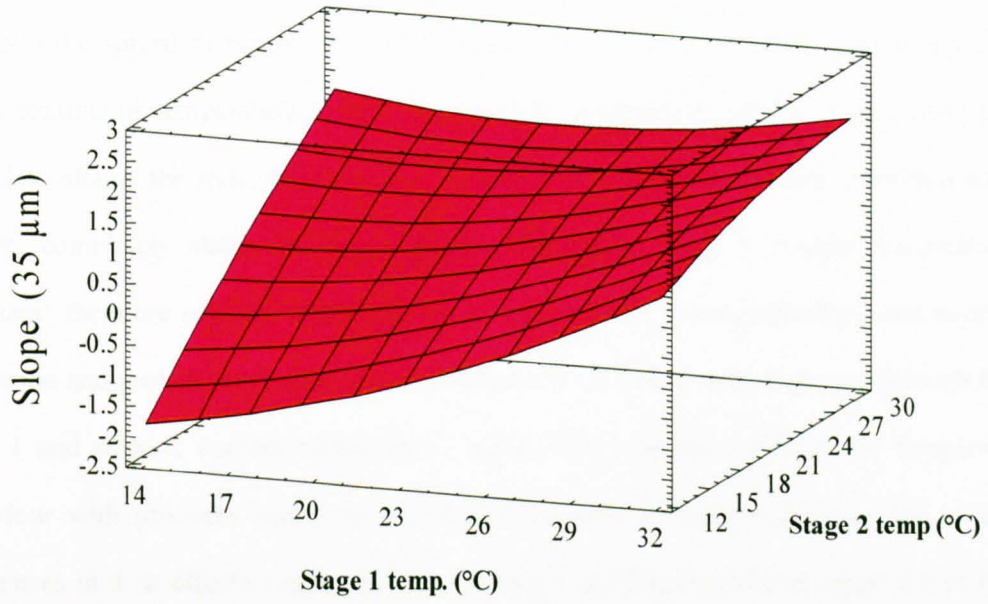


a.

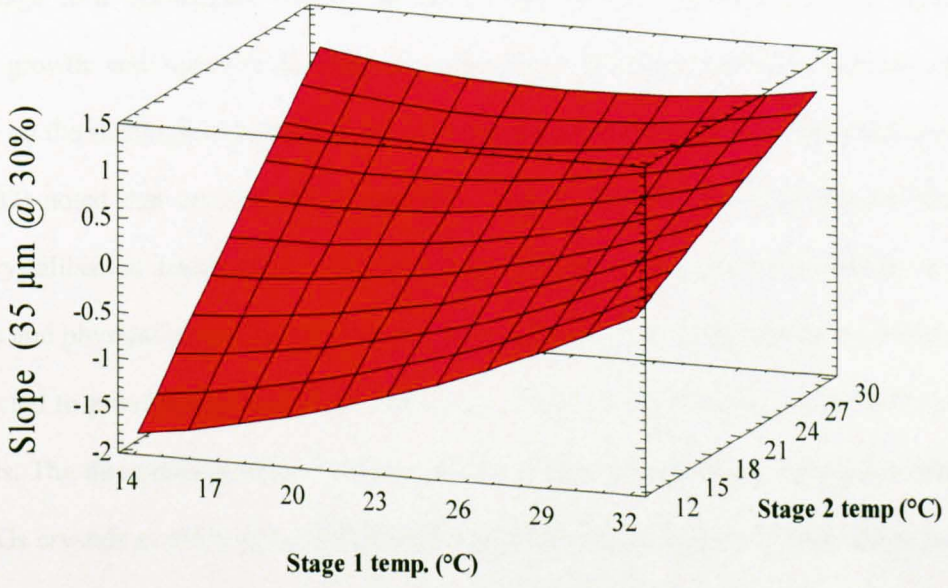


b.

Figure 5.8. Response plot showing chocolate temper slope for sample containing (a) 25 µm PS at 35% fat content and (b) 25 µm PS at 30% fat content



a.



b.

Figure 5.9. Response plot showing chocolate temper slope for sample containing (a) 35 µm PS at 35% fat content and (b) 35 µm PS at 30% fat content

During progression through the machine, agitation from scraping and mixing blades increases the spread of nuclei in a fine homogeneous structure of small crystals. At this stage, continuous temperature control is applied in conjunction with the time period the chocolate attains the transition from the unstable strike seed temperature condition to a mature, completely stable optimum temper. Making the stage 3 coolant temperature 'constant' therefore offers levelled chance of seed maturity, independent of seed crystal nucleation and growth previously occurred within the chocolate as they passed through the stage 1 and stage 2 coolant temperatures zones. The observed variations in tempering behaviour with products containing varying fat content is suspected to be due to the differences in their effective heat capacity exchanges and the rheological properties of the products (Afoakwa et al., 2008a) as these influence the heating or cooling rate through the multi-stage heat exchangers during the tempering process. Nucleation of fat crystals, crystal growth and maturity in dark chocolates are therefore dependent among other factors, on the rheological properties and thermal history of the samples. Pérez-Martınez et al. (2007) noted that crystallisation conditions such as cooling rate, and thermal history (i.e., crystallisation temperature and tempering process) have significant effects on the kinetics and physical properties of the crystallised systems. Fat crystallisation in chocolates is expected to provide the unique characteristics of texture and flavour release in finished products. The three-dimensional crystal network organisation and the polymorphic state of the TAGs crystals as affected by the crystallisation conditions have been reported as major factors determining physical (i.e., rheology) and functional (i.e., texture) properties of crystallised TAGS systems (Herrera & Hartel, 2000; Narine & Marangoni, 1999; Marangoni & McGauley, 2002; Toro-Vazquez et al., 2004). Following observations made

during the tempering processes, satisfactory and unsatisfactory temper regimes and their corresponding temper slopes and chocolate temper units were developed (Table 5.6).

Table 5.6. Satisfactory and unsatisfactory temper values and their temper regimes

Temper slope	Temper regime	Chocolate temper unit (CTU)
------		------
-1.0		10
-0.9	Over-tempered	9
-0.8	(Unsatisfactory tempered)	8
-0.7		7.5
-0.6		7.0
-0.5		6.8
-0.4		6.0
-0.3		5.6
-0.2		5.4
-0.1	Optimally-tempered	5.2
0		5.0
0.1		4.8
0.2		4.6
0.3		4.0
0.4		3.5
0.5		3.0
0.6	Under-tempered	2.5
0.8	(Unsatisfactory tempered)	2.0
1.0		1.0

This would enhance the knowledge base of chocolate manufacturers by providing greater understanding and guidance on temper index-temper regime relationships during tempering (pre-crystallisation) of dark chocolates.

5.6. CONCLUSION

Variations in PSD and fat content influenced the crystallisation behaviour causing wide variations in chocolate temper units during tempering of products. Differences in fat content exerted the greatest variability in temperature settings of the different zones for attaining well-tempered products. At 35% fat content, changes in PSD caused only minimal and non-significant effects on tempering behaviour. However, at 30% fat content, the effect of PSD was pronounced. No unique set of conditions was found to achieve good temper with a given chocolate in a specified tempering unit. The models developed showed that a wide range of optimal conditions exist, depending on the attainment of the appropriate temperature settings, all of which would result in tempered chocolate. Thus, different combinations of tempering temperatures could be employed to induce stable fat polymorph formation and are dependent greatly on fat content and partly PSD during dark chocolate manufacture. Optimal satisfactory and unsatisfactory temper regimes and their corresponding temper slopes and chocolate temper units have been provided. These would have great industrial significance for reducing processing (tempering) times during tempering of dark chocolates with assurance in quality control and shelf characteristics.

CHAPTER 6

EFFECTS OF TEMPERING AND FAT CRYSTALLISATION BEHAVIOURS ON MICROSTRUCTURE, MECHANICAL PROPERTIES, APPEARANCE AND MELTING CHARACTERISTICS IN DARK CHOCOLATE SYSTEMS

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Part of this chapter has been accepted for publication into Food Research International (Afoakwa et al., 2008; In Press).

Part of this chapter was presented orally at the Student Professional Development Session at the Institute of Food Technologists (IFT) Annual Meeting 2008, New Orleans LA, USA, June 28 – July 1, 2008.

Part of this chapter has been accepted for oral presentation at the 14th World Food Congress of the International Union of Food Science and Technology (IUFoST), Shanghai, China, October 19 – 23, 2008.

6.1 ABSTRACT

Fat crystallisation behaviours in dark chocolates varying in particle size distribution (PSD) (D_{90} of 18, 25, 35 and 50 μm) was studied, examining influence of temper regimes (optimal, over- and under-temper), and evaluating mechanical properties, appearance, microstructure and melting characteristics. Wide variations in mechanical properties and appearance were noted. Particle size (PS) was inversely related with texture and colour, with greatest effects noted in hardness, stickiness and lightness for all tempers. Over-tempering increased product hardness and stickiness but reduced gloss and darkening of surfaces. Under-tempering induced fat bloom yielding defects in texture, colour and surface gloss. The PSD had no influence on crystallinity at all tempers but limited effects on T_{onset}, T_{peak}, and ΔH_{melt} independent of temper but significantly influenced T_{end} and T_{index}. Contrary, temper influenced crystallinity and melting properties (T_{end}, T_{index} and ΔH_{melt}). Under-temper showed as widened crystal size distribution (CSD) with significant changes in T_{end}, T_{index} and ΔH_{melt} with whitening of both surface and internal structures with other effects on appearance and texture. Over-tempering caused moderate increases in CSD and melting properties, with significant effects on T_{end}, T_{index} and ΔH_{melt} but not on T_{onset}, or T_{peak}. Fat-sugar melting profiles were similar for all formulations and tempers. Micrographs revealed variations in surface and internal crystal network structures and inter-particle interactions related to temper. From scanning electron micrography, under-temper resulted in re-arrangements with re-crystallisation of unstable fat crystals to smaller numbers of larger agglomerates with formation of solid bridges between the crystalline network structures. Thus, optimizing temper regime is central to achievement of premium quality to avoid defects affecting mechanical properties, appearance and melting characteristics.

6.2 INTRODUCTION

Tempering is a technique of controlled pre-crystallisation employed to induce the most stable solid form of cocoa butter, a polymorphic fat in finished chocolates. The process consists of shearing chocolate mass at controlled temperatures to promote crystallisation of triacylglycerols (TAGs) in cocoa butter to effect good setting characteristics, foam stability, demoulding properties, product snap, contraction, gloss and shelf-life characteristics. Time - temperature protocols and shearing are employed to induce nucleation of stable polymorphs with the formation of three-dimensional crystal network structure influencing the microstructure, mechanical properties and appearance of products. The crystal network organisation and the polymorphic state of the TAGs crystals as affected by the crystallisation conditions are major factors determining rheological and textural properties of crystallised TAGs systems (Herrera & Hartel, 2000; Narine & Marangoni, 1999; Toro-Vazquez et al., 2004; Pérez-Martínez et al., 2007; Altimiras et al., 2007).

Cocoa butter, the only continuous fat phase in dark chocolates, consist of a mixture of ~ 40–50 different TAGs dominated by 2-oleyl glycerides of palmitic and stearic acids, mainly, 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS) 35%, 1,3-distearoyl-2-oleoylglycerol (SOS) 23% and 1,3-disaturated-2-oleoylglycerol type: 1,3-dipalmitoyl-2-oleoylglycerol (POP) 15% (Lipp & Anklam, 1998; Segall et al., 2005). These occur as symmetric triacyglycerols that contain a central monounsaturated fatty acid, with saturated fatty acids in the 1 and 3 positions, which dominate the crystallisation, polymorphism and phase transformations, thus provide chocolate with its unusual textural and other sensory properties.

Particle size distribution influences rheological and textural properties of both molten and tempered dark chocolates, with effects on microstructure, product spread, tempering and pre-crystallisation behaviour, hardness and sensorial qualities (Chevalley, 1999; Beckett, 2000; Do et al., 2007; Afoakwa et al., 2008a, 2008b, 2008c). Smaller particles improve sensory properties (Ziegler et al., 2001) but plastic viscosity and yield stress increase due to changes in surface area of particles in contact with fat phase. Chocolate production processes, such as refining, conching, tempering and crystallisation mechanisms result in physical and compositional attributes, influencing product quality and stability through the supply chain occurring during production, storage, distribution and ultimately sensory character in consumption and product identification.

Instrumental measurements can act as complements for sensory evaluations (Lawless & Heymann, 1998) with statistically significant correlations (Mohamed et al., 1982; Meullenet, Carpenter et al., 1997; Rosenthal, 1999; Ali, Selamat et al., 2001; Bourne, 2002). Appropriate strategies can objectively assess features of texture and appearance such as gloss, colour, shape, roughness, surface texture, shininess, and translucency (Leemans et al., 1998; Jahns et al., 2001; Hatcher et al., 2004; Briones & Aguilera, 2005; Briones et al., 2006; Altimiras, et al., 2007). Hartel (2001) noted that the control of crystallisation is critical for texture, melting properties and other quality characteristics. Melting profiles of chocolates have been studied using pulsed nuclear magnetic resonance (pNMR) and DSC (Tabouret, 1987; Walter & Cornillon, 2001; Walter & Cornillon, 2002; Smith et al., 2007). Knowledge of tempering effects on product texture and appearance attributes can have significant commercial implications.

With recent innovations and growth in chocolate confectionery industry, understanding the factors influencing chocolate microstructure, texture and appearance would be of value in predicting changes in quality. As well, information on cocoa butter isothermal phase behaviour is important for optimising production processes that maintain product quality. This study was therefore aimed at investigating effects of tempering and fat crystallisation behaviours on mechanical properties, appearance, melting characteristics and crystallised network microstructure in dark chocolates varying in particle size distribution.

6.3. MATERIALS AND METHODS

6.3.1 Materials

Cocoa liquor of Central West African Origin was obtained from Cargill Cocoa Processing Company (York, UK); sucrose (pure extra fine granulated) from British Sugar Company (Peterborough, UK); pure prime pressed cocoa butter and soy lecithin from ADM Cocoa Limited (Koog aan de Zaan, Netherlands) and Unitechem Company Ltd. (Tianjin, China) respectively.

The recipe, formulation and production of samples have been described under Section 5.4.1 above. Chocolates were formulated with total fat of 35% (w/w) from sucrose, cocoa liquor, cocoa butter and lecithin. Experimental samples (5 kg batch for each formulation) were produced by mixing sucrose (40.8%) and cocoa liquor (53.7%) in a Crypto Peerless Mixer (Model K175, Crypto Peerless Ltd, Birmingham, UK) at low speed for 2 min and then at high for 3 min, then using a 3-roll refiner (Model SDX 600, Buhler Ltd., CH-9240 Uzwil, Switzerland) to a specified particle size (D_{90} :18 ± 1 μm, 25 ± 1 μm, 35 ± 1 μm & 50 ± 1 μm) conducting particle size analysis, during refining, to ensure D_{90}

values. The refined chocolates were melted at 50 - 55 °C for 24 h and the chocolate mass conched in a Lipp Conche (Model IMC-E10, Boveristr 40-42, D-68309, Mannhein, Germany) at low speed for 3.5 h at 60 °C. Lecithin (0.5%) and cocoa butter (5%) were added and then conched at high speed for 30 min to effect adequate mixing and liquefaction. Samples were kept in sealed plastic containers at ambient (20 - 22 °C) and moisture and fat contents determined using Karl Fischer and Soxhlet methods (ICA, 1988) and (ICA, 1990).

6.3.2 Determination of particle size distribution

A MasterSizer® Laser Diffraction Particle Size Analyzer equipped with MS 15 Sample Presentation Unit (Refractive index 1.590) (Malvern Instrument Ltd., Malvern, England) was used. About 0.2 g of refined dark chocolate was dispersed in vegetable oil (Refractive index 1.450) at ambient temperature (20 ± 2 °C) until an obscuration of 0.2 was obtained. The sample was placed under ultrasonic dispersion for 2 min to ensure particles were independently dispersed and thereafter maintained by stirring during the measurement. Size distribution was quantified as the relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Micro Software v 2.19). PSD parameters obtained included specific surface area, largest particle size (D_{90}), mean particle volume (D_{50}), smallest particle size (D_{10}) and Sauter mean diameter (D[3,2]).

6.3.3 Tempering experiment

Samples were incubated at 50 °C for 4 h for melting and tempered using Aasted Mikrovert laboratory continuous three-stage tempering unit (Model AMK 10, Aasted

Mikroverk A/S, Farum, Denmark). Chocolate was pumped through the multi-stage units and a worm screw drove the product through the heat exchangers. Sensors located at specific points in the equipment measured the temperature of both the chocolate and the coolant fluid at each stage. Based on our earlier work on modelling temperature controls to study tempering behaviour (Afoakwa et al., 2008d), the temperature of each of the coolant fluids (Zones 1: 2: 3) were thus set as 26: 24: 32 °C, 21: 19: 32°C and 18: 16: 32°C respectively for attaining the under-tempered, optimally-tempered and over-tempered regimes. The degree of pre-crystallisation was measured using a computerized tempermeter (Exotherm 7400, Systech Analytics, Neuchâtel, Switzerland) and a built-in algorithm provided the tempering curves and temper readings in chocolate temper index (Slope), corresponding to optimal temper (Slope 0), under-temper (Slope 1.0) and over-temper regimes (Slope -1.0). The principle of this method has been described by Nelson (1999). Chocolate from the three regimes were moulded using plastic moulds: 80 mm length; 20 mm breadth; and 8 mm height. The final products were allowed to cool in a refrigerator (12 °C) for 2 h before de-moulding onto plastic trays and conditioned at 20 ± 2 °C for 14 days before analysis. Triplicate measurements were taken for each product composition and the mean values recorded.

6.3.4 Texture measurements

Mechanical properties of chocolates (hardness and stickiness) were measured using TA-HD Plus Texture Analyzer with a penetration probe (needle P/2) attached to an extension bar and a 50 kg load cell and a platform reported by Afoakwa et al. (2008c). Maximum penetration and withdrawal forces through a sample (80 x 20 mm, depth 8 mm)

were determined with 8 replications at a pre-speed of 1.0 mm/s, test of 2.0 mm/s, post speed of 10.0 mm/s, penetrating 6 mm at 20°C, converting mean values of the penetration force exerted by the 50 kg load cell into hardness (g force) and the withdrawal force with time into stickiness (g force.s) data respectively using XT.RA Dimension, Exponent 32 software (Stable Micro Systems, Godalming, Surrey, UK) as shown on Figure 4.3b.

6.3.5 Colour and gloss measurements

HunterLab Miniscan^{TM} XE Colorimeter Model 45/0 LAV (Hunter Associates Inc., Reston, VA) calibrated with white ceramic reference standard was used. Colour images of chocolate surfaces were converted into XYZ tristimulus values, which were further converted to CIELAB system: L*, luminance ranging from 0 (black) to 100 (white); and a* (green to red) and b* (blue to yellow) with values from -120 to +120. Information was obtained using a software algorithm (Matlab v. 6.5; The Math-Works, Inc., Natick, MA): hue angle (h°) = arctan (b*/a*); chroma (C*) = $[(a^*)^2 + (b^*)^2]^{½}$. Mean values from 5 replicate measurements and standard deviations were calculated.

Gloss of chocolate surface was measured using the multiple angle Tricor Gloss meter (805A/806H Gloss System, Elgin, IL). Reflectance was measured at an incidence light angle of 85° from the normal to the chocolate surface, in accordance with ASTM method D523. A polished black glass plate with a refractive index of 1.567 was used as standard surface (ASTM, 1995) and given a gloss value of 200. Gloss was reported as gloss units (GU) based on determinations (in triplicate) at six positions along a chocolate sample. As a reference, a surface with a gloss value less than 10 GU is considered a low gloss surface (BYK, 1997; Briones et al., 2006).

6.3.6 Image acquisition and capture

A colour digital camera (Canon Powershot, Model A70, MA, USA) was mounted on a stand inside a large box with internal black surface impervious to light. Images of the optimally-tempered, under-tempered and over-tempered samples were captured before storage and after 14 days in storage. The iris was operated in manual mode, with the lens aperture at f = 8 and speed 1/20 (no zoom, no flash) to achieve high uniformity and repeatability. The camera was gray balanced before each imaging session. Uniform diffuse lighting was used to illuminate the samples. The lighting system consisted of four CIE source D65 lamps (60 cm length and 18 W; Model TLD/965, Philips, Singapore) placed above the sample at a 45° angle to maximize diffuse reflection responsible for colour. The angle between the camera lens axis and the sample was around 90° to reduce gloss. A Kodak gray card with 18% reflectance was used as a white reference to standardize the illumination level. The gray-level image (1600 x 1200 pixels) of this card was divided into 192 blocks, each one of 100 x 100 pixels. After calibration, samples were placed in the field of view of the camera and an image of 1600 x 1200 pixels (approximately covering the whole area of the tablet) was acquired and stored in JPEG (Joint Photographic Experts Group, a standard for compressing digital photographic images) format of high resolution and superfine quality.

6.3.7 Determination of melting properties

Differential scanning calorimeter (DSC Series 7, Perkin Elmer Pyris, Norwalk, CT, USA) equipped with a thermal analysis data station was calibrated using indium and octadecane at a scan rate of 5 °C/min using an aluminium pan as reference. Samples (~ 5

mg) were loaded into 40 µl capacity pans with holes, which were sealed with lids using a sample press. Pans were heated at 5 °C/min from 15-55 °C in a N_2 stream. Onset temperature (T_{onset}), peak temperature (T_{peak}), end temperature (T_{end}) and enthalpy of melting (ΔH_{melt}) were calculated automatically by the software. Melting index (T_{index}) was computed as (T_{end} - T_{onset}), as described by Vasanthan and Bhatty (1996). Each sample was analyzed in triplicate and mean values and standard deviations reported.

Thermal behaviour of fat and sugar components in samples from the different temper regimes were analysed using DSC. Pans containing ~ 5 mg were heated at 10 °C/min from 15-200 °C in a N_2 stream and melting profiles of the fat and sugar calculated by the software. To calculate the ΔH_{melt} Sugar/ ΔH_{melt} Fat ratio, the melting enthalpy of the sugar was divided by the melting enthalpy of the fat peak, technique reported to provide information on the possible structural changes in the fat and/or sugar components in bloomed chocolates (Lonchampt & Hartel, 2006). Triplicate analyses were conducted and mean value and standard deviation reported.

6.3.8 Microstructural determinations

Chocolate samples were characterised using stereoscopic binocular microscope (Nikon, SMZ-2T, Tokyo, Japan) equipped with a variable removable lens. Micrographs (coloured images) were captured using a digital camera (Model 2.1 Rev 1, Polaroid Corporation, NY, USA) and observed using Adobe Photoshop (Version CS2, Adobe Systems Inc. NJ, USA). Triplicate experiments were conducted capturing 6 images per sample, and micrographs representing the surface of each temper regime captured and

presented. Samples were then sectioned (cut) into two pieces using a knife and the internal microstructures observed.

6.3.9 Scanning electron microscopy

Microstructural studies were carried out on optimally-, under and over-tempered chocolates after 14 days in storage using a 1200 EX JEM scanning electron microscopy (SEM; Joel Ltd., Akishima, Japan). Sectioned samples (20 x 20 mm) were lyophilized (Heto Model DW3, Allerød, Denmark), then transferred and separately placed on grids with the help of double-sided tape, sputter-coated with gold (2 min, 2 mbar). Microstructures were observed at 5 kV and 9.75 x 10^{-5} torr vacuum taking 12 micrographs for each section (500x, 1,500x and 5000x) showing typical micrographs for each temper regime.

6.3.10 Experimental design and statistical analysis

Two experimental variables comprising temper regime and PSD were used. Other variables including refiner temperature and pressure, conching time and temperature were held constant. A 3 x 4 factorial experimental design was used comprising;

i. Temper regime: optimal temper, under-temper and over-temper
ii. PSD (D_{90}): 18, 25, 35 and 50 μm

Statgraphics Plus 4.1 (Graphics Software System, STCC, Inc, Rockville, USA) examined mechanical properties (hardness and stickiness), appearance (colour [L, C*, h°] and gloss) and melting properties (T_{onset}, T_{end}, T_{index}, T_{peak}, ΔH_{melt}) using two-way analysis of variance (ANOVA) and multiple comparison tests to determine effects of factors and their

interactions. Tukey multiple comparisons (95% significance level) determined differences between levels. All experiments were conducted in triplicates and the mean values reported.

6.4. RESULTS AND DISCUSSION

6.4.1 Particle size distribution of dark chocolates

These findings (Fig. 4.1), previously reported (Afoakwa et al., 2008a), show volume histograms consisting of narrow (18 μm PS) and wide (25 μm PS) bimodal and narrow (35 μm PS), and wide (50 μm PS) multimodal size distributions. This PSD range 18 - 50 μm using D_{90} values (>90% finer) covers optimum minimum and maximum sizes with direct effects on texture and sensory character in manufacture (Ziegler & Hogg, 1999; Beckett, 2000). Data from the PSD as previously described (Afoakwa et al., 2008a) showed variations in specific surface area, mean particle volume D(v,50), Sauter mean (D[3,2]) and mean particle diameter (D[4,3]) with increasing D_{90} particle sizes. Specific surface area (SSA) was inversely correlated with the different component of PSD. Similar inverse relationships of SSA with all the other components of PSD have been reported (Beckett, 1999; Ziegler & Hogg, 1999; Sokmen & Gunes, 2006). Beckett (1999) concluded largest particle size and solids specific surface area are the two key parameters for chocolate manufacture. The former determines chocolate coarseness and textural character, the latter with desirable flow properties. Fat contents of the products were 35 ± 1% and moisture within the range of 0.90 - 0.98%.

6.4.2 Fat crystallisation behaviours during tempering of dark chocolate

Four different temper regimes (untempering, under-tempering, over-tempering and optimal-tempering) were characterised (Fig. 6.1) each with its unique characteristic crystallisation behaviour. In optimal tempering, the temperature of the chocolate remained constant for sometime during cooling, to initiate formation of stable fat crystals. The crystallisation heat released was then balanced by an equal amount of cooling energy causing the growth of stable crystal nuclei in adequate amounts, which during post-tempering conditioning mature to effect shelf stability of the product. The temperature of the chocolate dropped further when the liquid cocoa butter was transformed into solid crystals resulting in solidification of the products (Fig. 6.1). Beckett (2000) reported that properly tempered chocolate shows formation of Form V, the most desirable polymorphic form which confers appropriate product snap, contraction, gloss and shelf-life characteristics.

Under-tempering (insufficient tempering) was caused by the relatively higher temperatures released between the multi-stage heat exchangers during tempering. The process caused development of more crystallisation heat within the product during solidification, effecting quick cooling, as more liquid fat was transformed quickly into solid form, resulting in the formation of very few stable fat crystal nuclei (Fig. 6.1). Distinct increase in temperature was observed during the crystallisation period, which declined again after reaching a maximum point where most of the stable crystals formed were re-melted prior to cooling. Untempered chocolate, produced no stable fat crystals as the heat exchange system generated higher crystallisation heat during

cooling, resulting in quick cooling of the completely melted product with no inflexion point for stable fat crystal formation (Fig. 6.1).

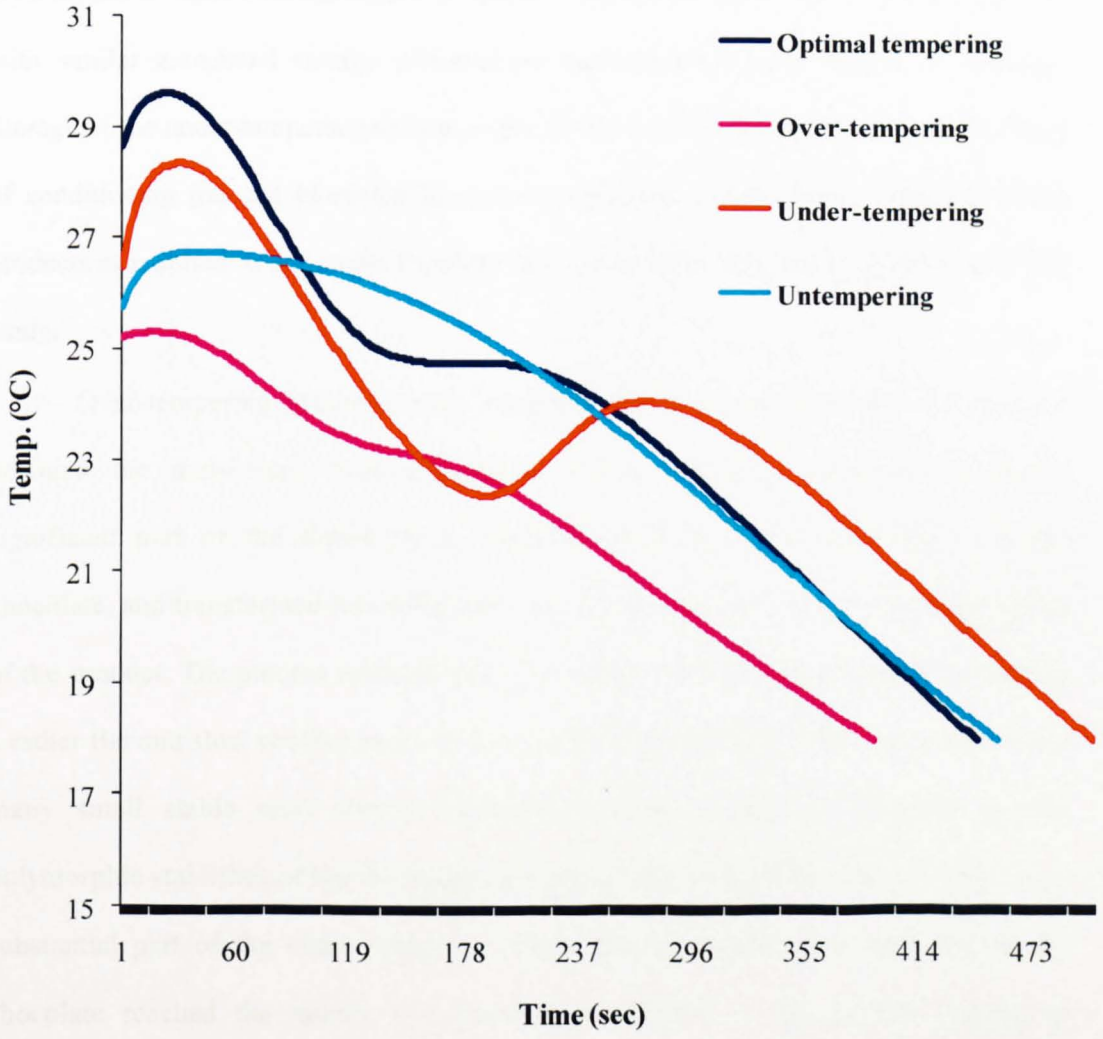


Figure 6.1. Pre-crystallisation (cooling) curves of different temper regimes from dark chocolate (18 μm PS)

Beckett (2000) explained that the crystallisation processes in both untempered and under-tempered chocolates lead to the formation of unstable crystal polymorph, which later transforms into more stable Form VI polymorph during storage. Preliminary studies showed that untempering and under-tempering regimes exhibits different crystallisation behaviours but results in similar unstable fat crystal nucleation and growth, with similar associated storage polymorphic transformations and defects in products. Storage of the under-tempered products under ambient temperature (20-22 °C) for 14 days of conditioning induced blooming in samples, effecting various quality changes in the products as reported in this study. Products from under-tempering regime were used in this study.

Over-tempering occurred when relatively lower temperatures were exchanged between the multi-stage heat exchangers of the tempering equipment, causing significant part of the liquid fat to withdraw from the continuous phase of the chocolate, and transformed into solid form leaving less liquid fat available for pumping of the product. The process released little crystallisation heat during cooling, rendering a rather flat and slow cooling curve (Fig. 6.1). This crystallisation process results in too many small stable seed crystal formation leading to reduced strengths in the polymorphic stabilities of the fat crystals formed during the process (Talbot 1999). As a substantial part of the phase transition (from liquid to solid) took place before the chocolate reached the mould, less contraction occurred in the mould, leading to demoulding problems with defects in final product quality and storage characteristics (Hartel, 2001; Lonchampt & Hartel, 2004).

6.4.3 Effect of temper regime and PSD on mechanical properties

Hardness showed an inverse relationship with particle sizes, with significant reductions at all temper regimes, and greatest in the under-tempered (bloomed) products (Fig. 6.2). Hardness of the optimally-tempered products decreased from 5318 g with 18 μm PS to 4259 g at 50 μm. Similar trends in hardness were noted with the over-tempered samples, decreasing from 6064 g with 18 μm PS to 4651 g at 50 μm, and from 6533 g with 18 μm PS to 5459 g at 50 μm in the under-tempered products (Fig. 6.2), suggesting differences in hardness with varying PS at all temper regimes. Particle sizes have been noted as an important parameter in the hardness of fat crystal networks in many confectionery products (Narine & Marangoni, 2002; Campos et al., 2002; Marangoni & Narine 2002; Pérez-Martínez et al., 2007). Earlier studies showed inverse relationships of hardness in tempered dark chocolates with particle sizes at varying fat and lecithin levels (Afoakwa et al., 2008c), attributed to the relative strengths of their particle-to-particle interactions (Campos et al., 2002; Afoakwa et al., 2008b).

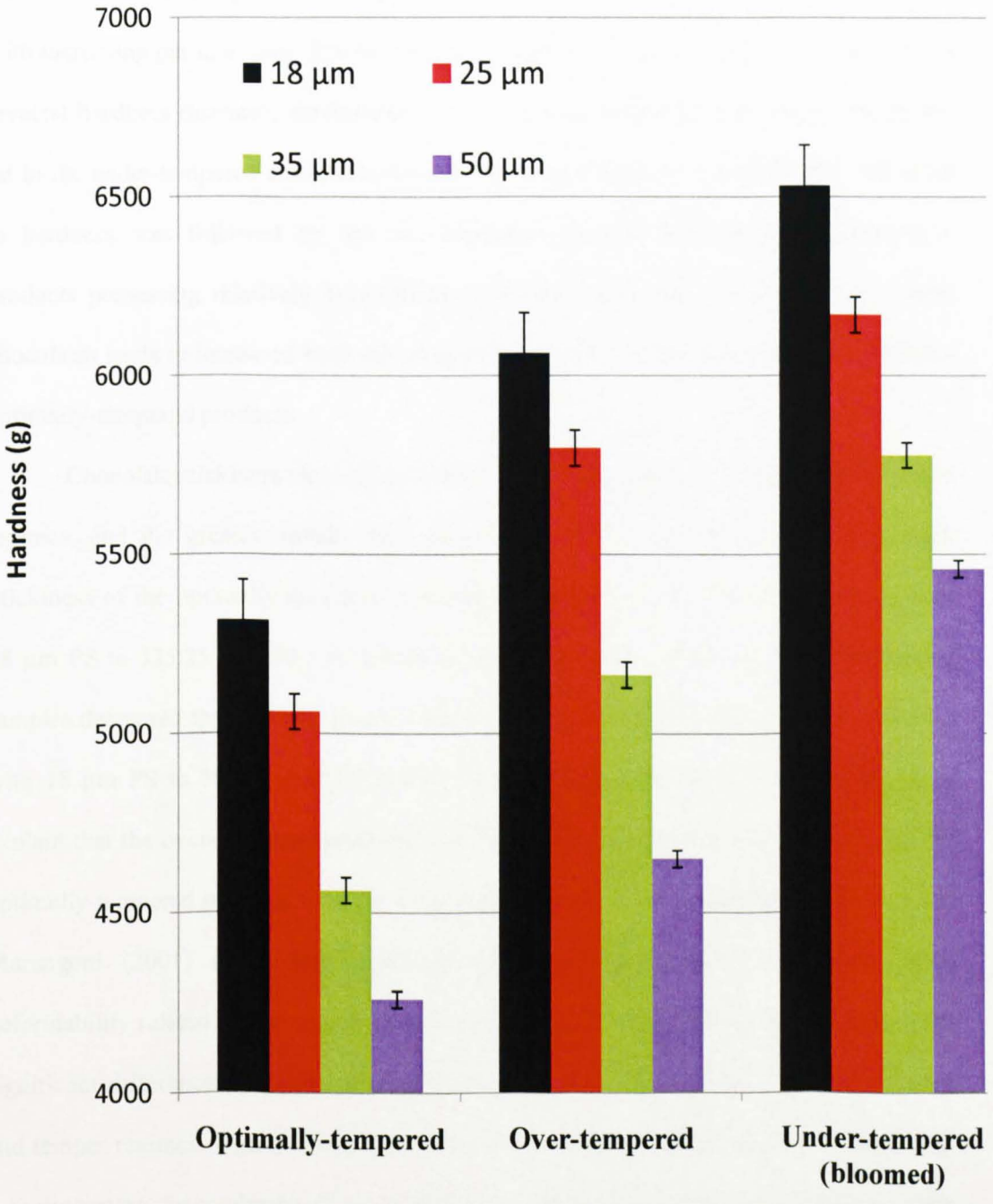


Figure 6.2. Effect of temper regime and PSD on hardness of dark chocolates

Do et al. (2007) also reported consistent reductions in hardness (texture) of milk chocolates with increasing particle sizes. The results showed that the under-tempered products had the greatest hardness (texture), attributable to the re-crystallisation process undergone by the fat in the under-tempered chocolates resulting in intense hardening of products. This trend in hardness was followed by the over-tempered samples with the optimal-tempered products possessing relatively lesser hardness levels, suggesting over-tempering of dark chocolates leads to increased hardness of samples at all PS as compared to their respective optimally-tempered products.

Chocolate stickiness showed an inverse relationship with particle sizes at all temper regimes, and the greatest trends were noted in the over-tempered products (Fig. 6.3). Stickiness of the optimally-tempered products decreased consistently from 380.67 g with 18 μm PS to 325.25 g at 50 μm. Likewise, the levels of stickiness in the over-tempered samples decreased from 447.92 g with 18 μm PS to 365.10 g at 50 μm, and from 336.86 g with 18 μm PS to 309.20 g at 50 μm in the under-tempered products (Fig. 6.3). These explain that the over-tempered products had the greatest stickiness levels, followed by the optimally-tempered products with the under-tempered samples having the least. Narine and Marangoni (2001) noted that stickiness of confectionery gives information about deformability related to oral sensory characters. Analysis of variance (ANOVA) suggested significant differences ($p \leq 0.05$) in both hardness and stickiness levels with particle sizes and temper regimes. Significant interactions were observed between all parameters (Table 6.1) suggesting the combined effects of PSD and tempering could be manipulated to reduce hardening and stickiness in dark chocolates. Multiple comparison tests showed over-

tempered products were significantly harder and stickier than the optimally-tempered – important for quality control and in new product development.

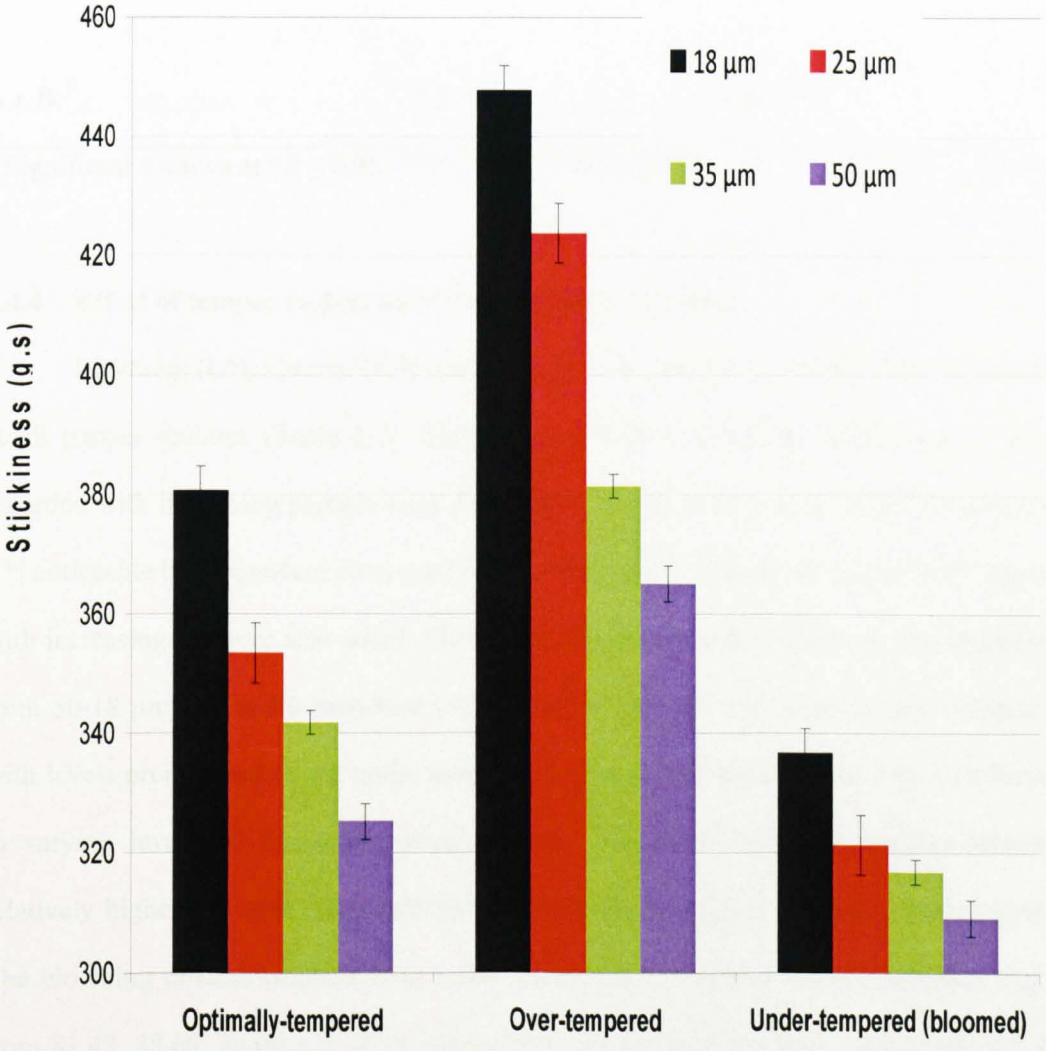


Figure 6.3. Effect of temper regime and PSD on stickiness of dark chocolates

Table 6.1. ANOVA Summary of F-values of texture measurements

Process variables	Hardness	Stickiness
A : Particle size (D_{90})	577.47***	5191.25***
B : Temper regime	419.16***	21562.10***
A x B	7.21**	22.51**

* Significant F-ratios at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

6.4.4 Effect of temper regime and PSD on colour and gloss

Lightness (L*), chroma (C*) and hue (h°) followed similar trends with varying PS at all temper regimes (Table 6.2). Significant ($p<0.001$) and linear effects on L* were recorded with increasing particle sizes from 18 to 50 μm, with consequential decreases in L*, noticeable but dependent on temper regime (Table 6.3). Similar decreases in C* and h° with increasing PS were also noted. Thus, dark chocolate became lighter as D_{90} decreased from 50-18 μm and as PS increased (18-50 μm) C* and h° were significantly decreased, with levels pronounced in the under-tempered samples. Similarly, temper regime affected to varying levels all the colour measurements. The under-tempered samples attained relatively higher L*-values than both the optimally-tempered and over-tempered samples. The blooming process resulted from under-tempering of samples caused decreases in L* from 81.47, 80.60, 80.09 and 78.76 respectively for the products with 18 μm, 25 μm, 25 μm and 50 μm, an indication that all the under-tempered samples had become whiter in colour within the 14 days conditioning period. As well, the blooming caused great reductions in C* and h° in the under-tempered products at all PS (Table 6.2). Hutchings

(1994) stated that L*, C* and h° respectively represent food diffuse reflectance of light, degree of saturation and hue luminance, which are dependent on particulate distribution, absorptivity and scattering factors or coefficients. In a densely packed medium, scattering factor is inversely related to particle diameter (Saguy & Graf, 1991). Chocolates with varying particle sizes differ in structure and particulate arrangements influencing light scattering coefficients and thus appearance (Afoakwa et al., 2008a).

Similar decreasing trends in L* were noted in both tempered and over-tempered samples with increasing PS. However, the over-tempered samples had relatively lower L* values at all PS as compared to their corresponding optimally-tempered products (Table 6.2). These suggest that over-tempering reduces the degree of lightness in dark chocolates, effecting product darkening and thus affecting quality. However, no noticeable effect on C* and h° were observed among the optimally- and over-tempered products (Table 6.2). Thus, changes in colour in dark chocolates were primarily dependent on PS and temper regime. Under-tempered (bloomed) dark chocolates tend to scatter more light, appear lighter and less saturated than over-tempered and optimally-tempered products. The blooming process resulted in higher scattering coefficients, with subsequent paleness (whitening) - higher L* values. Hartel (1999) reported that the whitish haze in bloomed chocolate is caused by the dispersion of light of fat crystals. Similar effects of PS on the degree of whitening during blooming have been reported (Altimiras et al., 2007). Colour of foods may be affected by various optical phenomena among them scattering and surface morphology, therefore an accurate understanding of the influence of appearance on measured colour is essential.

Table 6.2. Effects of temper regime and PS on gloss and colour measurements

Temper regime	Particle size (D_{90}) (μm)	Gloss (GU)	Colour measurements		
			L*	C*	h°
Optimally-tempered	18	158.6 ± 1.43	45.49 ± 0.42	17.57 ± 0.44	47.7 ± 0.12
	25	150.3 ± 1.78	44.79 ± 1.16	17.18 ± 0.27	46.4 ± 0.44
	35	146.6 ± 0.84	43.86 ± 0.40	17.02 ± 0.36	44.7 ± 1.06
	50	144.6 ± 1.27	42.19 ± 0.56	16.93 ± 0.17	44.5 ± 0.35
Over-tempered	18	142.0 ± 0.64	44.05 ± 0.40	17.36 ± 0.20	46.6 ± 0.21
	25	140.7 ± 2.07	43.43 ± 1.02	17.10 ± 0.18	45.7 ± 0.42
	35	129.0 ± 1.28	42.26 ± 0.21	17.04 ± 0.38	44.2 ± 0.26
	50	121.3 ± 1.36	41.87 ± 0.48	16.47 ± 0.17	44.0 ± 0.15
Under-tempered	18	7.3 ± 0.24	81.47 ± 1.44	10.45 ± 0.15	6.1 ± 0.16
	25	5.3 ± 0.32	80.60 ± 1.26	10.12 ± 0.18	5.9 ± 0.29
	35	5.0 ± 0.15	80.09 ± 0.83	9.98 ± 0.16	6.3 ± 0.13
	50	4.3 ± 0.28	78.76 ± 0.96	9.81 ± 0.27	6.2 ± 0.18

Means ± standard deviation from triplicate analysis

Gloss relates to capacity of a surface to reflect directed light at the specular reflectance angle with respect to the normal surface plane (ASTM, 1995). Significant ($p<0.001$) and linear effects on gloss were observed with increasing PS from 18 to 50 μm, with consequential decreases in gloss, greatly dependent on the temper regime (Table 6.3). Gloss of dark chocolates was reduced as D_{90} increased from 18-50 μm at all temper regimes. As well, differences in temper regime influenced the gloss measurements to varying levels. Blooming of the under-tempered samples caused drastic reduction in gloss of the products than their respective optimally-tempered and over-tempered samples. The under-tempered samples containing 18 μm PS had gloss value of 7.3 GU, while the corresponding tempered and over-tempered products had 158.6 GU and 142.0 GU respectively. Similar trends were noticed at all PS (Table 6.2). Beckett (2000) noted tempering was important for gloss, a key quality attribute in chocolate. In under-tempered chocolates light scattering is affected by reductions in surface regularity. Gloss stability of edible coating formulations of chocolates have been studied (Trezza & Krochta, 2000; Lee et al., 2002; Briones et al., 2006).

ANOVA showed that PS and temper regime both significantly ($p<0.001$) influenced L*, C*, h° and gloss, with significant ($p\leq0.05$) interactions (Table 6.3), all influencing appearance. Multiple comparison tests showed under-tempering had the greatest influence on appearance and gloss of products but differences between optimally- and over-tempered products were significant. Attention to tempering is important for consistency in dark chocolate appearance and quality control.

Table 6.3. ANOVA Summary of F-values of colour and gloss measurements

Process variables	L*	C*	h°	Gloss
A : Particle size (D_{90})	516.04***	80.99***	15.08**	111.46***
B : Temper regime	2960.75***	17482.54***	2302.96***	10183.49***
A x B	29.95**	43.86**	12.15*	23.01***

* Significant F-ratios at *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

6.4.5 Effect of temper regime and PSD on melting properties

6.4.5.1 Effects of temper regime

Figure 6.4 shows typical DSC thermograms used for evaluating the melting properties of dark chocolates manufactured from the optimally-tempered, over-tempered and under-tempered regimes. All the samples exhibited similar distinct single endothermic transitions between 15 and 55 °C, the range expected for chocolate melting profiles. McFarlane (1999) explained that peak onset corresponds to the temperature at which a specific crystal form starts to melt; peak maximum, that at which melting rate is greatest; and end of melting, completion of liquefaction – all these information are related to the crystal type. Peak height, position and resolution are dependent on sample composition and crystalline state distribution.

Data from the DSC (Fig. 6.4) showed that differences in temper regime produced changes in crystallinity and melting properties, observed in the differences in their peaks, suggesting that variations in crystallisation behaviour in dark chocolates during tempering influence the degree of crystallinity and crystal size distribution (CSD) of their derived products. Under-tempered (bloomed) chocolates showed the greatest peak width, followed by the over-tempered samples having slightly wider CSD than the optimally-tempered products with resultant variation in their melting profiles (Fig. 6.4).

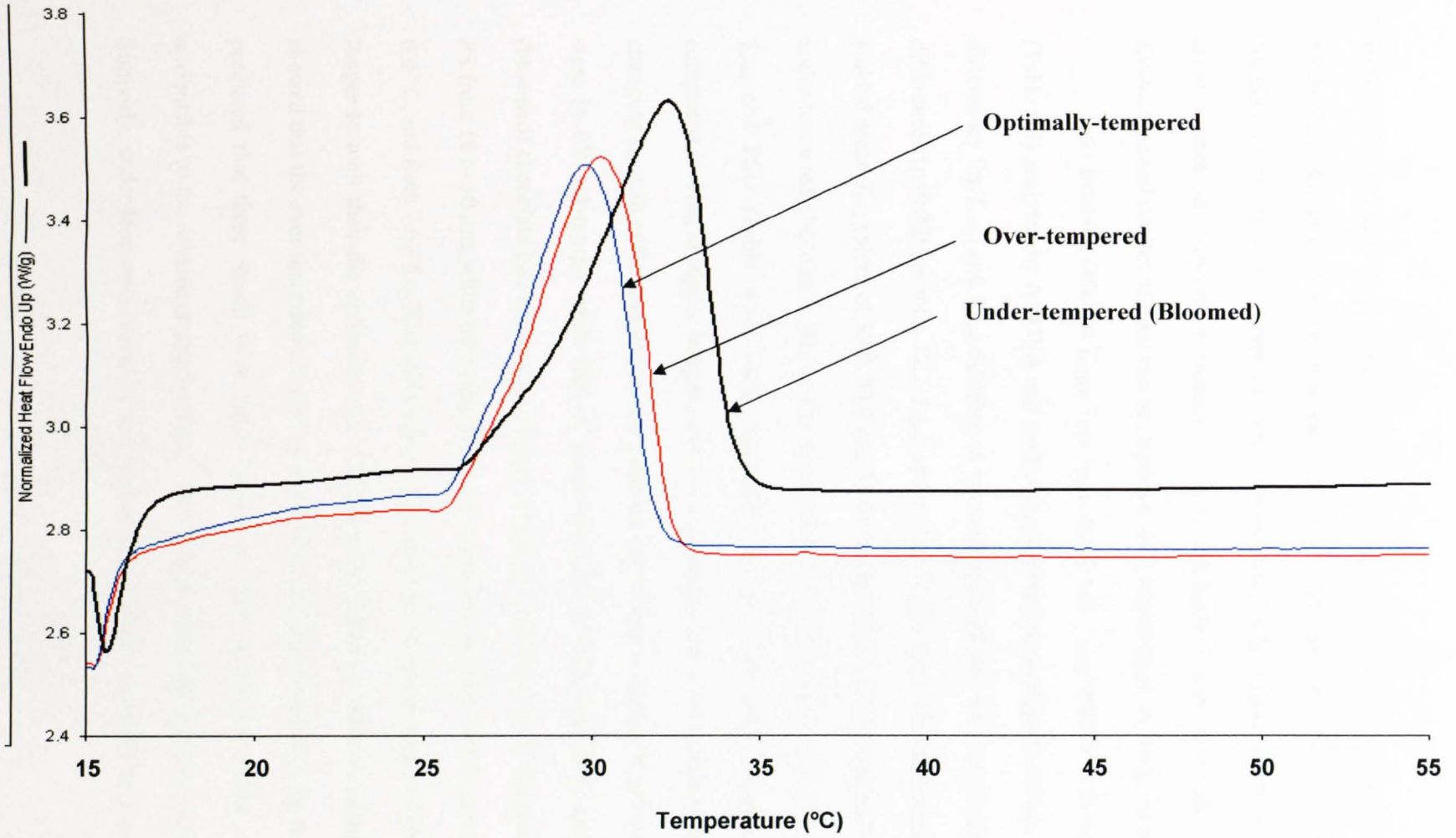


Figure 6.4. Typical DSC thermograms of fat melting profile showing optimally-tempered, over-tempered and under-tempered (bloomed) dark chocolates

Hartel (2001) concluded that distribution of crystal sizes in foods play key roles in final product quality, defined by the total and specific characteristics of their crystalline material. Number of crystals and range of sizes, shapes, and polymorphic stability, as well as arrangements in network structures dictates mechanical and rheological properties. Knowledge and control of CSD can be important for optimising processing conditions.

Data from the DSC on T_{onset}, T_{end}, T_{peak}, ΔH_{melt} and T_{index} in relation to temper regime (Table 6.4) analysed by ANOVA and multiple comparison tests showed significant ($p<0.05$) differences for T_{onset} and T_{peak} differing in temper regime (Table 6.4) and highly significant differences ($p<0.001$) among T_{end}, T_{index} and ΔH_{melt} (Table 6.5). The differences in temper yielded mean T_{end} values of 33.0, 33.7 and 35.9 °C respectively for the optimally-, over- and under-tempered chocolates. There was a significant ($p<0.05$) inverse relationship between T_{end} and PSD (Table 6.5). Such observations suggest that under-tempered chocolate completed melting at higher temperatures than optimally- and over-tempered products. The changing T_{end} values the samples revealed that the crystallites in optimally and over-tempered were in ßV polymorph while that of under-tempered in ßVI. Similarly, under-tempered (bloomed) chocolate had higher T_{index} values of 8.8, 8.7, 8.5 and 8.2 °C inversely related to PS from 18 to 50 μm, while the optimal and over-tempered products had T_{index} ranges of 7.1 - 6.0 °C, and from 7.6 - 6.6 °C respectively, suggesting that the under-tempered chocolate took longer to melt than the optimally and over-tempered products. Multiple comparison tests showed that the over-tempered samples took longer to melt than the optimally tempered. It is predicted that these would have likely impact on their behaviour during consumption, attributable to the relative strengths of their mechanical properties (hardness and stickiness). Similarly, under-tempered chocolate had higher ΔH_{melt} values at all PS than the optimally-

and over-tempered products (Table 6.4), with significant ($p<0.05$) interactions with PS. Multiple comparison test revealed that over-tempered chocolates showed higher T_{index} and ΔH_{melt} than the optimally-tempered, a significant finding for process quality control.

6.4.5.2 Effects of particle size distribution

In dark chocolate, PSD influences rheological and microstructural properties as well as texture in tempered products (Afoakwa et al., 2007b; 2008a; 2008b). In this study, peak shapes and sizes were similar with all values for PSD suggesting little differences in crystallinity between them. Examination of important DSC parameters (Table 6.4) - T_{onset}, T_{end}, T_{peak}, ΔH_{melt} and T_{index} – suggested increasing PS from 18 to 50 μm yielded no significant (P=0.2782) changes in T_{onset}, for any temper regime (Table 6.5), with values in the ranges of 26.5 – 26.6 °C, 26.5 – 26.7 °C and 27.2 – 27 4 °C respectively for the optimally, over- and under-tempered chocolate. Similar observations were made for T_{peak} (Table 6.4) - ranging from 31.7 – 31.9, 32.1 – 32.3 and 33.6 – 33.8 °C for the optimally, over- and under-tempered chocolates. Similarly, ΔH_{melt} in products with increasing PS from 18 μm to 50 μm decreased marginally from 37.73 – 36.76, 41.26 – 40.36 and 44.45 – 43.80 J/g in the optimally, over- and under-tempered products respectively (Table 6.4), with insignificant differences ($p>0.05$) in PS found at all temper regimes. The lack of significant relationship between PS and ΔH_{melt}, implies that enthalpy of melting was similar for chocolates at all PS independent of temper regime.

Table 6.4. Effects of temper regime and particle size distribution on melting properties

Temper regime	Particle size (D_{90}) (μm)	Melting properties				
		T_{onset} (°C)	T_{end} (°C)	T_{index} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)
Optimally-tempered	18	26.5 ± 0.4	33.6 ± 0.3	7.1 ± 0.2	31.9 ± 0.1	37.73 ± 0.65
	25	26.4 ± 0.3	33.3 ± 0.4	6.7 ± 0.4	31.7 ± 0.2	37.56 ± 0.92
	35	26.6 ± 0.2	32.7 ± 0.3	6.1 ± 0.2	31.7 ± 0.1	36.87 ± 0.58
	50	26.5 ± 0.4	32.5 ± 0.4	6.0 ± 0.4	31.8 ± 0.2	36.76 ± 0.72
Over-tempered	18	26.6 ± 0.2	34.2 ± 0.3	7.6 ± 0.2	32.6 ± 0.2	41.26 ± 0.61
	25	26.5 ± 0.4	33.8 ± 0.4	7.3 ± 0.4	32.7 ± 0.1	40.42 ± 0.88
	35	26.7 ± 0.2	33.5 ± 0.2	6.8 ± 0.2	32.5 ± 0.2	40.47 ± 0.57
	50	26.6 ± 0.3	33.2 ± 0.4	6.6 ± 0.4	32.6 ± 0.2	40.36 ± 0.52
Under-tempered	18	27.4 ± 0.2	36.2 ± 0.3	8.8 ± 0.2	33.8 ± 0.2	44.45 ± 0.88
	25	27.3 ± 0.4	36.0 ± 0.4	8.7 ± 0.4	33.7 ± 0.1	44.10 ± 0.51
	35	27.2 ± 0.2	35.7 ± 0.3	8.5 ± 0.2	33.6 ± 0.2	43.87 ± 0.86
	50	27.4 ± 0.3	35.6 ± 0.4	8.2 ± 0.4	33.6 ± 0.1	43.80 ± 0.58

Means ± standard deviation from triplicate analysis

In contrast, varying PS had significant effects on T_{end} and T_{index} with general inverse relationships between particle size and T_{end}, and T_{index}, at all temper regimes (Table 6.4). Optimally-tempered products with smaller PS (18 μm) had T_{end} value of 33.6°C, and decreased consistently to 32.5 °C in the 50 μm samples, representing a difference of 0.9°C. Similar marginal but significant ($p<0.05$) decreasing trends in T_{end} with increasing PS were observed with both the over-tempered and under-tempered (bloomed) samples (Table 6.5). These findings suggested that dark chocolates with larger PS (50 μm) require slightly lower temperatures to complete melting than the smaller PS (18 μm) products at all temper regimes. Similar inverse relationships were observed between T_{index} and PS at all temper regimes. The data (Table 6.4) showed that increasing PS for 18 μm to 50 μm in the optimally-tempered products caused significant ($p\leq0.05$) reductions in T_{index} from 7.1 °C to 6.0 °C respectively, and similar trends were noted with the over-tempered and under-tempered products. ANOVA showed significant ($p<0.001$) influence of PS on T_{end} and T_{index} with significant interactions with temper regime (Table 6.5). Multiple comparison test revealed significant differences ($P = 0.001$) between T_{end} of products containing 18 μm, 35 μm and 50 μm, suggesting that chocolates with finer particles (18 μm) would take relatively longer time to melt than their corresponding products with larger particles (35 and 50 μm) independent of temper regime, attributable to the relative strengths of the inter-particle aggregations and flocculation in the different PS products (Narine & Marangoni, 1999; Marangoni & McGauley, 2002; Afoakwa et al., 2008b). Do et al. (2007) noted that quantitative decreases in particle aggregation and structure in chocolate influence melting behaviour suggesting that in its crystallised state, structures with larger PS are less interconnected, providing less resistance to breakage and melting. This could be important

for predicting oral melting behaviour with impacts on temporal components of flavour release and oral epithelial sensations.

Table 6.5. ANOVA Summary of F-values of melting properties

Process variables	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	T_{index} (°C)	ΔH_{melt} (J/g)
A : Particle size (D_{90})	1.78	12.17*	3.74	34.73**	6.96
B : Temper regime	198.75***	261.19***	22.57**	1107.80***	462.78***
A x B	1.18	2.45*	7.26***	160.33***	3.67**

* Significant F-ratios at *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

6.4.5.3 Thermal behaviours and ratio of sugar/fat melting enthalpies in products

Thermal behaviours and ratio of sugar/fat melting enthalpies in chocolates differing in temper regime were studied using DSC to provide information on differences in structure. The DSC thermograms (Fig. 6.5) showed differences in fat melting profile, resulting from the widened peak width in the under-tempered (bloomed) sample; but no differences were noted in the sugar melting profiles, explaining the structural (polymorphic) transformations in the fat component in the under-tempered product.

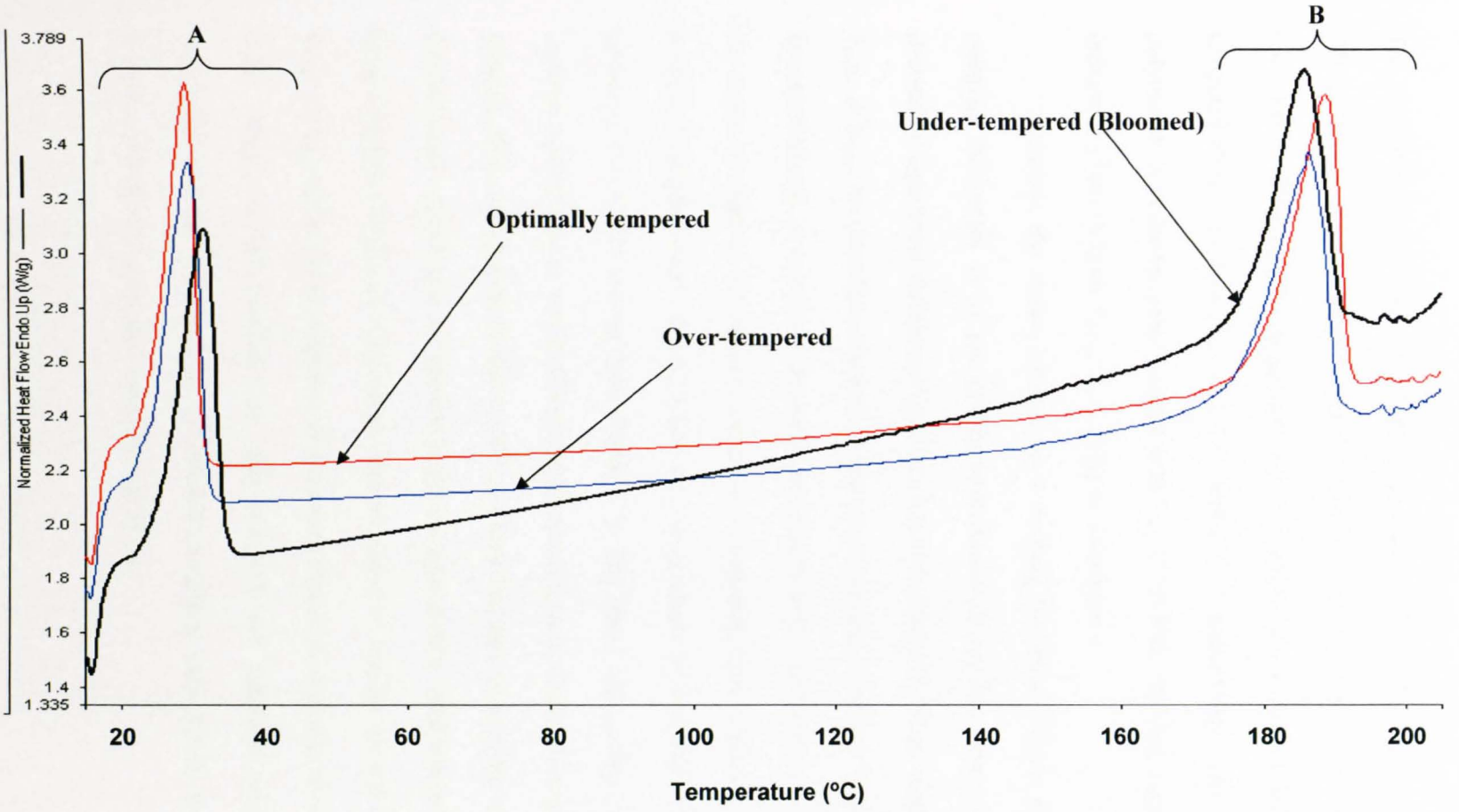


Figure 6.5. Typical DSC thermograms showing (A) fat and (B) sugar melting profiles of optimally-tempered, over-tempered and under-tempered (bloomed) dark chocolates at 18 µm PS

The DSC data on fat and sugar melting properties (T_{onset}, T_{end}, T_{peak}, ΔH_{fat}, ΔH_{sugar} and $\Delta H_{sugar}/\Delta H_{fat}$) related to temper regime (Table 6.6) were similar to the trends for fat (Table 6.4). Fat melting profiles suggested the ßV polymorph in both optimally- and over-tempered chocolates with T_{end} of 32.3 °C and 32.9 °C respectively, and a more stable ßVI polymorph in under-tempered sample with T_{end} of 35.8°C, showing significant ($p<0.001$) influences (Table 6.7) on T_{onset}, T_{peak}, ΔH_{fat} in chocolates.

Contrary, the results of the sugar melting properties (Table 6.6) showed only marginal differences in all the melting properties with varying temper regime. ANOVA showed no significant differences ($p>0.05$) in all the studied melting properties (T_{onset}, T_{end}, T_{peak}, ΔH_{sugar}) on chocolates from the three temper regimes (Table 6.7), suggesting that no structural change in sugar were found in products from the three temper regimes. Similarly, the ratios of sugar to fat melting enthalpies in products from optimal-, over- and under-tempered samples were 1.25, 1.24 and 1.17 respectively (Table 6.6), with no significant different ($P = 6.853$) among them (Table 6.7). The lower $\Delta H_{sugar}/\Delta H_{fat}$ ratio noted in the under-tempered sample resulted from the higher ΔH_{fat} as a result of recrystallization of fat (Hartel, 2001; Lonchampt & Hartel, 2004). These findings support the earlier finding that fat and sugar components are present in similar quantities in both bloomed and optimally-tempered dark chocolates, but contrast with the report of Lonchampt and Hartel (2006) that the melting peak of fat in untempered (bloomed) chocolate was almost non-existence with ΔH_{fat} being ten-fold smaller than that obtained for optimally-tempered chocolate, concluding that the whitish spots in bloomed chocolates were mainly composed of sugar crystals and cocoa powder and nearly devoid of fat.

Table 6.6. Thermal properties of fat and sugar components in dark chocolates from different temper regimes

Temper regime	Fat melting properties				Sugar melting properties				Sugar/fat relations
	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)	ΔH_{sugar} /ΔH_{fat}
Optimally-tempered	26.2 ± 0.24	32.3 ± 0.44	30.8 ± 1.04	37.60 ± 0.66	179.43 ± 0.43	191.98 ± 0.39	188.83 ± 0.52	50.64 ± 0.64	1.25 ± 0.21
Over-tempered	26.4 ± 0.18	32.9 ± 0.28	31.4 ± 0.83	39.58 ± 0.42	178.85 ± 0.18	191.34 ± 0.83	187.37 ± 0.74	49.13 ± 0.47	1.24 ± 0.34
Under-tempered	27.3 ± 0.53	35.8 ± 0.19	33.5 ± 0.71	42.07 ± 0.73	178.21 ± 0.47	190.82 ± 0.50	186.87 ± 0.58	49.16 ± 0.76	1.17 ± 0.28

Means ± standard deviation from triplicate analysis

Table 6.7. ANOVA Summary of F-values of fat and sugar thermal properties

Process variable	Fat melting properties				Sugar melting properties				Sugar/fat relations
	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)	ΔH_{sugar} /ΔH_{fat}
Temper regime	12.41*	42.83***	3.86*	32.89***	2.07	1.52	2.54	3.28	1.95

* Significant F-ratios at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

Kinta and Hatta (2005) also reported the presence of fat components in bloomed dark chocolate, suggesting mechanisms of bloom development in chocolate involves phase separation associated with the growth of xenomorphic fat crystals.

6.4.5.4 Effect of temper regime on product image

Digital images of dark chocolates (18 μm PS) were assembled to show surface appearances of the optimally-, under- and over-tempered products before and after the 14 days conditioning (Fig. 6.6). Initially surface appearances were similar and smooth but after 14 days, clear differences were apparent. Optimally- and over-tempered chocolates maintained their characteristic glossy appearance and dark brown colour but the under-tempered samples had bloomed, with appearance of surface whitish spots, rendering them dull and hazy in colour (Fig. 6.6). Similar increases in whiteness in under-tempered (bloomed) chocolates have been reported (Lonchampt & Hartel, 2004; Lonchampt & Hartel, 2006; Altimiras et al., 2007). Hartel (1999) explained this phenomenon as re-crystallisation of fats from a less stable Form IV to a more stable Form VI polymorph, with changes in light dispersion on small surface fat crystals ($\geq$ 5μm), consequently impacting on both appearance and textural attributes. Fat bloom development, mechanisms and effects on chocolate appearance, quality and marketability has been extensively studied (Bricknell & Hartel, 1998; Ali et al., 2001; Hartel, 2001; Timms, 2003; Walter & Cornillon, 2001, 2002; Lonchampt & Hartel, 2004, 2006; Altimiras et al., 2007; Smith et al., 2007).

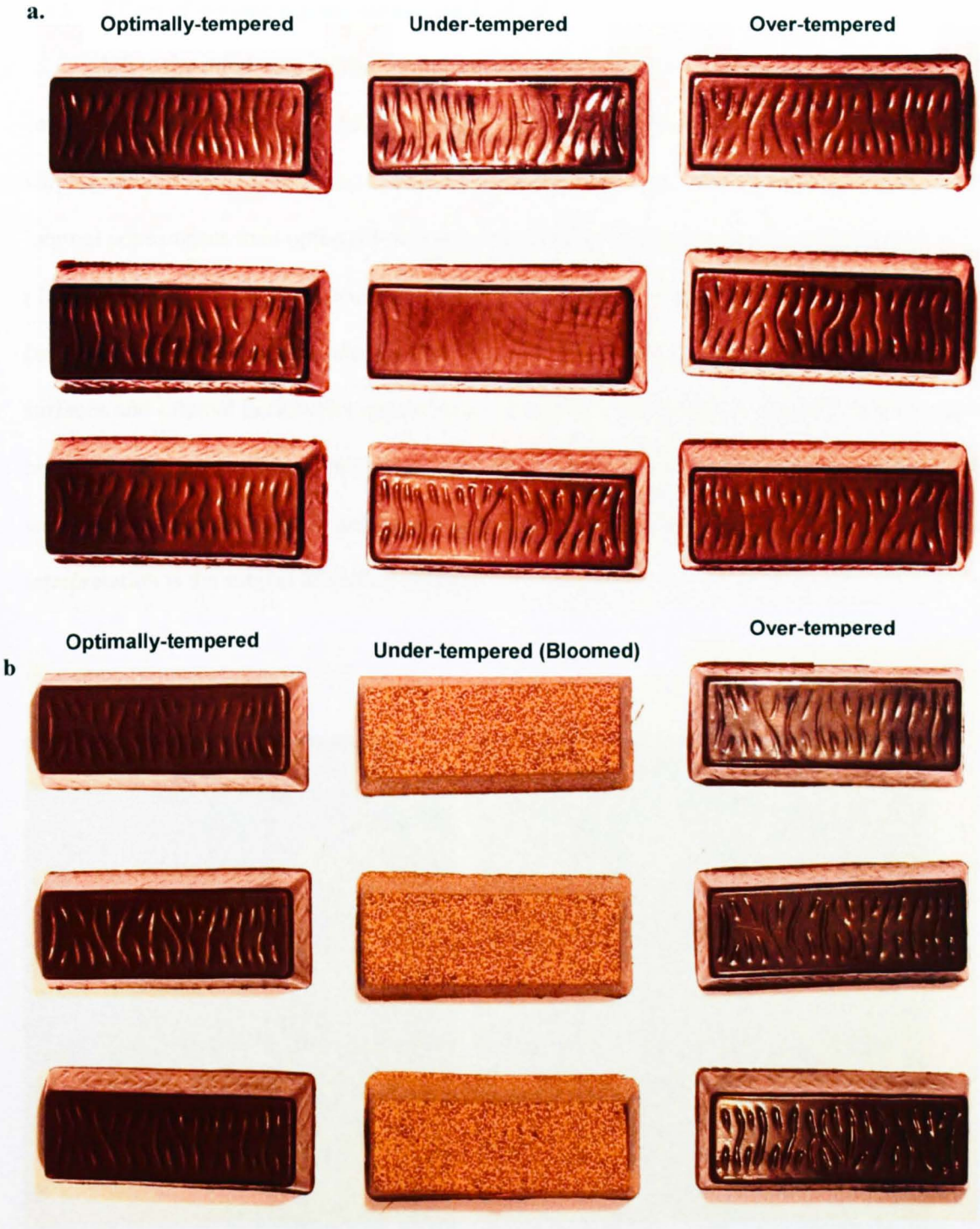
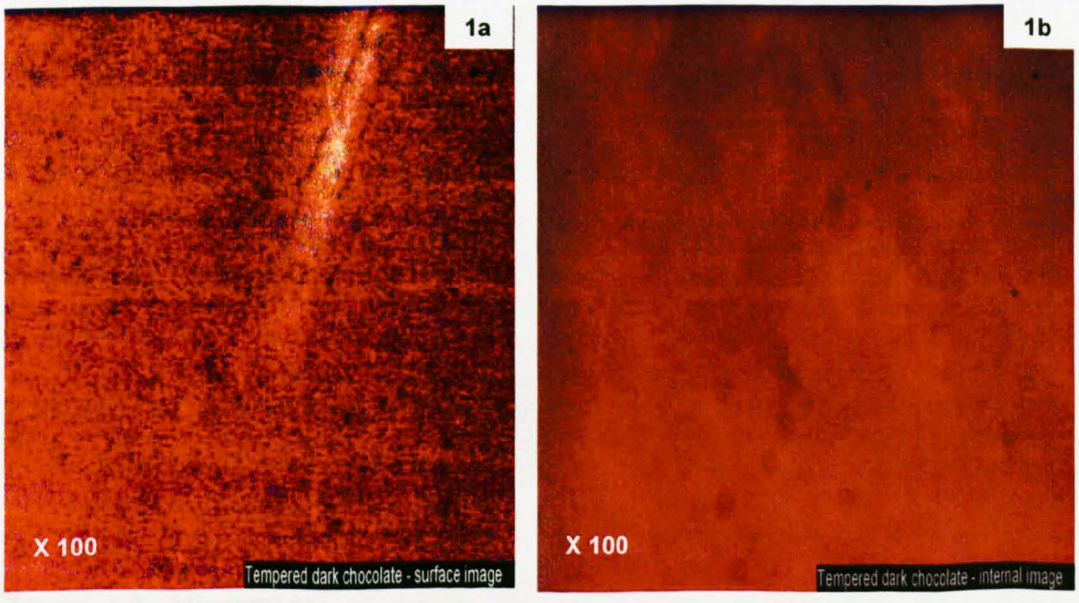


Figure 6.6. Photographic images of (a) fresh and (b) matured (conditioned) optimally-tempered, under-tempered and over-tempered dark chocolates (18 μm PS)

6.4.6 Effect of temper regime on microstructure

Microstructural examination using stereoscopic binocular microscopy after the 14 days conditioning showed clear variations in both surface and internal peripheries of products from varying temper regimes (Fig. 6.7). Over-tempered products had relatively darker surfaces and internal appearances than optimally-tempered confirming the reported relative differences in L* (Table 6.2). Under-tempered products showed both bloomed surface and internal periphery with large whitish, and distinct smaller brown spots (Fig. 6.7). The observed whitish appearance on surfaces and internal peripheries appear to be mixtures of fat and sugar crystals, and the small brown spots, cocoa solids. Lonchampt and Hartel (2004, 2006) suggested these whitish spots were primarily sugar crystals and cocoa powder and nearly devoid of fat. This difference in interpretation is the subject of further studies.



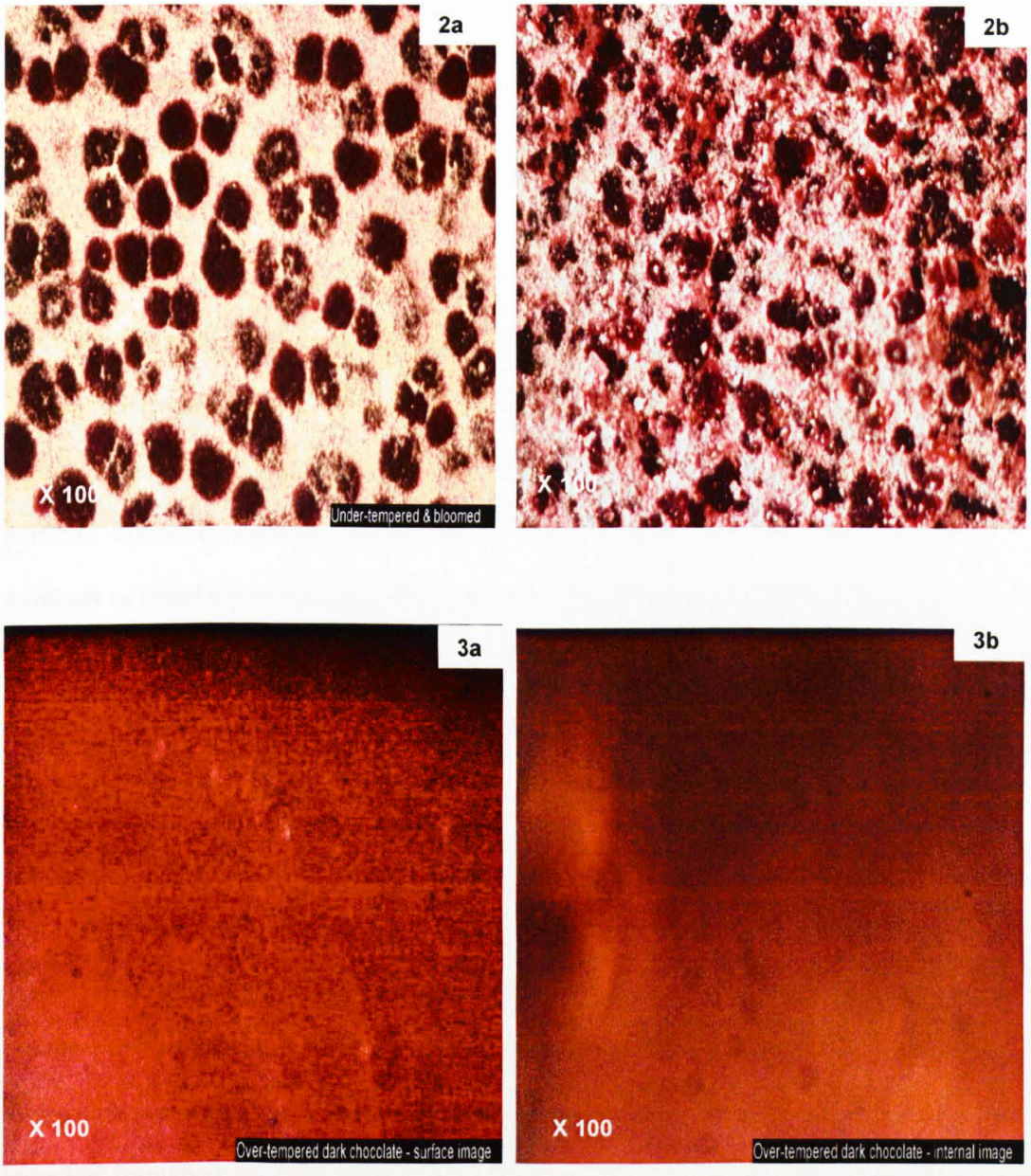


Figure 6.7. Micrographs of surface (a) and internal (b) structures respectively of (1) optimally-tempered, (2) under-tempered and (3) over-tempered dark chocolate (18 μm PS)

6.4.7 Effect of temper regime on scanning electron microstructure

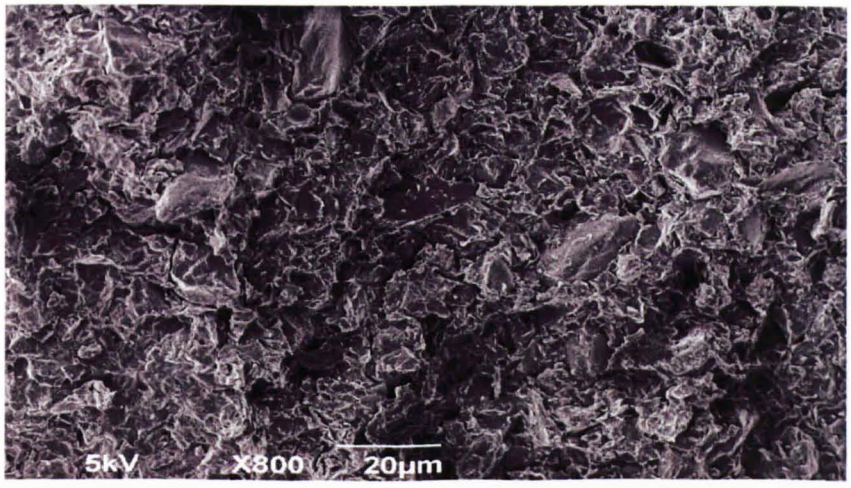
Microstructural examination using scanning electron microscopy after 14 days of conditioning showed clear variations in crystalline network structure, inter-particle interactions and spatial distributions of network mass among optimally-, over- and under-tempered samples, becoming well defined with increasing magnification from (i) x 800 (ii) x 1,500 to (iii) x 2,500 (Fig. 6.8a-c). Microscopy of the optimally-tempered chocolate showed an even spatial distribution of small number of dense crystalline network with well defined inter-particle connections among crystals suggesting stable β-polymorph (Fig. 6.8a). Similarly, micrographs of the over-tempered chocolate showed a spatial distribution of a dense mass of smaller crystals (relative those of the optimally-tempered) within a network structure of well defined particle-to-particles crystal connections suggesting their β-polymorph stability (Fig. 6.8b). These larger numbers of small crystalline networks noted in the over-tempered samples is suspected to result from early nucleation and growth of seed crystals due to the slow cooling (Fig. 6.1), leading to the formation of sub-micron primary crystallites from the melt, with the resulting fat crystal network stabilized by van der Waals forces, possibly with steric and Coulombic forces (deMam, 1999; Narine & Marangoni, 2002; Tang & Marangoni, 2008).

Under-tempered (bloomed) chocolates showed dissolution, re-arrangement and re-crystallisation of the numerous small crystals noted in the over- and optimally-tempered products to a smaller number of larger (lumps) fat crystals (Ostwald ripening), and polymorphic transformation, nucleation and growth of new large crystals in a more stable polymorphic form, inducing formation of solid bridges with weak and less inter-crystal connections within the crystalline structures (Fig. 6.8c). Hartel (2001) suggested this phenomenon is brought about by thermodynamic differences in equilibrium between large and small crystals within a network structure leading to re-crystallisation of unstable fat polymorphs. This hypothesis suggests that

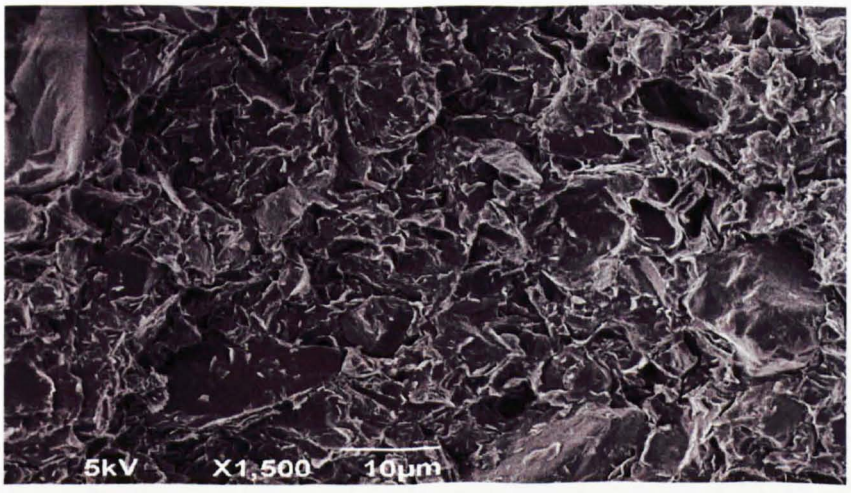
differences in crystallisation behaviour during tempering leads to formation of different microstructural organisations of crystal network structure with associated physical changes in chocolates. Characterising the nature of crystals in confectionery is an important step in quantifying the physical and sensory properties, as the resulting three-dimensional fat crystal network along with the phase behaviour and structural arrangements impact on mechanical, rheological, and melting properties and shelf life (Hartel, 2001; Campos et al., 2002; Perez-Martinez et al., 2007). Parameters such as cooling rate and thermal history (i.e., crystallisation temperature and tempering) influence kinetics and ultimate physical properties of the crystallised fat systems.

a.

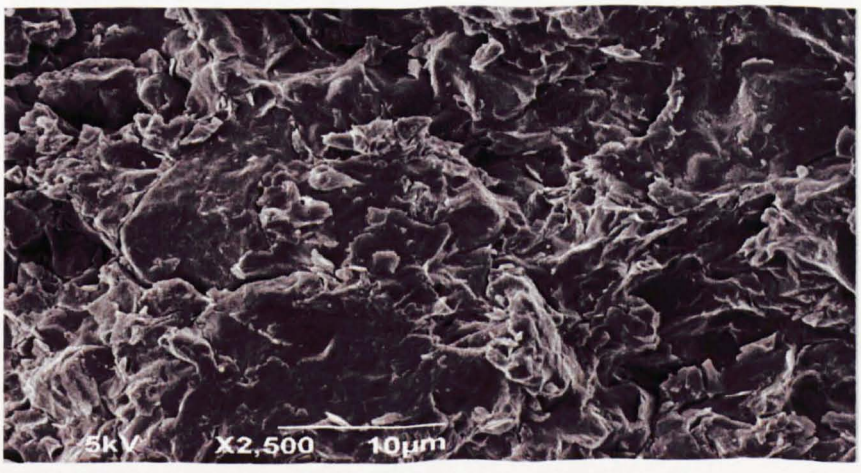
i.



ii.

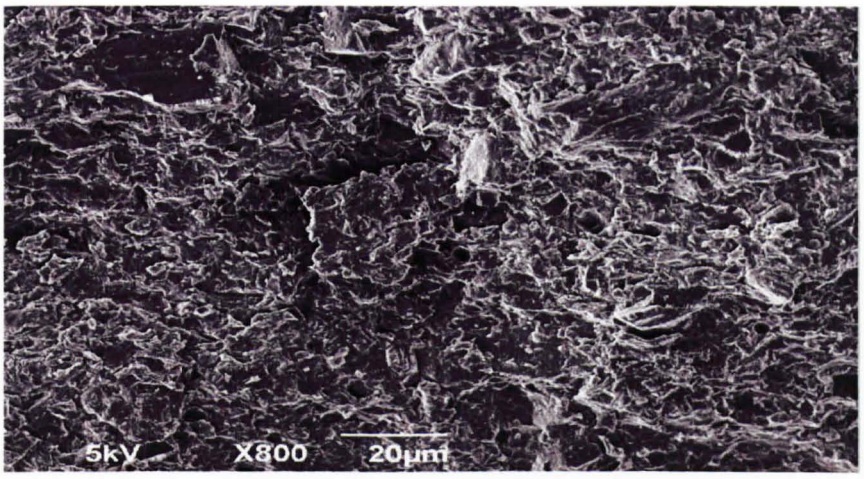


iii.

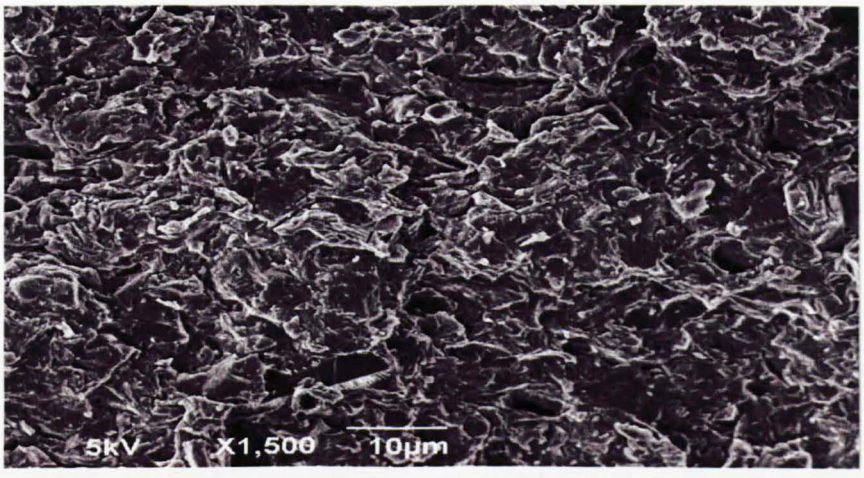


b.

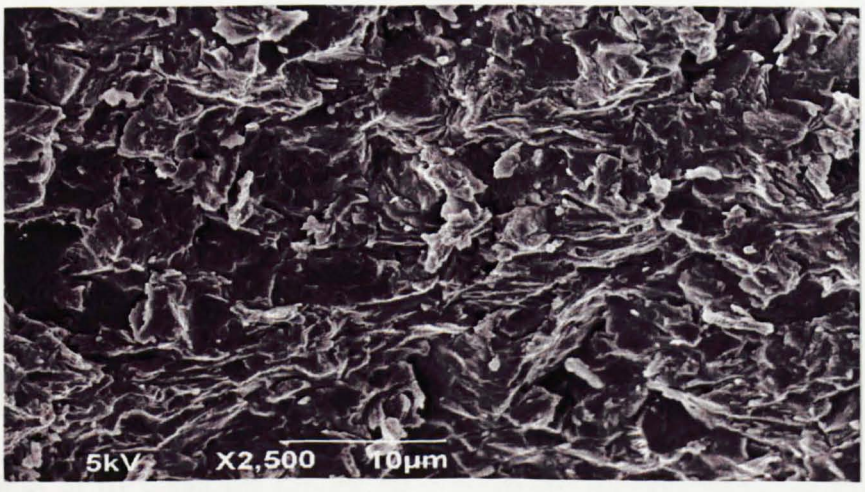
i.



ii.

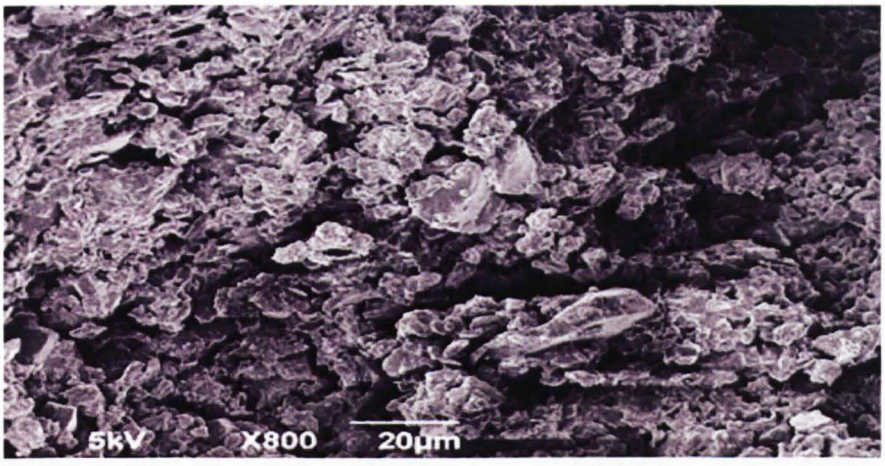


iii.

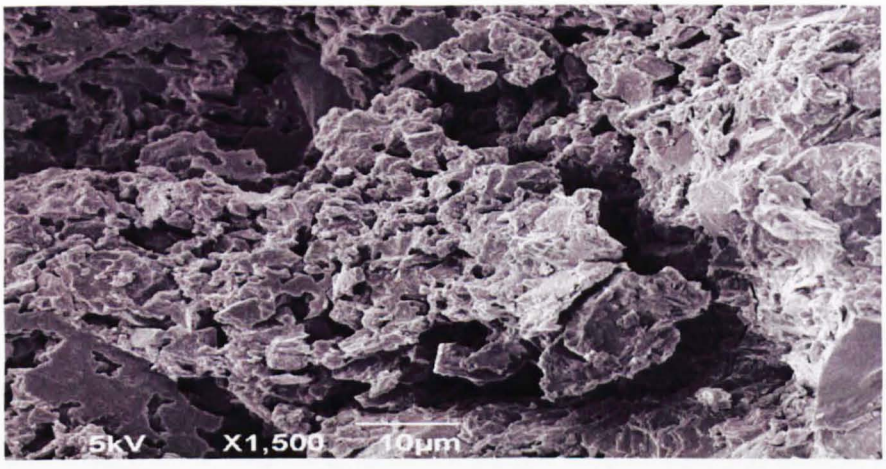


c.

i.



ii.



iii.

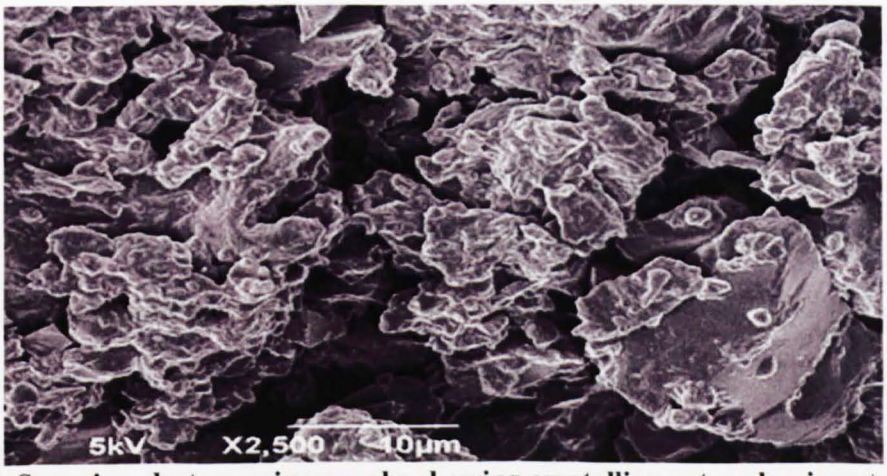


Figure 6.8. Scanning electron micrographs showing crystalline network microstructures at magnifications of (i) x 800 (ii) x 1,500 (c) x 2,500 for (a) optimally-tempered, b) over-tempered and (c) under-tempered (bloomed) dark chocolates at 18 µm PS

6.5 CONCLUSION

Fat crystallisation behaviour during tempering of dark chocolate play vital roles in defining the structure, mechanical properties and appearance of products. Wide variations in mechanical properties and appearance occurred in products from different PS and temper regimes. Particle size was inversely related with texture and colour, with the greatest effects noted with hardness, stickiness and lightness at all temper regimes. Over-tempering caused increases in product hardness, stickiness with reduced gloss and darkening of product surfaces. Under-tempering induced fat bloom in products with consequential quality defects in texture, colour and surface gloss. Variations in PSD had no influence on crystallinity of chocolates whether optimally-, over- or under-tempered. Particle size had a limited but significant direct relationship with certain melting parameters - T_{onset}, T_{peak}, and ΔH_{melt} - independent of temper but significant inverse relationship with others - T_{end} and T_{index}. Contrariwise, varying temper influenced crystallinity and chocolate melting properties (T_{end}, T_{index} and ΔH_{melt}). Under-tempering of chocolate resulted in widened crystal size distribution with significant changes in T_{end}, T_{index} and ΔH_{melt}. Over-tempering caused moderate increases in crystal size distribution, with significant effects on T_{end}, T_{index} and ΔH_{melt} but no changes were noted in T_{onset}, or T_{peak}. Fat-sugar melting profiles were similar in all chocolates independent of PS and temper regime.

Stereoscopic binocular micrographs revealed clear variations in surface and internal crystal network structure and inter-particle interactions among optimally-tempered-, over-tempered and under-tempered (bloomed) samples. Blooming caused whitening of both surface and internal periphery of products with consequential effects on texture and appearance. Electron micrographs showed an even spatial distribution of numerous small stable β-polymorph crystals in a network with well defined inter-particle connections in optimally-tempered chocolate. With over-tempered chocolate there were large numbers of very small crystals in

network with similar well defined particle-to-particle connections resulting from formation of a stable β-polymorph with early nucleation: the outcome was growth of seed crystals from the melt into sub-micron primary crystallites and a fat crystal network stabilised by van der Waals forces. Under-tempering resulted in dissolution of a large number of small crystals, re-arrangement and re-crystallisation into a small number of larger (lumps) fat crystals (Ostwald ripening). In this process there was polymorphic transformation, nucleation and growth of new large crystals in a more stable polymorphic form with formation of solid bridges with weak and fewer inter-crystal connections within the chocolate structure. Attainment of optimal temper regime during tempering of dark chocolate is necessary for the achievement of premium quality products and avoidance of defects in mechanical properties, appearance and melting character.

CHAPTER 7

FAT BLOOM FORMATION AND DEVELOPMENT DURING STORAGE OF UNDER-TEMPERED DARK CHOCOLATES

This chapter has been accepted for publication in the Journal of Food Engineering (Afoakwa et al., 2008; In Press)

7.1 ABSTRACT

Fat bloom development and associated changes in microstructure, texture, appearance and melting properties were studied. Dark chocolates varying in particle size (PS) (D_{90} of 18, 25, 35 and 50 μm) were processed and pre-crystallised to under-temper regime. Bloom was induced by storing products under ambient conditions (18 ± 2 °C, RH 50%) and changes in texture, surface whiteness, gloss and melting properties evaluated on cooling and after every 24 h in storage until reaching asymptotic values. Microstructure of products were characterised during blooming using stereoscopic binocular microscopy. Measurements on texture and surface whiteness showed initial rapid increases with consequential reductions in gloss within the first 96 h, followed by gradually decreasing gradient until reaching asymptotic levels. Storage influenced melting properties (T_{onset}, T_{end}, T_{peak} and ΔH_{melt}) in products causing polymorphic transformation from βIV to βVI within 72 h. Micrographs showed similar surface crystalline network structure and inter-particle interactions among products from different PS after tempering, and bloom initiation occurred within 24 h in storage resulting in appearance of both liquid and unstable fat on the surface of products. The unstable fat then recrystallised during storage into more stable polymorphs and crystal growth was promoted by Ostwald ripening (larger crystals growing at the expense of smaller ones), with the appearance of white crystalline structure which spread gradually throughout the chocolate mass after 96 h. Product containing the largest PS (50 μm) showed the fastest fat bloom rate, with the smallest PS (18 μm) the least, attributed mainly to hydrodynamic forces by capillary action. It was hypothesised that fat bloom development was initiated by capillarity, followed by growth of re-crystallised fat by diffusion across the entire chocolate mass until fully bloomed.

7.2. INTRODUCTION

Fat crystallisation in chocolates is a complex process induced by tempering (pre-crystallisation) during manufacture. The process promotes crystallisation of triacylglycerols (TAGs) in cocoa butter to effect formation of a large number of small crystals of the βV polymorph (2% to 3% of the initial fat content) that act as seeds for further crystal growth. The bulk of the TAGs are deposited on the seeds during cooling, forming crystals and eventually an interconnected fat crystal network. The crystal network organisation and the polymorphic state of the TAGs crystals as affected by the crystallisation conditions are major factors determining the microstructure, rheological and textural properties of the crystallised TAGs systems (Narine & Marangoni, 1999; Herrera & Hartel, 2000; Toro-Vazquez et al., 2004; Pérez-Martínez et al., 2007), the stability of which depends on the temper regime attained by the crystals during pre-crystallisation.

Cocoa butter, the only fat phase in dark chocolates is composed mainly of TAGs of the 1,3-disaturated-2-oleoylglycerol type with 3 fatty acids accounting for almost 95% of the attachments to the glycerol backbone. These fatty acids and their approximate proportions are oleic acid (C 18:1, 35%), stearic acid (C 18:0, 34%), and palmitic acid (C 16:0, 26%) (Beckett 2008). Main TAGs in cocoa butter are POS, POP, and SOS, according to the esterification position of fatty acids in the glycerol molecule. Cocoa butter can exist in six polymorphic forms of which the β forms V and VI are the most stable. Form V predominates in well-tempered chocolate and slowly transforms into Form VI, during prolonged storage of over-tempered chocolate with the physical appearance of fat bloom (Lipp & Anklam, 1998; Talbot 1999; Aguilera et al., 2004; Segall et al., 2005).

Fat bloom in chocolate products is a major quality defect in modern confectionery industry. This physical phenomenon is manifested by the appearance of whitish haze on the

surface of chocolates due to re-crystallisation of cocoa butter when chocolate is either insufficiently tempered or exposed to high temperatures during storage and/or distribution in supply chain, depriving it from its smooth appearance, bright colour and gloss (Hartel, 1999; Beckett, 2008). Several studies have attributed this to fat migration, mainly induced by insufficient formation of stable polymorphs (Form V) in cocoa butter during tempering, polymorphic crystalline transition from Form V to VI during prolonged storage of products, melting and re-crystallisation of low melting point crystals without re-tempering during fluctuating storage temperatures, and in composite structures such as chocolates with nut-based filings, consequently impacting on microstructure, visual appearance and textural properties (Hartel, 1999; Talbot, 1999; Briones & Aguilera, 2005; Lonchampt & Hartel, 2006; Afoakwa et al., 2008c; Beckett, 2008).

Many hypothesis and mechanisms have been published to explain the kinetics of fat migration in different chocolate and confectionery products, most of which have been attributed to diffusion and capillary rise due to the particulate nature of chocolate structure (Ziegleder et al., 1996; Miquel et al., 2001; Ghosh et al., 2002; Aguilera et al., 2004; Quevedo et al., 2005). Ghosh et al. (2002) explained that the driving force for diffusion was assumed to be effected by the difference in liquid fat content, but recently diffusion has been attributed to a gradient in TAG concentration within some domains of the product, with the explanation that differences in TAGs are less likely to occur in chocolate made with a homogeneous liquid phase of cocoa butter but may occur in composite structures. Though the physical changes associated with fat bloom is well known, available information on the mechanism of the crystallisation phenomenon is confusing, and on the actual complex crystal structures that are formed and the rate of bloom development with products from different particle sizes as occurs in under-tempered dark chocolate systems remain unclear.

To enhance understanding of the mechanism of fat bloom development in dark chocolate systems, it is important to evaluate the structure-appearance relationships leading to the formation and development of fat bloom in products during post-processing handling and storage. Thus, the objectives of this work were to investigate changes in microstructure, appearance, texture and melting characteristics during blooming in under-tempered dark chocolates varying in particle size distribution and to explain the possible mechanism leading to fat bloom development in products.

7.3 MATERIALS AND METHODS

7.3.1 Materials

Cocoa liquor of Central West African Origin was obtained from Cargill Cocoa Processing Company (York, UK); sucrose (pure extra fine granulated) from British Sugar Company (Peterborough, UK); pure prime pressed cocoa butter and soy lecithin from ADM Cocoa Limited (Koog aan de Zaan, Netherlands) and Unitechem Company Ltd. (Tianjin, China) respectively.

The recipe, formulation and production of samples have been described previously under Section 4.31 but limited only to products containing 35% fat and 0.5% lecithin. Chocolates were formulated with total fat of 35% (w/w) from sucrose, cocoa liquor, cocoa butter and lecithin. Experimental samples (5 kg batch for each formulation) were produced by mixing sucrose (40.8%) and cocoa liquor (53.7%) in a Crypto Peerless Mixer (Model K175, Crypto Peerless Ltd, Birmingham, UK) at low speed for 2 min and then at high for 3 min, then using a 3-roll refiner (Model SDX 600, Buhler Ltd., CH-9240 Uzwil, Switzerland) to a specified particle size (D_{90} :18 ± 1 μm, 25 ± 1 μm, 35 ± 1 μm & 50 ± 1 μm) conducting particle size analysis, during refining, to ensure D_{90} values. The refined chocolates were melted at 50 - 55 °C for 24 h and the

chocolate mass conched in a Lipp Conche (Model IMC-E10, Boveristr 40-42, D-68309, Mannhein, Germany) at low speed for 3.5 h at 60 °C. Lecithin (0.5%) and cocoa butter (5%) were added and then conched at high speed for 30 min to effect adequate mixing and liquefaction. Samples were kept in sealed plastic containers at ambient (20 - 22 °C) and moisture and fat contents determined using Karl Fischer and Soxhlet methods (ICA, 1988) and (ICA, 1990).

7.3.2 Determination of particle size distribution

A MasterSizer® Laser Diffraction Particle Size Analyzer equipped with MS 15 Sample Presentation Unit (Refractive index 1.590) (Malvern Instrument Ltd., Malvern, England) was used. About 0.2 g of refined dark chocolate was dispersed in vegetable oil (Refractive index 1.450) at ambient temperature (20 ± 2 °C) until an obscuration of 0.2 was obtained. The sample was placed under ultrasonic dispersion for 2 min to ensure particles were independently dispersed and thereafter maintained by stirring during the measurement. Size distribution was quantified as the relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Micro Software v 2.19). PSD parameters obtained included specific surface area, largest particle size (D_{90}), mean particle volume (D_{50}), smallest particle size (D_{10}) and Sauter mean diameter (D[3,2]).

7.3.3 Tempering experiment

Samples were incubated at 50 °C for 4 h for melting and tempered using Aasted Mikrovert laboratory continuous three-stage tempering unit (Model AMK 10, Aasted Mikroverk A/S, Farum, Denmark). Chocolate was pumped through the multi-stage units and a worm screw drove the product through the heat exchangers. Sensors located at specific points in the

equipment measured the temperature of both the chocolate and the coolant fluid at each stage. Based on our earlier work on modelling temperature controls to study tempering behaviour (Afoakwa et al., 2008b), the temperature of each of the coolant fluids (Zones 1: 2: 3) were thus set as 26: 24: 32 °C respectively for attaining the under-tempered regime. The degree of pre-crystallization was measured using a computerized tempermeter (Exotherm 7400, Systech Analytics, Neuchâtel, Switzerland) and a built-in algorithm provided the tempering curves and temper readings in chocolate temper index (Slope), corresponding to under-temper (Slope 1.0). The principle of this method has been described by Nelson (1999). Chocolates were moulded using plastic moulds: 80 mm length; 20 mm breadth; and 8 mm height, allowed to cool in a refrigerator (12 °C) for 2 h before de-moulding onto plastic trays. Bloom was induced by storing the products under ambient condition (18 ± 2 °C, RH 50%), and samples evaluated on cooling and after every 24 h in storage until reaching asymptotic values. Triplicate measurements were conducted and the mean values recorded.

7.3.4 Texture measurements

Hardness of products were measured using TA-HD Plus Texture Analyzer with a penetration probe (needle P/2) attached to an extension bar and a 50 kg load cell and a platform as reported by Afoakwa et al. (2008c). Maximum penetration forces through a sample (80 x 20 mm, depth 8 mm) was determined with 8 replications at a pre-speed of 1.0 mm/s, test of 2.0 mm/s, post speed of 10.0 mm/s, penetrating 6 mm at 20°C, converting mean values of the penetration force exerted by the 50 kg load cell into hardness (g) using XT.RA Dimension, Exponent 32 software (Stable Micro Systems, Godalming, Surrey, UK) as shown on Figure 4.3b.

7.3.5 Surface colour and gloss measurements

HunterLab Miniscan^{TM} XE Colorimeter Model 45/0 LAV (Hunter Associates Inc., Reston, VA) calibrated with white ceramic reference standard was used. Colour images of chocolate surfaces were converted into XYZ tristimulus values, which were further converted to CIELAB system: L*, luminance ranging from 0 (black) to 100 (white); and a* (green to red) and b* (blue to yellow) with values from -120 to +120. Mean surface whiteness (L*-values) from 5 replicate measurements and standard deviations were reported.

Gloss of chocolate surface was measured using the multiple angle Tricor Gloss meter (805A/806H Gloss System, Elgin, IL). Reflectance was measured at an incidence light angle of 85° from the normal to the chocolate surface, in accordance with ASTM method D523. A polished black glass plate with a refractive index of 1.567 was used as standard surface and given a gloss value of 200 (ASTM, 1995). Gloss was reported as gloss units (GU) based on determinations (in triplicate) at six positions along a chocolate sample. As a reference, a surface with a gloss value less than 10 GU is considered a low gloss surface (BYK, 1997; Briones, Aguilera & Brown, 2006).

7.3.6 Determination of melting properties

Differential scanning calorimeter (DSC Series 7, Perkin Elmer Pyris, Norwalk, CT, USA) equipped with a thermal analysis data station was calibrated using indium and octadecane at a scan rate of 5 °C/min using an aluminium pan as reference. Samples (~ 5 mg) were loaded into 40 µl capacity pans with holes, which were sealed with lids using a sample press. Pans were heated at 5 °C/min from 15-55 °C in a N_2 stream. Onset temperature (T_{onset}), peak temperature (T_{peak}), end temperature (T_{end}) and enthalpy of melting (ΔH_{melt}) were calculated automatically by

the software. Each sample was analysed in triplicate and mean values and standard deviations reported.

7.3.7 Microstructural determinations

Chocolate samples were characterized using stereoscopic binocular microscope (Nikon, SMZ-2T, Tokyo, Japan) equipped with a variable removable lens. Micrographs (coloured images) were captured using a digital camera (Model 2.1 Rev 1, Polaroid Corporation, NY, USA) and observed using Adobe Photoshop (Version CS2, Adobe Systems Inc. NJ, USA). Triplicate experiments were conducted capturing 6 images per sample, and micrographs representing the surface of samples during storage were captured and presented. Samples of products containing 50 μm were also sectioned (cut) into two pieces after every 24 h during blooming using a knife and the internal microstructures observed.

7.3.8 Experimental design and statistical analysis

Two experimental variables comprising storage time (on cooling until reaching asymptotic levels) and PSD (D_{90}): 18, 25, 35 and 50 μm were used. Other variables including refiner temperature and pressure, conching time and temperature were held constant. Statgraphics Plus 4.1 (Graphics Software System, STCC, Inc, Rockville, USA) examined textural properties (hardness) and appearance (L* and gloss) and melting properties (T_{onset}, T_{end}, ΔH_{melt}) using two-way analysis of variance (ANOVA), and multiple comparison tests to determine effects of factors and their interactions. Tukey multiple comparisons (95% significance level) determined differences between levels. All experiments were conducted in triplicates and the mean values reported.

7.4 RESULTS AND DISCUSSION

7.4.1 Particle size distribution (PSD) of dark chocolates

These findings (Fig. 4.1), previously reported under Section 4.4.1, show volume histograms consisting of narrow (18 μm PS) and wide (25 μm PS) bimodal and narrow (35 μm PS), and wide (50 μm PS) multimodal size distributions. This PSD range 18 - 50 μm using D_{90} values (>90% finer) covers optimum minimum and maximum sizes with direct effects on texture and sensory character in chocolate manufacture (Ziegler & Hogg, 1999; Beckett, 2008). Data from the PSD as previously described (Afoakwa et al., 2008a) showed variations in specific surface area, mean particle volume D(v,50), Sauter mean (D[3,2]) and mean particle diameter (D[4,3]) with increasing D_{90} particle sizes. Specific surface area (SSA) was inversely correlated with the different components of PSD. Similar inverse relationships of SSA with all the other components of PSD have been reported (Ziegler & Hogg, 1999). Beckett (1999) concluded largest particle size and solids specific surface area are the two key parameters for chocolate manufacture. The former determines chocolate coarseness and textural character, the latter with desirable flow properties. Fat contents of the products were 35 ± 1% and moisture within the range of 0.90 - 0.98%.

7.4.2 Changes in textural properties during blooming

Changes in texture (hardness) in the under-tempered products with varying PS were investigated during storage to provide information on their rate of hardening during bloom development. Hardness showed an inverse relationship with particle sizes, prior to storage with the 18 μm sample showing the highest hardness (1081.24 g) and the 50 μm sample the least (1008.75 g) (Fig. 7.1), attributed mainly to the relative strengths of the particle-to-particle interactions within the different particulate structures of products containing different PSD

(Campos et al., 2002; Do et al., 2007; Afoakwa et al., 2008b). These values revealed that the under-tempered products on cooling were very soft, compared to hardness values of 5318.23 g and 4259.48 g for 18 and 50 μm PS respectively noted for optimally-tempered dark chocolates as previously reported (Afoakwa et al., 2008d).

Storage of the products caused consistent and significant ($p<0.05$) increases of ~ 4-fold in hardness levels within the first 72 h (Table 7.1), within which period over 90% of the textural changes that occurred during the bloom development of the products were noted at all particle sizes, before attaining asymptotic levels where only gradual increases were noted. Products with the largest PS (50 μm) showed the fastest increase in hardness levels within 72 h of storage, followed by the 35 μm PS, and then 25 μm PS, with those containing 18 μm PS showing the least increases (Fig. 7.1), an indication that the rate of structural changes during blooming are directly related to the magnitude of product particle sizes (D_{90}).

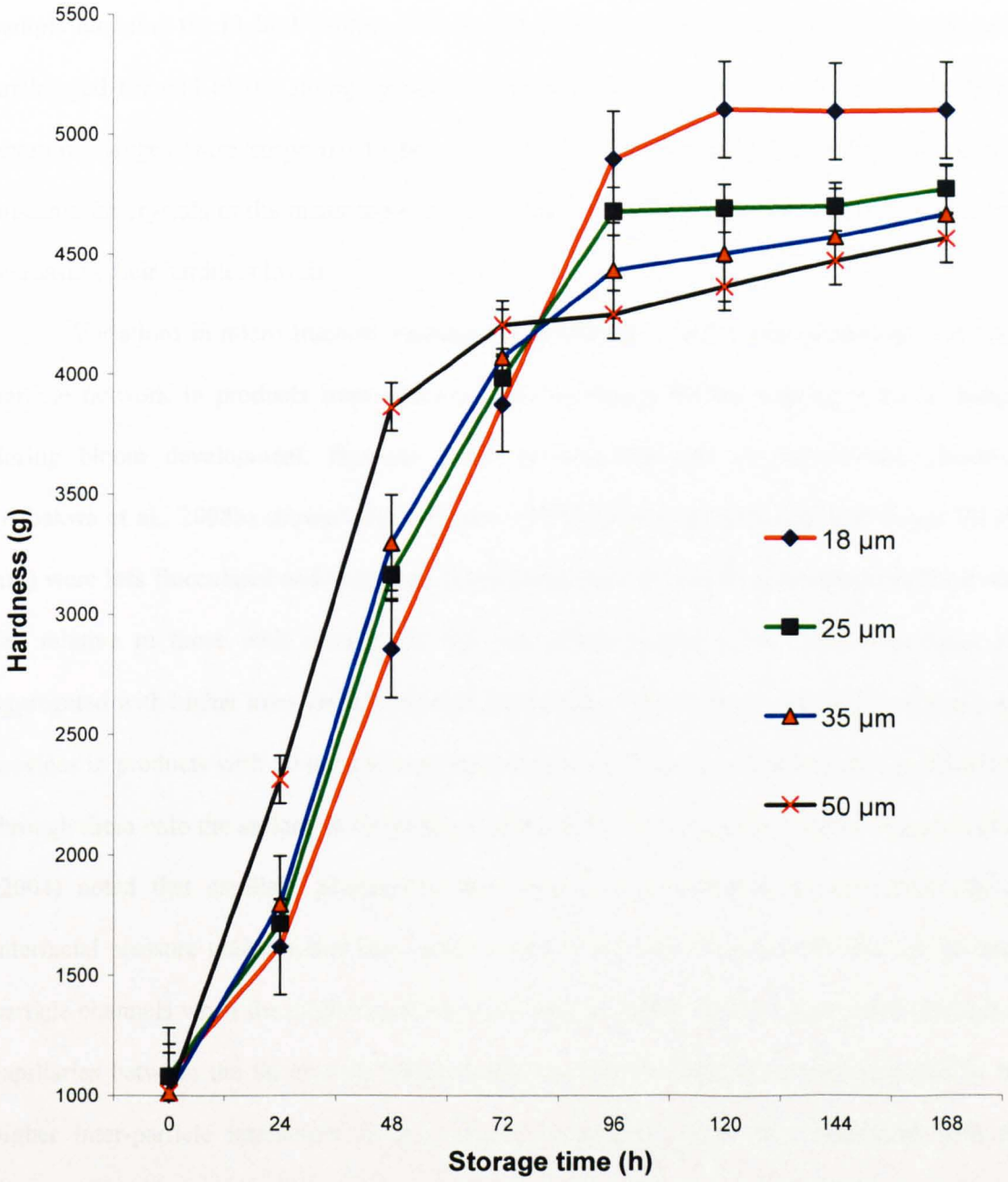


Figure 7.1. Changes in hardness during blooming of dark chocolates

However, this hardening trend was reversed between 72 h and 96 h in storage with the 18 μm PS sample attaining the highest hardness values and the 50 μm PS the least after 96 h, remaining unchanged the end of the storage period where asymptotic values were noted. The observed textural changes were suspected to be caused by the re-structuring and re-crystallisation of unstable fat crystals in the under-tempered chocolates inducing blooming in products, and thus increasing their hardness levels.

Variations in microstructure regarding the structural arrangements of particles and inter-particle network in products from different PSD accounted for the varying textural changes during bloom development. Previous work on microstructure of molten dark chocolate (Afoakwa et al., 2008b) showed that at higher (35%) fat content, products with larger PS (50 μm) were less flocculated and contained larger pores and crevices between particles filled with fat, relative to those with smaller PS (18 μm) which tended to be more flocculated and aggregated with higher inter-particle network interaction. The presence of these larger pores and crevices in products with 50 μm PS were suspected to facilitate movement of re-crystallised fat through them onto the surface of the product, suspected to be by capillary action. Aguilera et al. (2004) noted that capillary penetration into pores is a spontaneous process driven by an interfacial pressure gradient, and may occur at two pore scales in chocolates: that of the inter-particle channels when the migrating mass is the total fat phase (liquid and crystals) and that of capillaries between the fat crystals for the liquid fat. The flocculated network provided by the higher inter-particle interaction in the 18 μm PS product might have interfered with the migration of re-crystallised fat onto the product surface and consequently reducing their rate of bloom development.

Table 7.1. ANOVA Summary of F-values of texture, whiteness, gloss and melting properties

Process variables	Hardness	Whiteness (L*)	Gloss	T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)
A : Particle size (D_{90})	11.80*	134.78***	51.73***	0.55	11.41***	0.65	10.85**
B : Storage time	198.49***	2673***	3312.39***	429.73***	1227.66***	707.84***	53.47***
A x B	3.75*	45.04***	9.54***	0.91	5.72***	0.72	6.59**

* Significant F-ratios at *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

7.4.3 Changes in appearance (surface whiteness and gloss) during blooming

Significant ($p<0.001$) and linear effects on surface whiteness (L*) were recorded with increasing storage time, noticeable at all particle sizes (Table 7.1). Prior to storage, whiteness of products varied between 42.26 for 50 μm PS and 45.54 for the 18 μm PS, suggesting that products with smaller PS (18 μm) appear lighter, decreasing consistently with increasing PS. This confirmed previous findings that chocolates with varying particle sizes differ in structure and particulate arrangements influencing light scattering coefficients and thus appearance (Afoakwa et al., 2008c). Generally, storage induced blooming in products causing initial rapid increases in whiteness until reaching asymptotic levels after 96 h, trends observed in all particle sizes (Fig. 7.2). Increases in whiteness from 45.54-82.46 and 42.26-87.62 were noted in the 18 μm and 50 μm PS products respectively, showing that over 95% of the change in whiteness occurring during blooming of products took place within 96 h after processing, rendering the products surface whitish in appearance. Similar increases in whiteness in fat bloomed chocolates have been reported (Lonchampt & Hartel, 2004; Lonchampt & Hartel, 2006; Altimiras et al., 2007). Hartel (1999) attributed this phenomenon to re-crystallisation of fats, causing changes in light scattering and dispersion effects on small surface fat crystals ($\geq$ 5μm), consequently impacting on their appearance attributes.

Contrariwise, gloss of products showed significant ($p<0.001$) and inverse effects with increasing storage time at all particle sizes (Table 7.1). Gloss of under-tempered dark chocolates prior to storage showed slightly reducing levels as D_{90} increased from 18-50 μm with values of 146.6-130.4 GU respectively, explaining that differences in particulate arrangements in dark chocolate structure influence final product gloss after tempering.

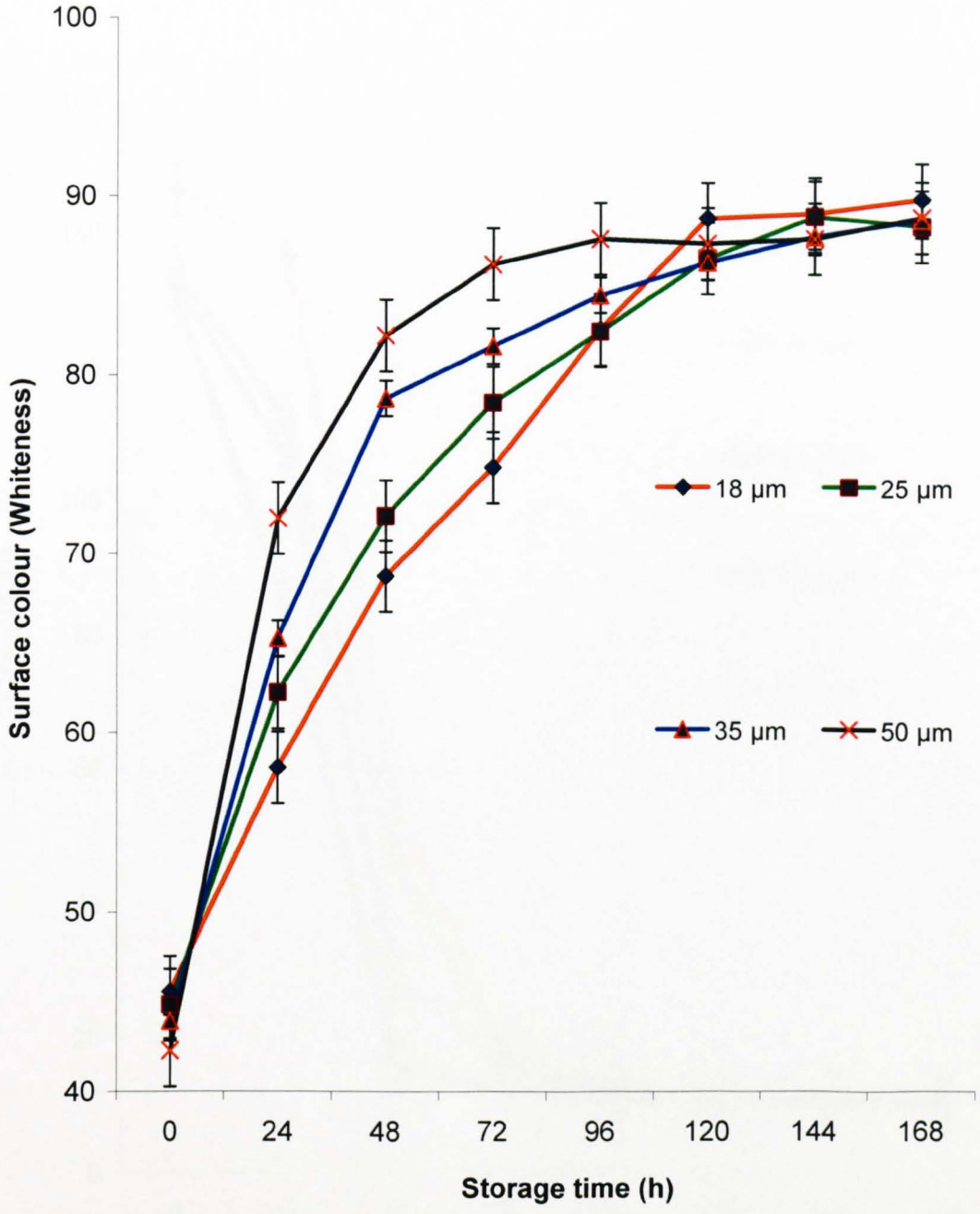


Figure 7.2. Changes in surface whiteness during blooming of dark chocolates

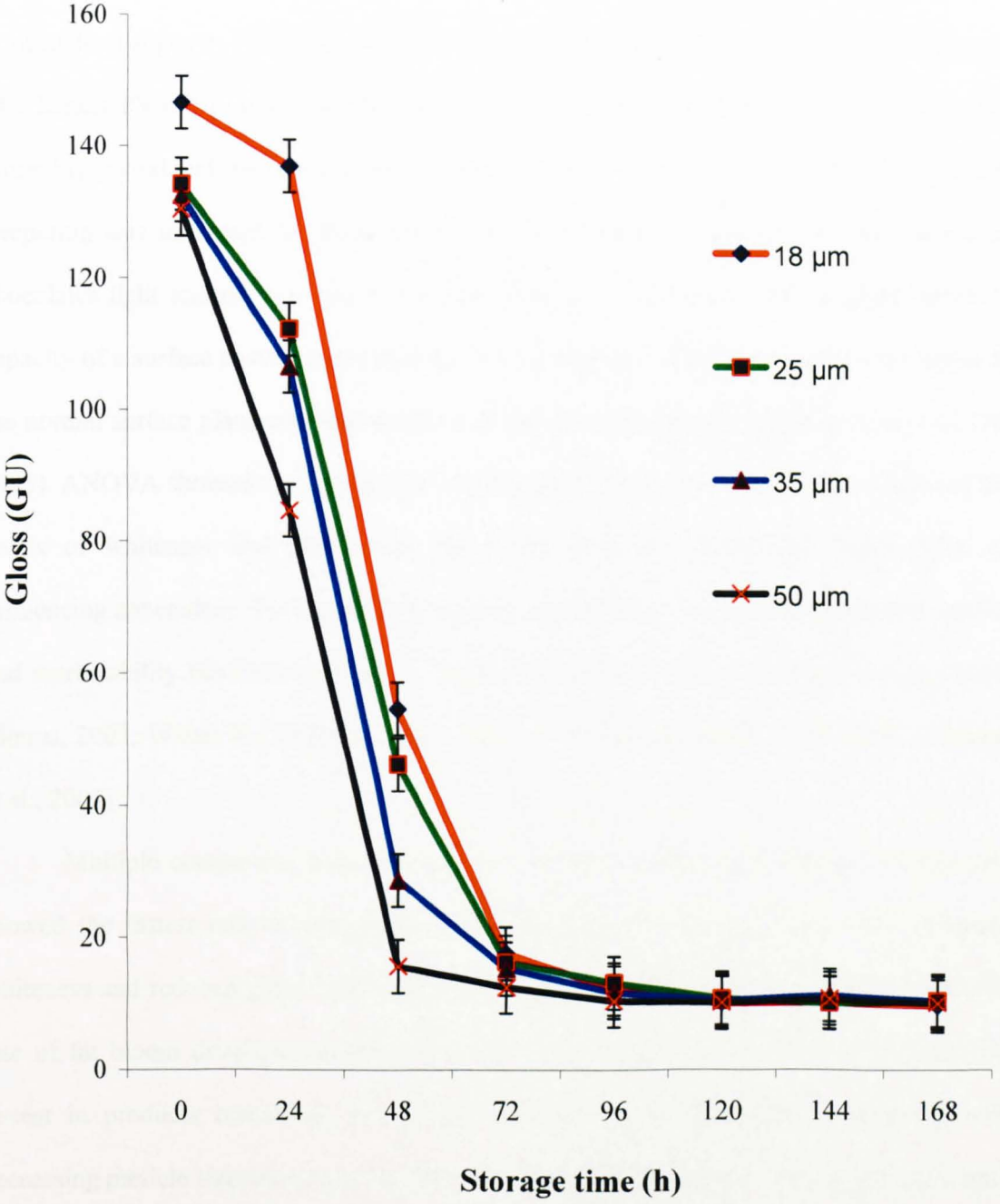


Figure 7.3. Changes in gloss during blooming of dark chocolates

Storage of the products induced blooming within 24 h causing drastic consistent reduction in gloss until 96 h in storage where decreases in gradient were observed (Fig. 7.3). No noticeable changes in gloss occurred after 96 h and this trend was noted in all particle sizes. The largest PS (50 μm) showed the fastest rate of reduction in gloss within the 96 h fast blooming period and the smallest PS (18 μm) the least (Fig. 7.3). Beckett (2008) noted that tempering was important for gloss, a key quality attribute in chocolate. In under-tempered chocolates light scattering is caused by reductions in surface regularity, as gloss relates to capacity of a surface to reflect directed light at the specular reflectance angle with respect to the normal surface plane and any interference on this plane influences gloss levels (ASTM, 1995). ANOVA showed that both PS and storage time significantly ($p=0.001$) influenced the levels of whiteness and gloss, with significant ($p\leq0.05$) interactions (Table 7.3), all influencing appearance. Fat bloom development and effects on chocolate appearance, quality and marketability has been extensively studied (Bricknell & Hartel, 1998; Ali et al., 2001; Timms, 2003; Walter & Cornillon, 2001, 2002; Lonchampt & Hartel, 2004, 2006; Altimiras et al., 2007).

Multiple comparison tests revealed that products containing the largest PS (50 μm) showed the fastest rate of change on surface appearance with regards to both increased whiteness and reduced gloss, whilst those with smallest PS (18 μm) showed the least. The rate of fat bloom development in under-tempered dark chocolate found in this study was fastest in products containing the largest (50 μm) PS, which tended to decrease with decreasing particle size with the smallest PS (18 μm) showing the slowest blooming rate until 96 h when no further changes in appearance occurred. These suggested that bloom development in under-tempered dark chocolates is caused by hydrodynamic forces exerted on the liquid fat content of unstable fat crystals, forcing its movement under capillary action through inter-particle passages and connected pores onto the product surface. The relatively

larger capillary pores created within the particulate structures of products containing 50 μm PS facilitated their rate of bloom development, relative to the smaller pores of their respective smaller PS products (Afoakwa et al., 2008b). Beckett (2008) noted that fat bloom may occur due to insufficient formation of stable polymorphs (Form V) in cocoa butter during tempering leaving a liquid fraction that is propelled to the surface, particularly if the chocolate have cracks and crevices. These findings confirm predictions from capillary theory that higher migration rates would occur through bigger capillaries (at short times), reaching asymptotic levels with long storage periods (Aguilera et al., 2004). Contrary to these findings, Altimiras et al. (2007) reported that the rate of fat bloom development was fastest in chocolate with smaller particle size as compared to those with medium and larger particle sizes and attributed the effects to Brownian motion. However, Genovese et al. (2007) explained that three kinds of forces coexist to various degrees in flowing dispersions: Brownian, colloidal and hydrodynamic forces, and their relative magnitude of bulk flow depend on the particle sizes within the products. Brownian motion and inter-particle forces equilibrate for sub-nanometer-size dispersion (1 nm-10 μm), while hydrodynamic forces dominate in particles between 10-100 μm, such as chocolate, sauces and fruit purees. For such particles as in chocolate, Brownian motion and inter-particle forces are negligible compared to hydrodynamic forces, thus defeating Brownian motion as the resultant force during structural-fat migration relationships in chocolates. These differences in findings require further studies into the microstructural changes occurring during bloom development in products to clarify the blooming rates and associated mechanisms.

Multivariate regression and correlation analyses were conducted between changes in texture, surface whiteness and gloss to help establish the structural-appearance relationships during blooming of under-tempered dark chocolates. The output showed the results of fitting

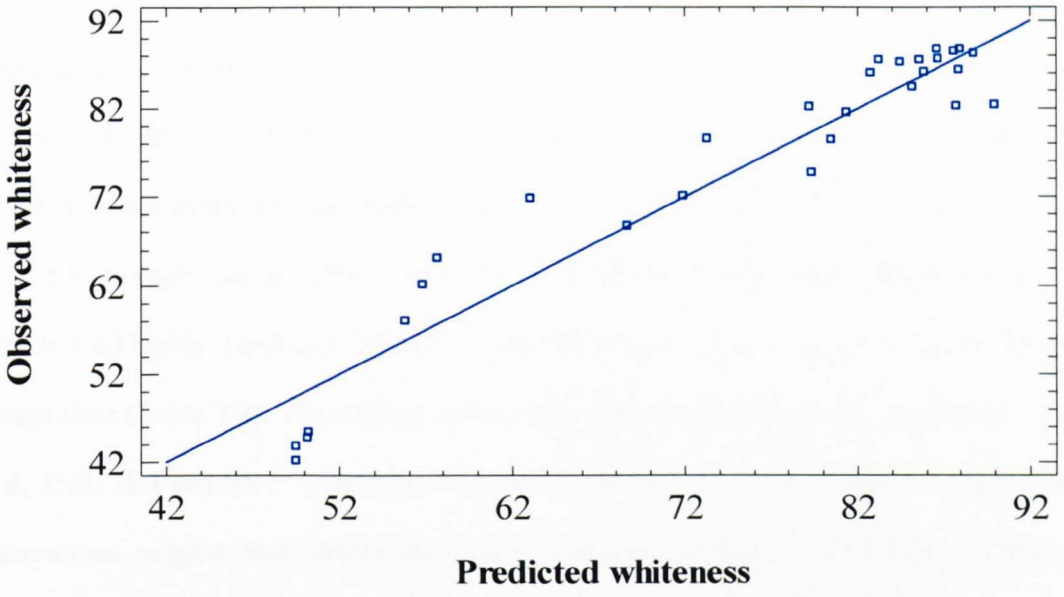
linear models to describe the relationship between surface whiteness and hardness, and gloss and hardness during blooming are as follows:

Whiteness = 38.9304 + 0.0104*Hardness ..... Eq. 7.1

Gloss = 166.534 - 0.0342*Hardness .....Eq. 7.2

Good fit of the models was confirmed graphically by scatter plots, in which high regression coefficient of determination, R^2 = 94.0%, (p = 0.001) for whiteness and hardness with very high correlation coefficient, R = 0.96 (p = 0.001), and also R^2 = 92.5% (p = 0.001) for gloss and hardness with very high correlation coefficient R = -0.97 (p = 0.001) were found between the predicted and experimental values (Fig. 7.4). These explained that during bloom development in under-tempered dark chocolates, changes in textural properties (hardness) could be used to predict the rate of change in surface whiteness and gloss reduction (blooming) in products. These developments are important in bringing greater understanding on structure-appearance relationships during blooming of dark chocolates and would be useful for further studies on the prevention of fat bloom in chocolates.

a.



b.

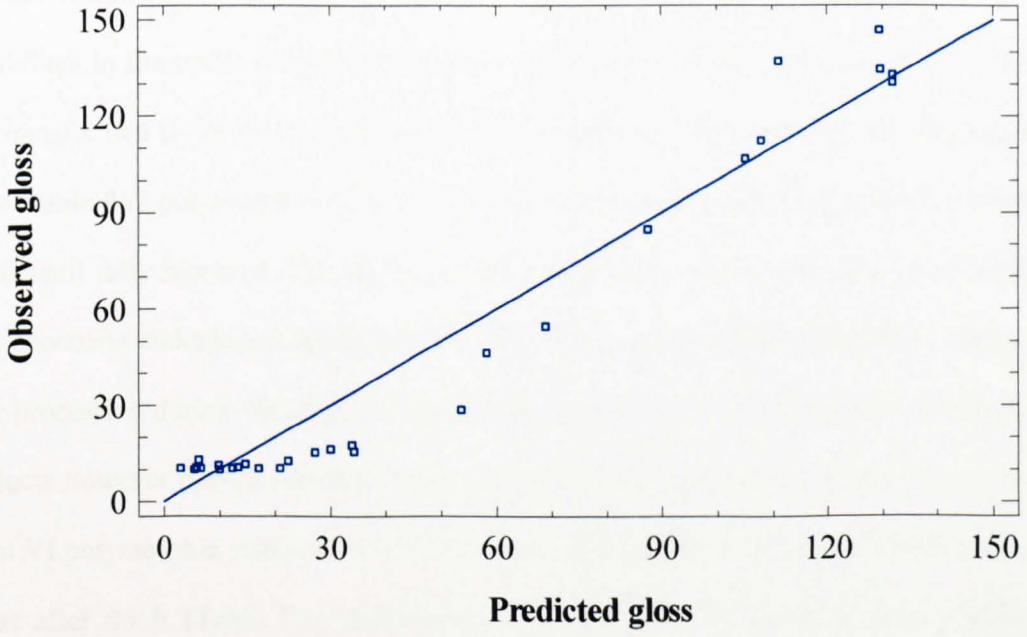


Figure 7.4. Scatter plots of (a) observed and predicted whiteness (b) observed and predicted gloss with changes in hardness during blooming of dark chocolates

7.4.4 Changes in melting behaviour during blooming

Typical DSC thermograms used for characterising the melting properties of the under-tempered dark chocolates during blooming are as shown in Figure 7.5. All the samples exhibited similar distinct single endothermic transitions between 15 and 55 °C, the range expected for chocolate melting profiles. Data from the DSC on T_{onset}, T_{end}, T_{peak}, and ΔH_{melt} in relation to storage time and PSD (Table 7.2) analysed by ANOVA and multiple comparison tests showed highly significant differences ($p<0.001$) T_{onset}, T_{peak}, T_{end} and ΔH_{melt} for differing storage time (Table 7.2). The differences in storage time yielded mean T_{end} values of ~ 28.7, 32.4, 33.0, 35.3 and 35.7 °C respectively after 0, 24, 48, 72 and 96 hours in storage. These observations suggest that bloom development in under-tempered chocolate significantly influences their melting temperatures as a result of polymorphic transformations of fat crystals within the products. The changing T_{end} values of the samples revealed that the crystallites in the under-tempered products were in ßIV polymorph (~28.7 °C) on cooling, and transformed to ßV (~32.4 °C) within 24 h in storage, with further transformation to the more stable ßVI polymorph (~35.5°C) after 72 h in storage, stabilising at that polymorphic status until fully bloomed. This finding is very significant as it reveals that under-tempered dark chocolate undergoes a three-stage (β'_1 - β_2 - β_1) polymorphic transformation within 72 h after processing during which period fat bloom occurs (Fig. 7.5). Particle size distribution in products however played significant role as products containing 25, 35 and 50 µm reached Form VI polymorphic status after 72 h, those with the smallest PS (18 µm) attained Form VI status after 96 h (Table 7.2) influencing T_{end} and ΔH_{melt} of products during blooming. Similarly, the blooming process influenced the melting enthalpies (ΔH_{melt}) during storage (Table 7.2), with significant ($p<0.05$) interactions with PS (Table 7.1).

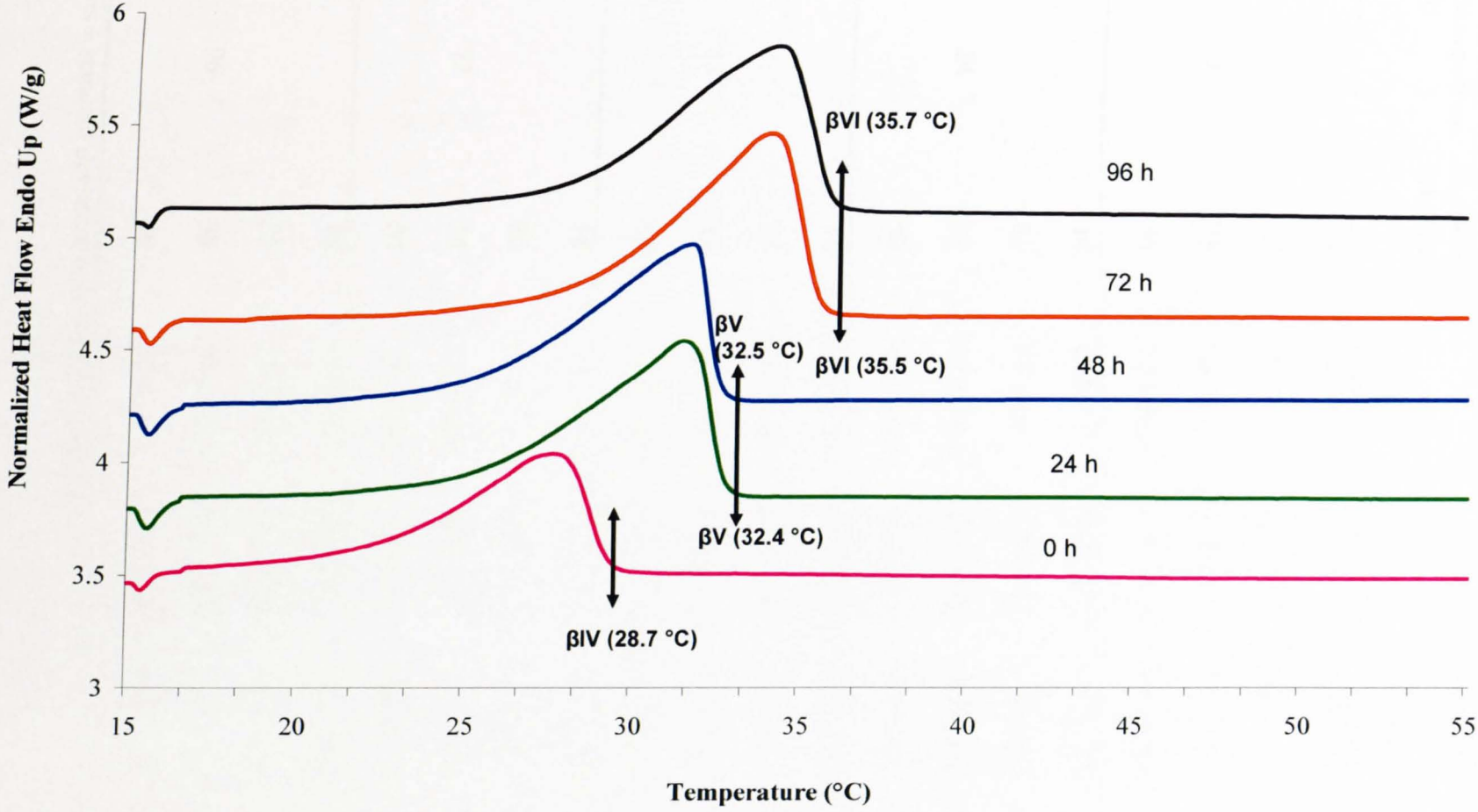


Figure 7.5. Typical DSC thermograms showing changes in fat melting profile during blooming of dark chocolates with 25 µm PS

Table 7.2. Changes in melting properties during storage

Storage time (h)	Particle size (D_{90}) (μm)	Melting properties			
		T_{onset} (°C)	T_{end} (°C)	T_{peak} (°C)	ΔH_{melt} (J/g)
0	18	22.8 ± 0.3	28.9 ± 0.3	27.2 ± 0.2	35.66 ± 0.42
	25	22.9 ± 0.3	28.7 ± 0.2	27.4 ± 0.1	34.74 ± 0.39
	35	22.9 ± 0.4	28.6 ± 0.2	27.6 ± 0.2	34.56 ± 0.25
	50	22.8 ± 0.4	28.6 ± 0.4	27.4 ± 0.2	34.72 ± 0.63
24	18	25.1 ± 0.4	32.3 ± 0.3	31.7 ± 0.1	36.76 ± 0.68
	25	25.4 ± 0.3	32.4 ± 0.2	31.8 ± 0.2	37.17 ± 0.71
	35	25.3 ± 0.2	32.5 ± 0.4	31.8 ± 0.1	37.09 ± 0.43
	50	25.5 ± 0.4	32.4 ± 0.3	31.9 ± 0.2	38.26 ± 0.78
48	18	26.1 ± 0.2	32.3 ± 0.3	31.6 ± 0.1	37.72 ± 0.42
	25	26.2 ± 0.3	32.5 ± 0.2	31.4 ± 0.2	37.23 ± 0.38
	35	26.4 ± 0.4	33.2 ± 0.3	31.9 ± 0.1	38.31 ± 0.45
	50	26.5 ± 0.3	33.8 ± 0.4	32.3 ± 0.2	38.82 ± 0.48
72	18	27.7 ± 0.4	33.6 ± 0.3	32.1 ± 0.2	38.36 ± 0.27
	25	27.3 ± 0.2	35.5 ± 0.4	33.2 ± 0.1	39.27 ± 0.54
	35	27.6 ± 0.2	35.8 ± 0.3	33.6 ± 0.2	40.41 ± 0.04
	50	27.8 ± 0.2	36.4 ± 0.4	33.8 ± 0.1	40.37 ± 0.47
96	18	27.4 ± 0.2	35.2 ± 0.3	33.8 ± 0.2	40.63 ± 0.52
	25	27.7 ± 0.4	35.7 ± 0.2	33.7 ± 0.1	40.36 ± 0.43
	35	27.7 ± 0.2	35.8 ± 0.4	33.6 ± 0.2	41.07 ± 0.26
	50	27.9 ± 0.3	36.3 ± 0.3	33.7 ± 0.1	41.82 ± 0.75

Means ± standard deviation from triplicate analysis

Multiple comparison tests showed that ΔH_{melt} of products measured soon after tempering were relatively lower, and increased with increasing storage time, attributable to the relative strengths of the re-crystallised fat network formed during blooming of products, thereby requiring higher enthalpies for melting.

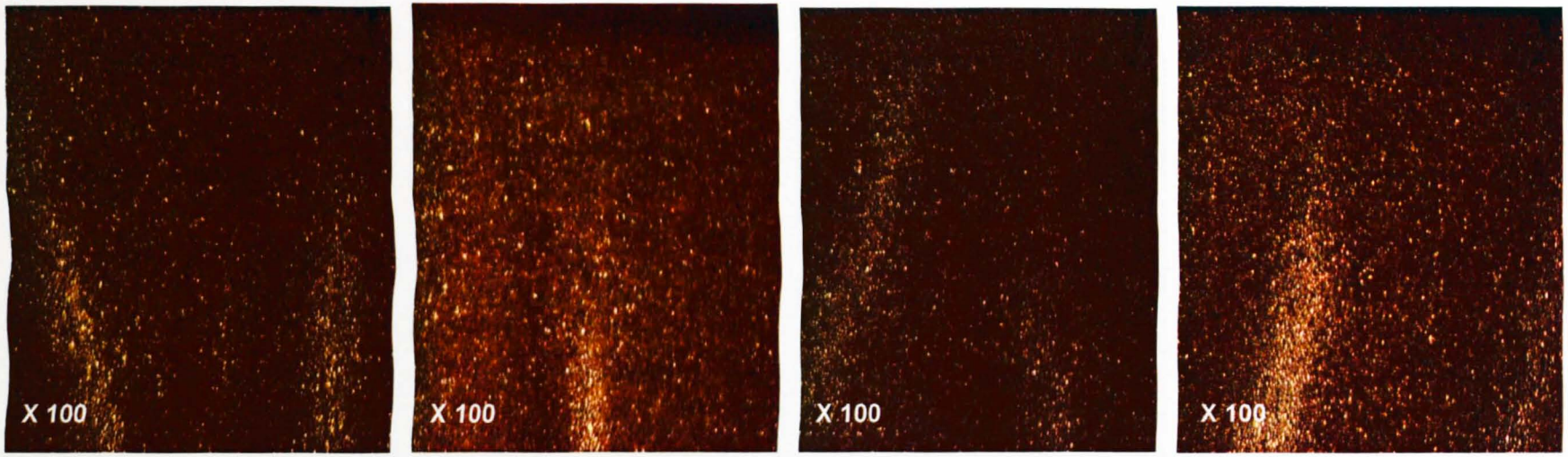
7.4.5 Changes in microstructure during blooming

Microstructural examination using stereoscopic binocular microscopy captured soon after cooling showed similarity in structure on the surface image of products with varying PS (Fig. 7.6). Storage of the products induced bloom development within 24 h with the the release and appearance of a colourless fluid and small spots of whitish haze suspected to be liquid and re-crystallised fat respectively onto the product surfaces. These were more obvious on products containing 50 μm, with the 35, 25 and 18 μm showing only little apparent surface changes (Fig. 7.6b). Bomba (1993) and Beckett (2008) stated that liquid fat originated from less stable crystals with lower melting points are likely to melt at fairly modest temperatures during chocolate blooming and are separated from the crystal structure migrating to the product surfaces. After 48 h in storage, blooming was physically induced in all products with the appearance of whitish crystal structures on their surfaces, growing across high-low concentration gradients with storage time and eventually spreading throughout the product surface until 96 h where no further change on surface appearances were noticeable in all products, hence the conclusion that the products were fully bloomed. Microscopic images captured after 120-168 h in storage on products were not shown as they were similar to those presented for 96 h (Fig. 7.6).

The micrographs showed that the fastest rate of bloom was noticeable in samples containing 50 μm PS, followed by the 35 and 25 μm PS, with those with 18 μm PS showing the least rate of bloom development, confirming the reported relative rates of changes in

texture, whiteness and gloss during storage (Figs. 7.1 – 7.3). The relatively larger capillary pores created within the particulate structures of products containing 50 μm PS were suspected to be the key factor facilitating their rate of bloom development, relative to the smaller pores with decreasing PS in products. These differences were attributed to the variations in amounts of hydrodynamic forces exerted on the liquid fat content of the unstable fat crystals in the under-tempered chocolate, forcing them to move under capillary action through the inter-particle passages and connected pores onto the product surface. The unstable fat portion then re-crystallised during storage into more stable polymorphs initiating the physical appearance of bloom (whitish haze) in products and crystal growth was promoted by Ostwald ripening (re-crystallisation and re-distribution of larger crystals growing at the expense of smaller ones). Microstructures showing the internal periphery of the 50 μm samples revealed that during bloom development crystal growth were noted to be facilitated by mass movement of re-crystallised fat through high-low concentration gradient by diffusion as the re-crystallised fat re-distributes itself across the entire chocolate mass during storage. The surface and internal periphery of the fully bloomed products showed whitish crystals which appear to be mixtures of fat and sugar components of the product, and distinct smaller brown spots made up of cocoa solids (Figs. 7.6 & 7.7).

A.



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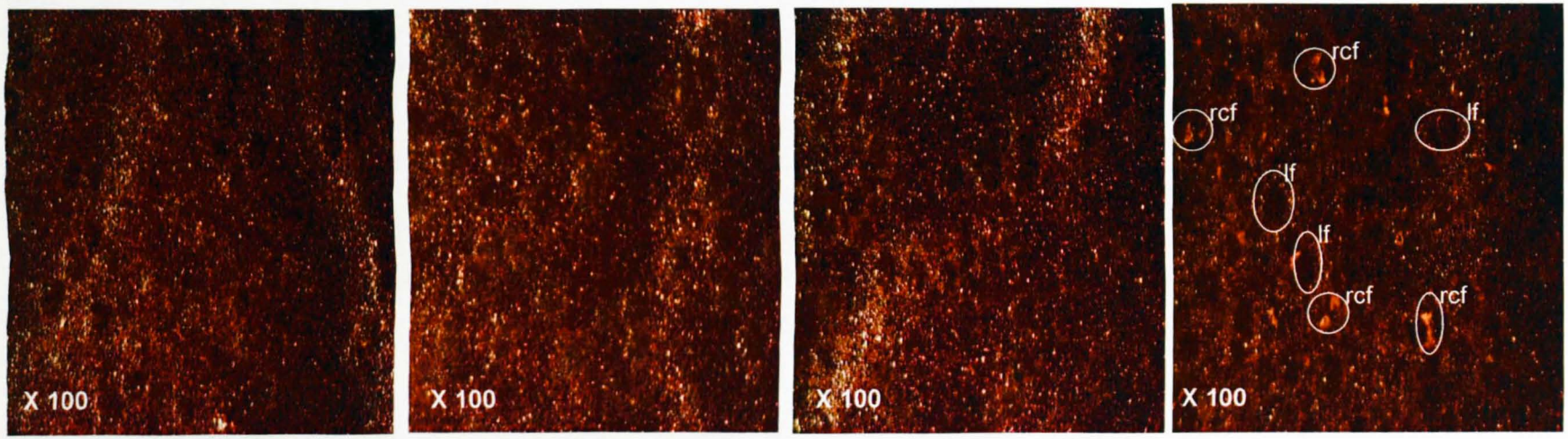
B.



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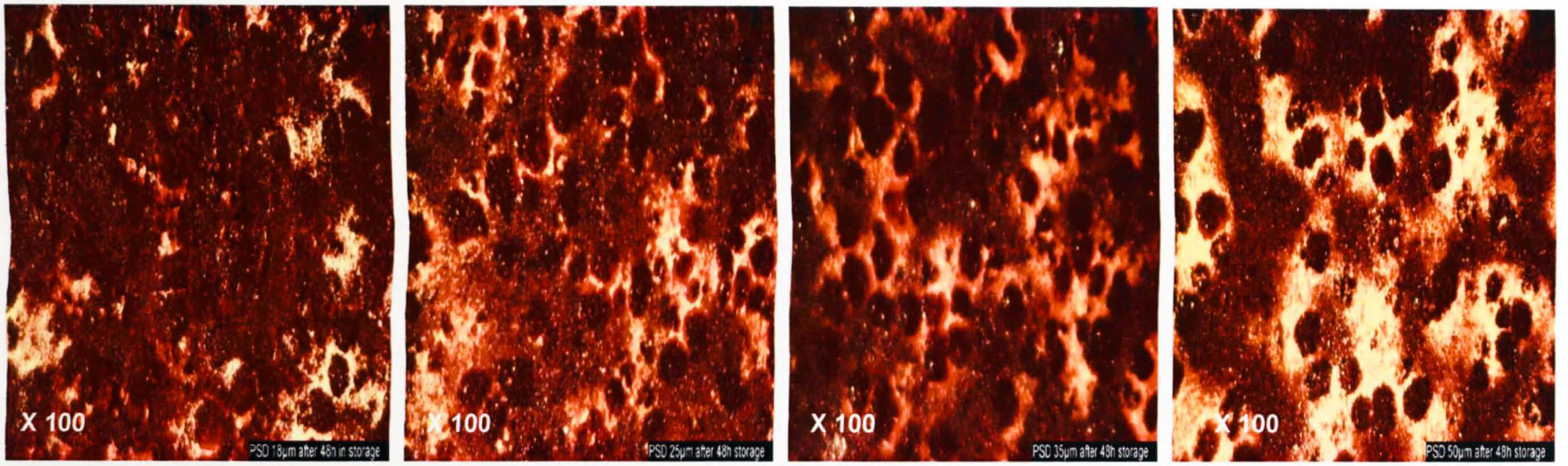
C.



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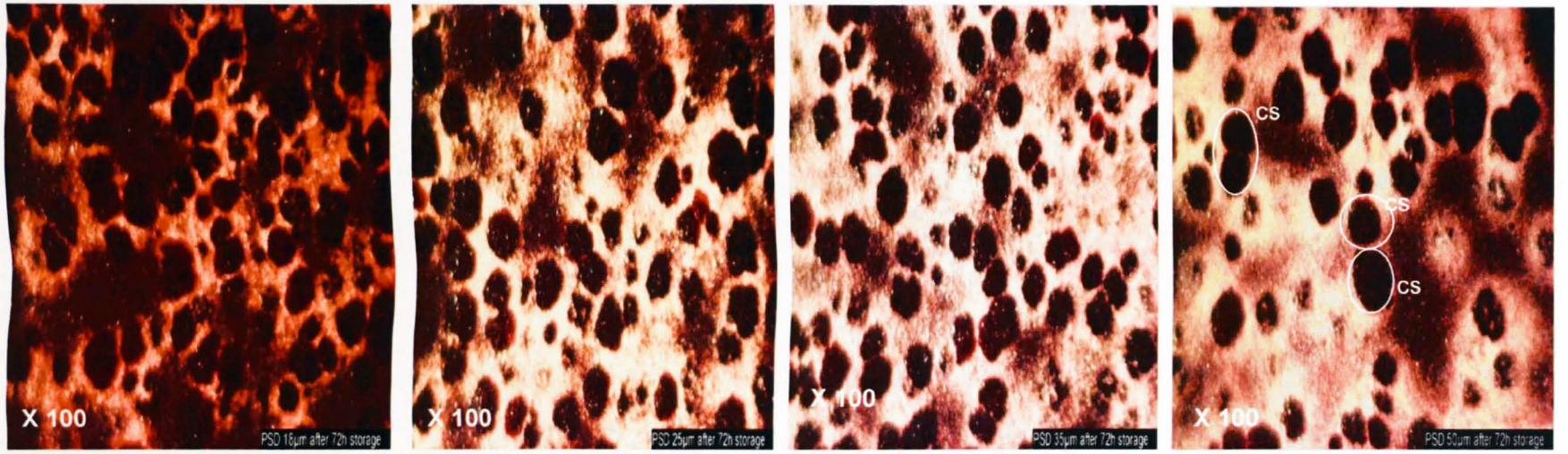
D.



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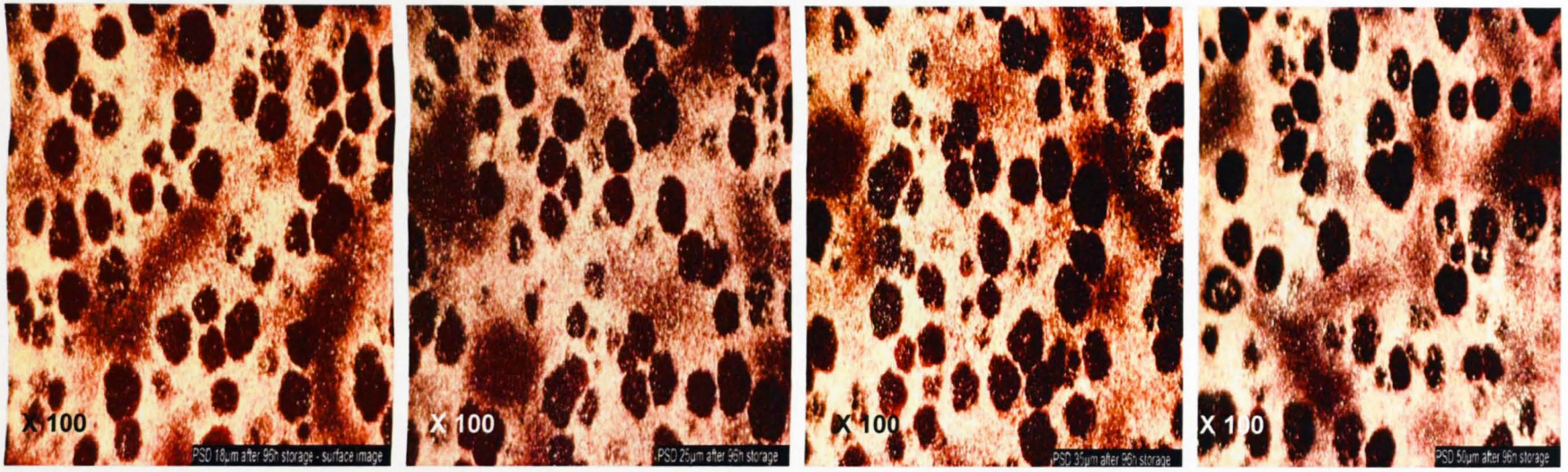
E.



i ii iii iv

Figure 7.6. Micrographs showing changes in surface appearance of dark chocolate with (i) 18 µm, (ii) 25 µm, (iii) 35 µm and (iv) 50 µm after (A) on cooling (0 h) (B) 24 h (C) 48 h (D) 72 h (E) 96 h in storage, showing liquid fat (lf), re-crystallised fat (rcf) and cocoa solids (cs)

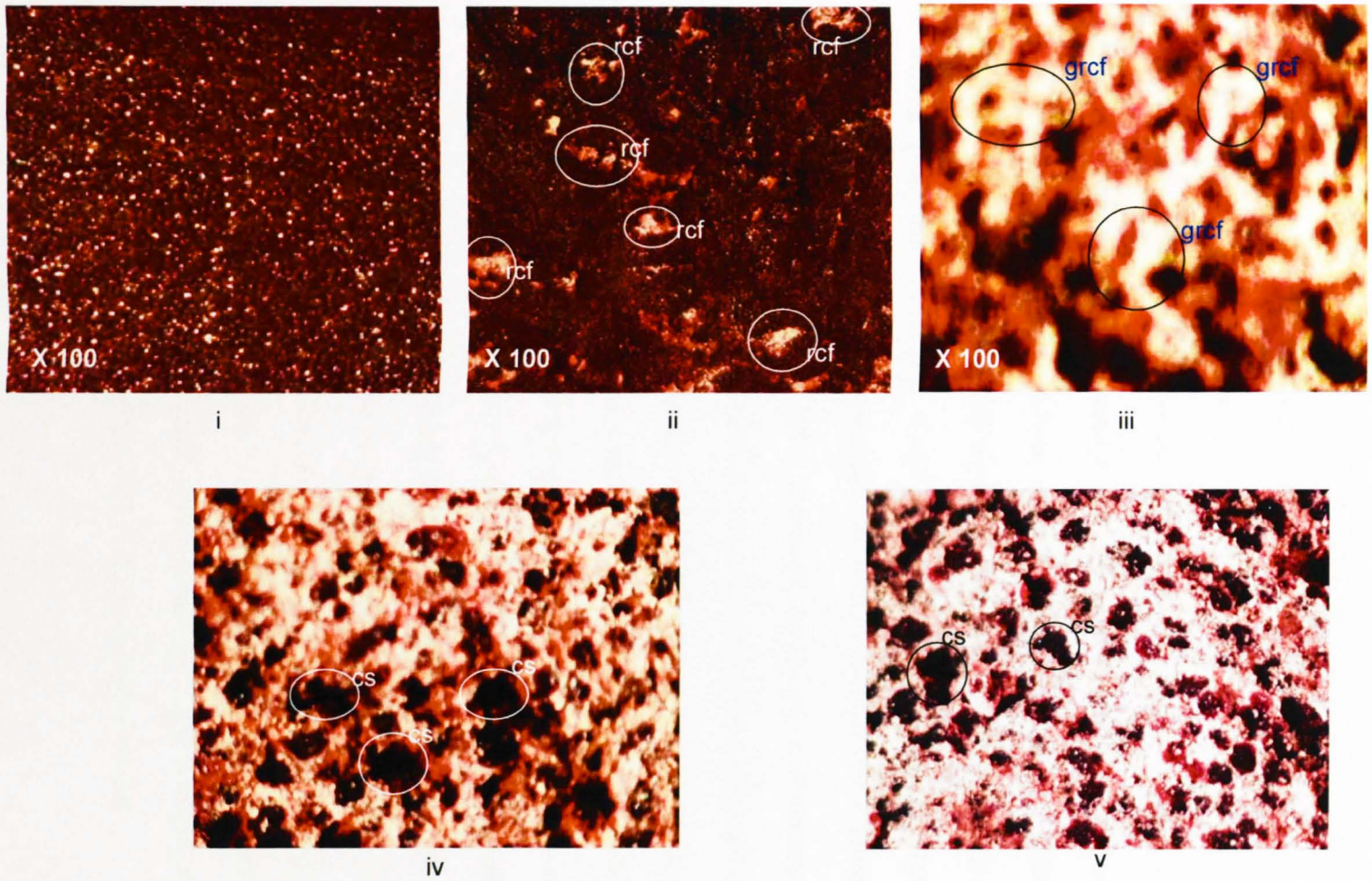


Figure 7.7. Micrographs showing changes in internal appearance of dark chocolate with 50 µm PS after (i) 0 h (ii) 24 h (iii) 48 h (iv) 72 h (v) 96 h in storage, showing liquid fat (lf), re-crystallised fat (rcf), growing re-crystallised fat (grcf) and cocoa solids (cs)

7.5 CONCLUSION

The rate of bloom development in under-tempered dark chocolate was dependent on solids particle size distribution and storage time. Hardness and surface whiteness showed initial rapid increases with parallel reductions in gloss in the first 96 h, with subsequent decreases in rate until asymptotic values were reached. Blooming was initiated in products within 24 h and essentially complete by 96 h. Changes during blooming were attributed primarily to growth of new fat crystals within the structural network with changes in light reflections yielding increases in surface whiteness and in hardness. From diferential scanning calorimetry on melting properties values for T_{onset}, T_{end}, T_{peak} and ΔH_{melt} suggested polymorphic transformation from βIV to βV within 24 h and further to βVI after 72 h. Micrographs showed similar crystal network structure and inter-particle interactions in chocolates of different PS immediately after tempering. Within 24 h, liquid and unstable re-crystallised fat had appeared on surfaces with initiation of bloom. Unstable fat re-crystallised during storage into more stable polymorphs and crystal growth was promoted by Ostwald ripening with the appearance of white crystalline structure that had spread gradually throughout entire chocolate masses after 96 h. Chocolate of largest PS (50 μm) showed most rapid fat bloom, smallest PS (18 μm) slowest, attributed mainly to hydrodynamic forces of capillary action. It was concluded that bloom development was initiated by movement of liquid and unstable fat onto product surfaces through capillarity created by hydrodynamic forces within the inter-particle pores and crevices, followed by growth of new fat crystals promoted by diffusion gradients across the mass until chocolate was fully bloomed. Understanding fat bloom formation and development in dark chocolate has potential applications in new product development.

CHAPTER 8

MATRIX EFFECTS ON FLAVOUR VOLATILES RELEASE IN DARK CHOCOLATES VARYING IN PARTICLE SIZE DISTRIBUTION AND FAT CONTENT USING GC-MASS SPECTROSCOPY AND GC-OLFACTOMETRY

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8.1 ABSTRACT

Influences of matrix particle size distribution (PSD) (18, 25, 35, 50 μm) and fat content (25, 30, 35%) on flavour release of dark chocolate volatiles were quantified by static headspace gas chromatography using GC-MS. Sixty-eight (68) flavour compounds were identified comprising: alcohols, aldehydes, esters, ketones, furans, pyrans, pyrazines, pyridines, pyroles, phenols, pyrones, and thiozoles. From GC-olfactometry 2-methylpropanal, 2-methylbutanal and 3-methylbutanal had chocolate notes. With cocoa/roasted/nutty notes were: trimethyl-, tetramethyl-, 2,3-dimethyl-, 2,5-dimethyl-, 3(or 2),5-dimethyl-2(or 3)-ethyl- and 3,5(or 6)-diethyl-2-methylpyrazine and furfuralpyrrole. Compounds with fruity/floral notes included: 3,7-dimethyl-1,6-octadien-3-ol, 5-ethenyltetrahydro-R,R,5-trimethyl-cis-2-furanmethanol. Caramel-like, sweet and honey notes were conferred by: 2-phenylethanol, phenylacetaldehyde, 2-phenylethylacetate, 2,3,5-trimethyl-6-ethylpyrazine, 2-carboxaldehyde-1H-pyrrole, furancarboxaldehyde, furfuryl alcohol and 2,5-dimethyl-4-hydroxy-3(2H)furanone. There were direct relationships between fat content and 3-methylbutanal and branched pyrazines but inverse with 2-phenylethanol, furfuryl alcohol, methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-H-pyrrole. Particle size influenced higher alcohols, aldehydes, esters, ketones and pyrazines concentrations at all fat contents. A multivariate product space suggested flavour effects of the interacting factors.

Keywords: Chocolate; cocoa; flavour release; pyrazines; acetic acid; GC-MS; GC-Olfactometry

8.2 INTRODUCTION

Flavour is central to acceptability in chocolate and is influenced not only by volatile aroma compounds but also by non-volatiles and behaviour of the continuous fat phase influencing release of volatiles into the mouth headspace and taste perception. Precursor composition depends on bean genotype and environmental effects particularly on contents of storage proteins and polyphenols (Kim & Keeney, 1984; Schwan & Wheals, 2004). Cocoa beans are rich in antioxidants - including catechins, epicatechin, and procyanidins - polyphenols similar to those found in wine and tea (Carnesecchia et al., 2002; Hatano et al., 2002; Grassi et al., 2005; Lamuela-Raventos et al., 2005; Hermann et al., 2006). Chocolate manufacture involves complex physical and chemical processes determining rheological characteristics, flavour development, melting properties and ultimately sensory perceptions of character (Ziegler & Hogg, 1999; Ziegler, Mongia & Hollander, 2001; Afoakwa et al., 2007; 2008a; Do et al., 2007). There are a number of studies of precursors for flavour formation in cocoa and chocolate (Misnawi et al., 2003; Counet et al., 2004; Kyi et al., 2005).

An appropriate cocoa bean composition can be converted through controlled post-harvest treatments and subsequent processing technologies to a high quality chocolate flavour character (Clapperton, 1994). Fermentation is crucial not only to the formation of key volatile fractions (alcohols, esters, and fatty acids) but also provision of Maillard flavour precursors (amino acids and reducing sugars) (Buyukpamukcu et al., 2001; Luna et al., 2002; Kyi et al., 2005). Drying reduces levels of acidity and astringency in cocoa nibs decreasing volatile acids and total polyphenols. Maillard reactions during roasting convert these flavour precursors into two main classes of flavour-active component - pyrazines and aldehydes (Gill et al., 1984; Oberparleiter & Ziegleder, 1997; Dimick & Hoskin, 1999; Stark et al., 2005; Ramli et al.,

2006; Granvogl et al., 2006). Flavour development continues during conching following the elimination of volatile acids, and moisture with associated viscosity changes due to emulsification and tannin oxidation (Mermet et al., 1992; Plumas et al., 1996; Reineccius, 2006). Afoakwa et al. (2008f) reviewed relationships between initial composition and post-harvest treatments of cocoa beans and subsequent processing (roasting and conching) and technological effects on final flavour character in chocolate.

Particle size distribution influences dark chocolate structure - specifically inter-particle interactions and network microstructure, rheology and texture - with specific surface area and mean particle size influencing yield stress, plastic viscosity, product spread and hardness (Chevalley, 1999; Beckett, 2008; Afoakwa et al., 2008a, 2008b; 2008c). Genovese et al. (2007) suggested that non-hydrodynamic parameters such as particle shape, particle size and size distribution, particle deformability, and liquid polarity influence food structure and flow behaviours. Such factors dictate the space dimension of a suspension, whether strongly or weakly flocculated, with influence on yield stress and plastic viscosity. Although key flavour compounds of milk and dark chocolates have been reported (Cerny, & Fay, 1995; Schnermann & Schieberle, 1997; Schieberle & Pfnuer, 1999; Counet et al., 2002; Taylor, 2002; Taylor & Roberts, 2004; Reineccius, 2006), their abundancy, release and contribution to product character and matrix effects remains unclear.

Modern healthier foods – less fat and low sugar products require modifications in ingredients and recipe formulation with impacts on flavour release and product rheology, structure and texture. Knowledge of how variations in PSD and continuous phase fat content would influence flavour would be useful for product development and manufacture. The objectives of this study were to characterise and quantify volatile flavour constituents in dark

chocolates, and to evaluate matrix effects from varying PSD and fat content on release of flavour volatiles using headspace HRGC, identifying components with GC-MS and flavour notes by GC- Olfactometry.

8.3 MATERIALS AND METHODS

8.3.1 Materials

Cocoa liquor of Central West African Origin was obtained from Cargill Cocoa Processing Company (York, UK); sucrose (pure extra fine) from British Sugar Company (Peterborough, UK); pure prime pressed cocoa butter and soy lecithin from ADM Cocoa Limited (Koog aan de Zaan, Netherlands) and Unitechem Company Ltd. (Tianjin, China) respectively.

Recipe (Table 8.1) and sample formulations have been described previously (Afoakwa et al., 2008a). Chocolates were formulated with total fat of 25 - 35% (w/w) from cocoa liquor and cocoa butter with >34% total cocoa: composition as specified for dark chocolate (European Commission Directive, 2000; Codex Revised Standard, 2003). Sucrose and cocoa liquor (5 kg per formulation) were mixed in a Crypto Peerless Mixer (Model K175, Crypto Peerless Ltd, Birmingham, UK) at low speed for 2 min and then at high for 3 min, then using a 3-roll refiner (Model SDX 600, Buhler Ltd., CH-9240 Uzwil, Switzerland) to a specified particle size (D_{90} :18 ± 1 μm, 25 ± 1 μm, 35 ± 1 μm & 50 ± 1 μm) conducting particle size analysis, during refining, to ensure D_{90} values. Refined chocolate flakes were placed in plastic containers and conditioned at 50 - 55 °C for 24 h to ensure melting of fat prior to conching in a Lipp Conche (Model IMC-E10, Boveristr 40-42, D-68309, Mannhein, Germany) at low speed for 3.5 h at 60 °C.

Table 8.1. Recipes used for the formulation of the dark chocolate

Ingredient	25% Fat [% (w/w)]	30% Fat [% (w/w)]	35% Fat [% (w/w)]
Sucrose (%)	59.0	49.9	40.8
Cocoa liquor (%)	35.5	44.6	53.7
Cocoa butter (%)	5.0	5.0	5.0
Lecithin (%)	0.5	0.5	0.5

Lecithin and cocoa butter were added and mixtures conched at high speed for 30 min to effect adequate mixing and liquefaction. Samples were stored in sealed plastic containers at ambient temperature (20 - 22 °C) and moisture and fat contents determined using Karl Fischer and Soxhlet methods (ICA, 1988; 1990) respectively.

8.3.2 Tempering procedure

Samples were melted at 50 °C for 4 h and tempered using a continuous three-stage tempering unit (Model AMK 10, Aasted Mikroverk A/S, Farum, Denmark) pumping chocolate through multi-stage units with a worm screw driving product through heat exchangers. Sensors in equipment measured temperature of both chocolate and coolant fluid at each stage. Based on our previous work on modelling temperature controls to study tempering behaviour (Afoakwa et al., 2008d), the temperature of each of the coolant fluids were thus set and controlled independently to obtain a final chocolate at ~ 27 °C to promote crystal growth of the desired triacylglyceride fractions. Pre-crystallisation was measured using a computerised tempermeter (Exotherm 7400, Systech Analytics, SA, Switzerland) using a

built-in algorithm to ensure an optimal temper regime of Slope 0 ± 0.3 (Nelson, 1999). Tempered chocolate was formed using plastic moulds: 80 by 20 by 8 mm, allowed to cool at 12 °C for 2 h before de-moulding onto plastic trays and conditioned at 20 ± 2 °C for 14 days before analysis.

8.3.3 Determination of particle size distribution

A MasterSizer® Laser Diffraction Particle Size Analyzer equipped with MS 15 Sample Presentation Unit (Refractive index 1.590) (Malvern Instrument Ltd., Malvern, England) was used. About 0.2 g of refined dark chocolate was dispersed in vegetable oil (Refractive index 1.450) at ambient (20 ± 2 °C) until an obscuration of 0.2 was obtained. Samples were placed under ultrasonic dispersion for 2 min to ensure particles were independently dispersed and suspensions thereafter maintained by stirring. Size distribution was quantified as the relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Micro Software v 2.19). PSD parameters obtained included specific surface area, largest particle size (D_{90}), mean particle volume (D_{50}), smallest particle size (D_{10}) and Sauter mean diameter (D[3,2]).

8.3.4 Quantification of flavour volatiles by gas chromatography

Static headspace isolation of volatile compounds was performed using solid phase micro extraction (SPME) for 30 min at 55°C onto a polydimethylsiloxane-divinylbenzene, 65µm fibre (Supelco, Bellafonte, PA, USA). Chocolate (~ 4 g) was previously heated to 55°C and intermittently stirred for 60 min for headspace equilibration. Each experiment had a system control sample, made by stirring an empty vial under the same conditions. Volatile

compounds were desorbed (5 min) into the splitless injector (220°C) of an Agilent Technologies 6890N-5793 Network GC-MS system (Agilent Technologies, Santa Clara, CA, USA) and separated on a J&W 60m DB-Wax capillary column (0.22 mm i.d., 0.25 μm film thickness). The temperature program was: 5 min at 40°C; 3°C min^{-1} to 230°C; finally 15 min at 230°C. Compounds were fragmented using Electron-Impact ionisation (70 eV), with a source temperature of 200°C, a scan range of 30-300 amu and a scan rate of 5 s^{-1}. Components were identified based on comparison of mass spectra with those of spectral libraries NIST 05 and Wiley 7N Registry of GC Mass Spectral Data, (John Wiley, New York, USA).

8.3.5 Gas chromatography-olfactometry analytical conditions

The GC–O analyses were conducted using an Agilent Technologies (6890N Network Systems, CA, USA) with analyses as before diverting the effluent to a humidified sniffing port. Two chromatographic runs were assessed by two trained assessors (alternating for 20 min periods). Only matching descriptors for an aroma attribute were retained.

8.3.6 Experimental design and statistical analysis

Two experimental variables comprising PSD and fat contents were used with other variables including refiner temperature and pressure, conching time and temperature held constant. A 4 x 3 factorial experimental design was used with: PSD (D_{90}): 18, 25, 35 and 50 μm; fat: 25, 30 and 35% (w/w). Statgraphics Plus 4.1 (Graphics Software System, STCC, Inc, Rockville, USA) examined quantitative data using two-way analysis of variance (ANOVA) and multiple range tests to determine effects of factors and interactions. Multivariate techniques comprising principal component analysis and multiple regression analysis were

used to evaluate relationships between selected flavour volatiles obtained by quantification of GC–MS data and influential factors. Tukey multiple comparisons at 95% significance level were conducted to determine differences between factor levels. All process treatments and analysis were conducted in duplicates and the mean values reported.

8.4 Results and Discussion

8.4.1 Particle size distribution of dark chocolates

Particle size distributions (Figure 4.1), previously reported show volume histograms consisting of narrow (18 μm PS) and wide (25 μm PS) bimodal and narrow (35 μm PS), and wide (50 μm PS) multimodal size distributions. This PSD range 18 - 50 μm using D_{90} values (>90% finer) covers optimum minimum and maximum sizes with direct effects on texture and sensory character in manufacture (Ziegler & Hogg, 1999; Beckett, 2008). Data from PSD showed variations in specific surface area, mean particle volume D(v,50), Sauter mean (D[3,2]) and mean particle diameter (D[4,3]) with increasing D_{90} particle sizes. Beckett (1999) concluded largest particle size and solids specific surface area were two key parameters in manufacture. The former determines chocolate coarseness and textural character, the latter desirable flow properties. Specific surface area was inversely correlated with the different component of PSD (Beckett, 1999; Ziegler & Hogg, 1999; Sokmen & Gunes, 2006). Fat contents were 25, 30 and 35 ± 1% (each) and moisture in the range 0.90 - 0.98%.

8.4.2 Characterisation of flavour compounds in dark chocolates

Criteria for selection of the key volatiles were presence in headspaces at $>10^6$ abundant units) quantified by GC–MS and also detection and intensities by the GC-

Olfactometric techniques. In all, 68 flavour compounds (Table 8.2) comprising nitrogen and oxygen heterocycles, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles, and sulphides were identified by GC-MS in dark chocolates. A typical chromatogram is as shown in Figure 7.1.

Compounds quantified included: 1-pentanol (1), 3-(methylthiol)-propionaldehyde (12), methylbenzene (38), methylpyrazine (41), ethenylpyrazine (47), pyridine (55), 2-methylpyridine (56), 1-(2-furanylmethyl)-1H-pyrrole (62), 1H-indole (63) and dimethyl disulphide (67) (Table 7.2). Two others, benzyl alcohol (5) and dihydro-2-methyl-3(2H)-furanone (30) were only recently reported in dark chocolates (Counet et al., 2002).

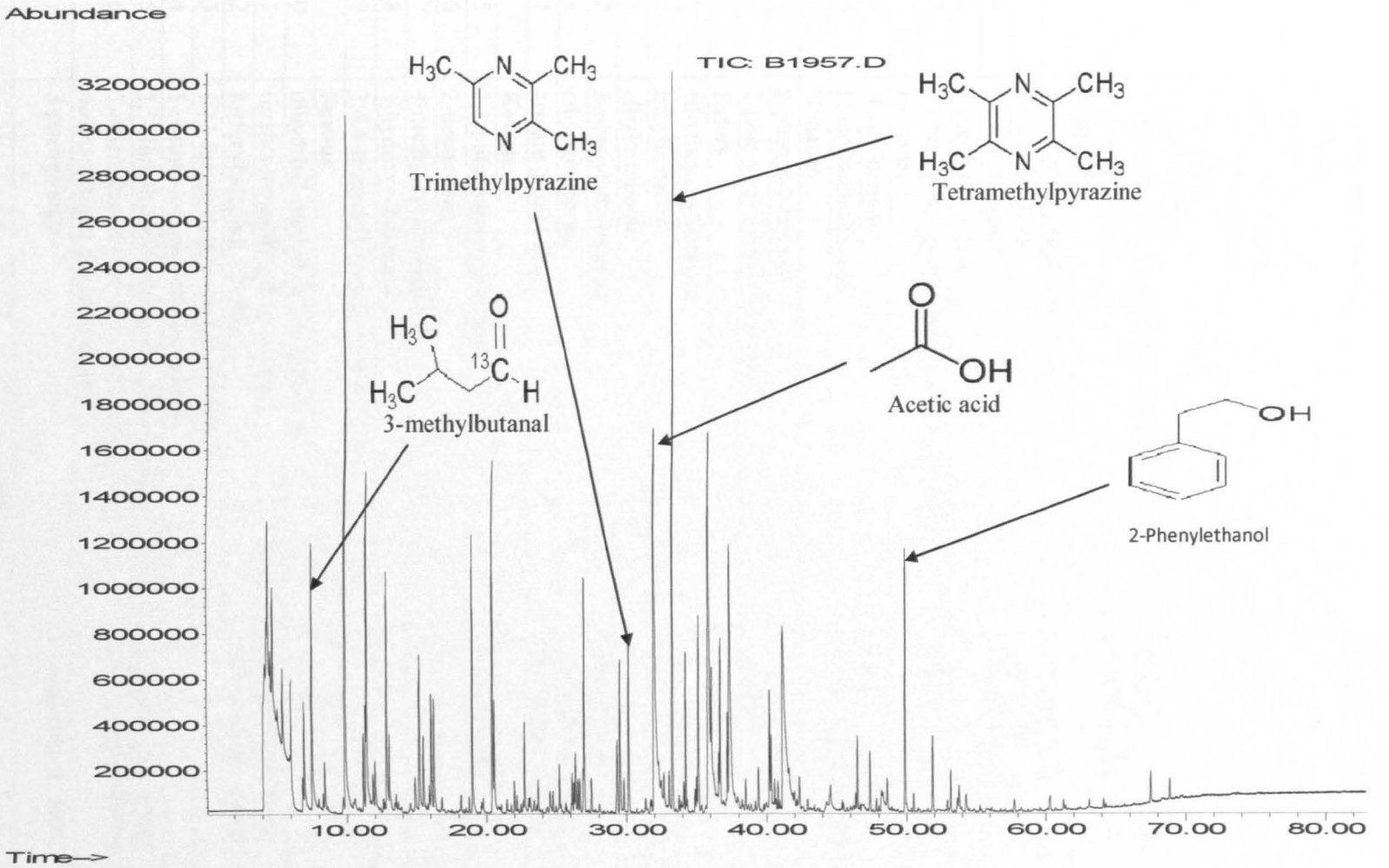

Figure 8.1. Typical GC-MS chromatogram used to identify flavour volatiles

Table 8.2. Key flavour volatiles identified in dark chocolate

no.	Flavour compound	Odour description[a]
	Alcohols	
1	1-pentanol	
2	2,4-hexadien-1-ol	
3	3-methyl pentanol	
4	2-heptanol	
5	benzyl alcohol	
6	3,7-dimethyl-1,6-octadien-3-ol (*linalool)*	*flowery, floral, fruity* (low)
7	2-phenylethanol	*caramel-like, sweet, honey*
	Aldehydes	
8	2-methylpropanal (*isobutanal)*	*chocolate*
9	2-methylbutanal	*chocolate*
10	3-methylbutanal	*chocolate*
11	2-methyl-2-butenal	
12	3-(methylthio)propionaldehyde (*methional)*	*potato*
13	Heptanal	
14	Benzaldehyde	*nutty*
15	Phenylacetaldehyde	*flowery, sweet, honey*
16	Nonanal	
17	2-phenyl-2-butenal	*cocoa, roasted*
18	2-phenyl-5-methyl-2-hexenal	*roasted*
	Esters	
19	ethylbenzoylformate	
20	Ethylbenzoate	
21	ethyloctanoate	
22	2-phenylethylacetate	*honey, sweet*
23	ethyl cinnamate	*fruity, floral* (low)
24	acetate (acetic acid)	*astringent, vinegar*
	Ketones	
25	2,3-butanedione (*diacetyl)*	*buttery* (low)
26	2-heptanone	
27	4-methylcyclohexanone	
28	3-hydroxy-2-butanone	
29	3,4,4-trimethyl-2-cyclopenten-1-one	
	Furans	
30	dihydro-2-methyl-3(2*H)-furanone*	
31	furancarboxaldehyde (*furfural)*	*caramel-like, sweet*
32	furfuryl alcohol (*furfurol)*	*caramel-like, sweet*
33	1-(2-furanyl)ethanone (*acetylfuran)*	
34	5-methyl-2-furancarboxaldehyde	
35	2,5-dimethyl-4-hydroxy-3(2*H)furanone (Furaneol)*	*caramel-like, sweet*
36	5-ethenyltetrahydro-R,R,5-trimethyl-*cis-2-furanmethanol (linalool oxide)*	*fruity, floral/flowery* (low)
37	3-phenylfuran	*cocoa, green, nutty*

	Hydrocarbons	
38	Methylbenzene (*toluene*)	
	Nitrogen compounds	
39	Benzonitrile	
	Pyrans	
40	3,4-dihydro-8-hydroxy-3-methyl-1*H-2-benzopyran-1-one*	
	Pyrazines	
41	Methylpyrazine	*nutty, green*
42	2,5-dimethylpyrazine	*roasted, cooked*
43	2,6-dimethylpyrazine	*roasted, cooked*
44	Ethylpyrazine	*nutty, roasted*
45	2,3-dimethylpyrazine	*cooked, nutty*
46	2-ethyl-5(or 6)-methylpyrazine	*cocoa, roasted, green*
47	Trimethylpyrazine	*cocoa, roasted, cooked*
48	2-ethyl-3-methylpyrazine	*hazelnut, roasted*
49	2-ethenyl-6-methylpyrazine	*roasted, smoky*
50	3(or 2),5-dimethyl-2(or 3)-ethylpyrazine	
51	Tetramethylpyrazine	*milk-coffee, roasted,*
52	2,3-dimethyl-5-ethylpyrazine	*cocoa, chocolate*
53	3,5(or 6)-dimethyl-2-ethylpyrazine	*cocoa, praline, chocolate*
54	2,3,5-trimethyl-6-ethylpyrazine	*candy, sweet,*
	Pyridines	
55	pyridine	
56	2-methylpyridine	*caramel-like, sweet*
57	2-pyridinamine	
58	1-(2-pyridinyl)-1-propanone	
	Pyrroles	
59	2,3-dimethyl-1*H-pyrrole*	*cocoa, praline, chocolate*
60	2-carboxaldehyde-1*H-pyrrole*	*honey, candy* (low)
61	3-ethyl-2,5-dimethyl-1*H-pyrrole*	*cocoa, coffee*
62	1-(2-furanylmethyl)-1*H-pyrrole (furfurylpyrrole)*	*cocoa, roasted* (low)
63	1*H-indole*	*chocolate, green* (low)
	Phenols	
64	phenol	
65	4-methylphenol	
	Pyrones	
66	*3-hydroxy-2-methyl-4-pyrone (maltol)*	
	Sulphur compounds	
67	Dimethyl disulphide	*meaty* (low)
	Thiozoles	
68	4,5-dihydro-2-methylthiazole	

[a] Odour quality and intensity at GC-O outlet

Specific nitrogen heterocycles from Maillard reactions included: 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine (50), 3,5-(or 6)-diethyl-2-methylpyrazine (53), 2,3-dimethyl-1H-pyrrole (59), 3-ethyl-2,5-dimethyl-1H-pyrrole (61) and 10(2-furanylmethyl)-1H-pyrrole (furfurylpyrrole) (62) (Table 8.2). All had *cocoa, praline, chocolate* and *roasted* notes identified as important. The ethyl group in two pyrazine compounds suggests key roles for alanine and/or its Strecker aldehyde, acetaldehyde, in dark chocolate flavour (Cerny & Fay, 1995).

Flavour-active compounds identified as having strong *chocolate* characters included 2-methylpropanal (8), 2-methylbutanal (9) and 3-methylbutanal (10). Compounds derived from Maillard reactions were 2,3-dimethylpyrazine (45), 2,5-dimethylpyrazine (42), 2,6-dimethylpyrazine (43), trimethylpyrazine (47), tetramethylpyrazine (51), 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine (50), 3,5(or 6)-diethyl-2-methylpyrazine (53), and furfurylpyrrole (60) exhibiting cocoa/roasted/nutty/cooked notes. Counet et al. (2002) identified such flavour volatiles in dark chocolates after conching, suggesting these are formed during cocoa processing.

Volatiles such as 2-phenylethanol (7), phenylacetaldehyde (15) and 2-phenylethylacetate (22), 2,3,5-trimethyl-6-ethylpyrazine (54), 2-carboxaldehyde-1*H-pyrrole* (60) were characterised by *sweet, candy* and *honey* flavours. Furancarboxaldehyde *(furfural)* (31), furfuryl alcohol *(furfurol)* (32), 2,5-dimethyl-4-hydroxy-3(2*H)furanone (Furaneol)* (35) were also characterised by *caramel-like, sweet* and *honey* notes likely derivatives of Strecker degradation and caramelisation reactions developed during cocoa processing and transformed during chocolate flavour synthesis in conching (Cerny & Fay, 1995).

Eight heterocyclic compounds including 2,3-dimethylpyrazine (45), 2,5-dimethylpyrazine (42), 2,6-dimethylpyrazine (43), trimethylpyrazine (47), tetramethylpyrazine (51), 3(or 2),5-dimethyl-2-(3)-ethylpyrazine (50), 3,5(or 6)-diethyl-2-methylpyrazine (53) and 2,3,5-trimethyl-6-ethylpyrazine (54) were identified (Table 8.2). Characteristic key chocolate flavours such as *fruity*

and *floral* likely derived from cocoa were found in 3,7-dimethyl-1,6-octadien-3-ol *(linalool)* (6) and 5-ethenyltetrahydro-R,R,5-trimethyl-*cis-2*-furanmethanol *(linalool oxide)* (36). Ethyl cinnamate (23) and acetic acid (24), not previously reported important in dark chocolates were characterised by *fruity-spicy* and *astringent-vinegar* notes respectively. Tetramethylpyrazine (51), the most abundant flavour compound in dark chocolate, exhibited *milk coffee-roasted-cooked* notes, and trimethylpyrazine (47) had cocoa-*roasted-cooked* characters (Table 8.2).

8.4.3 Effects of particle size distribution (PSD) on flavour volatile release

Effects of PSD and fat content on the release of selected abundant ($>10^6$ units) flavour volatiles characterised by distinct aroma were evaluated using SPME-HRGC with GC-MS detection and reported (Tables 8.3 & 8.4). Data from ANOVA indicated that with the exception of 3,7-dimethyl-1,6-octadien-3-ol *(linalool)* and 2-carboxaldehyde-1-H-pyrrole ($p = 0.965$ and 0.854 respectively), increasing particle size (PS) caused significant reduction on the release of all selected compounds measured in the sample headspace with $p<0.001$ for 3-methylbutanal, 2-phenylethanol, furfuryl alcohol *(furfurol)*, acetic acid, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine; and $p<0.05$ for 2-phenylethylacetate, 2-methylbutanal and 5-ethenyltetrahydro-R,R,5-trimethyl-*cis-2*-furanmethanol *(linalool oxide)*, with significant interactions noted with fat content (Table 8.5).

The decreasing flavour volatiles release with increasing PS could be related to increased matrix retention through structural, rheological and textural differences (Afoakwa et al., 2008a, 2008b, 2008c). Beckett (2008) noted that movement of volatiles was related to an initial concentration gradient between phases, and refining (degree of particle sizes) in production may influence release during manufacture. Beckett (2008) also noted correlated compositional and sensory analyses showed differences in flavour profile with preference for lower PS (*thick* and

pasty) chocolates, rather than the higher PS (*thin* and *runny*). These suggest that dark chocolates with finer PS (18 and 25 μm) would release more *cocoa-chocolate-praline* and *caramel-like-sweet-honey* notes than those with larger PS (35 and 50 μm), predicting perceived differences in flavour with varying PS. The increase in surface area with deceasing PS (D_{90}) would be predicted to facilitate volatiles release. The lack of significant effects of 3,7-dimethyl-1,6-octadien-3-ol (*linalool)* and 2-carboxaldehyde-1-H-pyrrole ($p = 0.965$ and 0.854 respectively) would not be predicted to influence flavour character from headspace contents and odour intensities (Tables 8.3 – 8.4). Voltz and Beckett (1997) and Ziegler et al. (2001) reported that finer (smaller PS) chocolate tend to be sweeter in taste than coarser (larger PS) ones, attributed to relative crystals sizes and melting behaviour. Particle size influences perceptions of *creaminess* and flavour release in soft model systems (Kilcast & Clegg, 2002; Engelen et al., 2005; Engelen & Van der Bilt, 2008). Concentration of flavour volatiles in headspaces has been reported as a function of diffusion in the solid phase (Carr et al., 1996; Guinard & Marty, 1995; Engelen et al., 2003; Kersiene et al., 2008).

Table 8.3. Flavour volatiles in dark chocolates varying in PSD and fat content[a]

Volatile compound (Abundance x 10^6)	25%				30%				35%			
	18 μm	**25 μm**	**35 μm**	**50 μm**	**18 μm**	**25 μm**	**35 μm**	**50 μm**	**18 μm**	**25 μm**	**35 μm**	**50 μm**
3,7-dimethyl-1,6-octadien-3-ol *(linalool)*	1.93	1.74	1.24	1.03	2.07	1.72	1.37	0.99	2.66	1.91	1.85	1.26
2-phenylethanol	46.84	32.42	26.24	25.22	27.84	24.45	22.33	22.01	26.43	24.01	22.02	17.14
2-methylbutanal	2.64	2.24	2.02	2.01	2.92	2.24	2.14	1.99	3.04	2.23	2.25	1.99
3-methylbutanal	8.12	8.17	7.24	7.11	8.82	8.26	7.46	7.16	9.42	9.19	8.86	7.14
Phenylacetaldehyde	20.85	12.79	10.21	10.01	9.78	9.44	7.99	7.86	6.70	6.35	6.37	3.99
2-phenylethylacetate	11.89	8.75	7.09	6.82	6.74	6.08	5.86	5.56	6.14	6.01	5.80	5.32
furfuryl alcohol *(furfurol)*	4.29	3.21	2.44	2.24	4.08	2.84	2.17	2.05	2.89	2.67	1.99	1.82
5-ethenyltetrahydro-R,R,5-trimethyl-*cis*-*2-furanmethanol (linalool oxide)*	5.58	5.22	4.63	4.60	5.45	5.05	4.73	4.52	5.87	5.32	5.16	5.01
2-carboxaldehyde-1-H-pyrrole	2.74	1.53	1.02	1.02	1.21	0.89	0.75	0.68	1.01	0.68	0.54	0.40
Acetic acid	130.43	78.64	58.83	56.08	112.36	65.26	48.76	42.01	30.77	28.74	28.07	27.15

[a]Quantification was by GC-MS expressed as mean peak area

Table 8.4. Abundant pyrazines in dark chocolates varying in PSD and fat content[a]

Volatile compound (Abundance x 10^6)	25%				30%				35%			
	18 μm	25 μm	35 μm	50 μm	18 μm	25 μm	35 μm	50 μm	18 μm	25 μm	35 μm	50 μm
Methylpyrazine	5.48	5.08	3.78	2.92	4.25	3.30	2.94	2.60	3.51	3.09	2.78	2.46
2,3-dimethylpyrazine	5.77	6.20	6.16	5.57	6.56	5.88	5.49	5.39	6.89	7.10	7.02	5.46
2,5-dimethylpyrazine	9.27	9.04	8.43	8.43	10.09	8.95	7.43	6.60	10.24	9.65	9.43	8.88
Trimethylpyrazine	28.63	28.87	28.60	28.54	29.50	29.10	28.81	28.80	33.14	32.78	32.05	30.42
Tetramethylpyrazine	109.61	105.37	96.69	96.43	98.89	96.88	96.79	96.05	112.61	107.17	106.82	96.87
2,3-diethyl-5-methylpyrazine	4.66	4.59	4.29	3.79	4.80	4.54	4.51	3.89	4.89	4.64	4.59	3.89
2,3,5-trimethyl-6-ethylpyrazine	3.86	3.05	2.68	1.93	2.25	2.11	1.82	1.78	1.89	1.90	1.78	1.56

[a]Quantification was by GC-MS expressed as mean peak area

8.4.4 Effects of fat content on flavour volatile release

Fat content influenced headspace concentration of volatiles independent of PSD (Table 8.3). Data from ANOVA showed 3,7-dimethyl-1,6-octadien-3-ol (*linalool),* 2-methylbutanal, 2-phenylethylacetate and 2-carboxaldehyde-1-H-pyrrole lacked significant effects ($p>0.05$). Fat content significantly influenced headspace concentrations of all other quantified volatiles ($p<0.001$) at all PSD with significant interactions among factors studied (Table 8.5). Volatiles characterised by *cocoa, chocolate, praline, fruity* and *roasted* notes included: trimethypyrazine, 3-methylbutanal, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, linalool-oxide and 2,3,5-triethyl-5-methylpyrazine. All showed a direct relationship with fat content at all PS (Tables 8.3 and 8.4). Volatiles release data suggested chocolates of higher fat content would exhibit greater release of components with *cocoa-chocolate-praline* notes than those with lower fat. This decreased matrix retention, could be related to differences in (micro)structure as inter-particle flocculation and aggregates are reduced with higher fat contents (Afoakwa et al., 2008b), releasing more Strecker degradation compounds with *cocoa-chocolate* notes. Concentrations of less volatile heterocyclic compounds were increased, notably polysubstituted ethyl- and isobutylpyrazines, tri- and tetramethylpyrazine, and furans (linalool oxide) suggesting structural and rheological effects major determinants of chocolate character (Do et al., 2007; Afoakwa et al., 2008a; 2008b).

Contrariwise, volatiles with *caramel-like, sweet, honey* and *candy* notes included: 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-H-pyrrole. All showed an inverse relationship with fat content at all PS (Tables 3 & 4), primarily due to lipophilic matrix–

flavour interactions. The major influence of fat content was observed with the most lipophilic compounds (Tables 8.3 and 8.4), particularly with fat contents above 25%. These results are consistent with earlier reports (Doyen et al., 2001; Jo & Ahn, 1999) and are also consistent with the suggestion that the more lipophilic the volatile, the less lipid is needed to reduce its headspace concentration (Roberts et al., 2003). More lipids generally reduce volatility of lipophilic components such as long-chain aldehydes and esters (Kersiene et al., 2008). Lack of a-significant effect on overall flavour character from 3,7-dimethyl-1,6-octadien-3-ol *(linalool)*, 2-methylbutanal, 2-phenylethylacetate and 2-carboxaldehyde-1-H-pyrrole would be predicted (Tables 8.2 and 8.3). Studies from emulsions showed that release of lipophilic compounds is decreased with limited amounts of lipid (Carey et al., 2002; Roberts et al., 2003). Factors such as lipophilicity or hydrophobicity of compounds could modulate the effect of fat content on release, specifically in confectionery (Barylko-Pikielna & Szczesniak 1994; Hyvönen et al., 2003), as well as mouth-feel (de Wijk et al., 2006) and thermal perceptions (Engelen et al., 2002).

A further key finding was related to headspace acetic acid contents in the products. The data showed very high values in dark chocolates containing 25% and 30% fat at lower (18 and 25 μm) PS, and inversely related to fat content. Greatest reduction in acetic acid (~ 4-fold) was noted with 35% fat at all PS, relative to similar products with 25% and 30% fat (Table 8.3). Similarly, increasing PS from 25 – 50 μm reduced contents by ~2-3 fold with 25% and 30% fat, whereas only minimal (5%) reduction were noted with 35% fat. From ANOVA there were highly significant effects of PSD and fat content ($p = 0.001$) on acetic acid release with significant interactions (Table 8.5).

Table 8.5. ANOVA summary showing F-values and regression coefficients of flavour compounds identified in dark chocolates with varying PSD and fat content

Volatile compound	PSD (D_{90}): A	Fat: B	Interaction s: A x B	R^{2b}
3,7-dimethyl-1,6-octadien-3-ol *(linalool)*	1.81	2.64	1.03	7.11
2-phenylethanol	1305.56 ***	1906.11***	265.68***	75.21***
2-methylbutanal	5.83*	0.48	4.76*	21.2
3-methylbutanal	32.36***	20.79***	27.92***	84.3***
Phenylacetaldehyde	8.62***	29.46 ***	10.28**	81.8***
2-phenylethylacetate	3.23*	1.67	3.28*	9.47
furfuryl alcohol *(furfurol)*	70.57***	19.16 ***	27.82***	87.7***
5-ethenyltetrahydro-R,R,5-trimethyl-*cis-2-furanmethanol (linalool oxide)*	4.89*	5.34*	5.71*	7.86
2-carboxaldehyde-1-H-pyrrole	1.15	1.77	0.95	17.9
Acetic acid	13.67***	26.31 ***	12.62***	75.0***
Methylpyrazine	30.15***	34.81 ***	28.63***	86.4***
2,3-dimethylpyrazine	8.93 ***	11.26 ***	10.56**	51.6*
2,5-dimethylpyrazine	15.62 ***	12.32 ***	18.63***	61.4*
Trimethylpyrazine	13.01***	795.09 ***	18.52***	81.9***
Tetramethylpyrazine	13.68***	17.20 ***	12.29***	51.0*
2,3-diethyl-5-methylpyrazine	312.88 ***	19.24***	48.78**	86.9***
2,3,5-trimethyl-6-ethylpyrazine	9.67 ***	29.59 ***	13.36**	76.6***

* Significant F-ratios at *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$; [b] R-squares from multiple regression

Acetic acid in 25 and 30% fat chocolate headspaces may be related to higher plastic viscosity and yield values (Afoakwa et al., 2008a), and greater flocculation and aggregation of inter-particle network structure (Afoakwa et al., 2008b), influencing release and volatilisation in conching. High acetic acid levels in low (25%) fat chocolates may reduce acceptability scores: effective elimination of volatile free fatty acids (e.g. acetic acid) and moisture during conching is crucial for development of final flavour character and texture in chocolates (Mermet et al., 1992; Pontillon, 1995; Plumas et al., 1996; Kealey et al., 2001; Beckett, 2008). As demand for healthier (low fat) chocolate has increased in recent years, good process optimisation to effect adequate release of acetic acid during manufacture of low (~25%) fat dark chocolates would be necessary to obtain well-balanced flavour characters.

8.4.5 Relating flavour volatiles release to PSD and fat content: product spaces

Multivariate principal component analysis (PCA) generated a product space exploring influence of PSD and fat content on headspace volatiles data on dark chocolates. The PCA space (Fig. 8.2) explained >91% variance in two factors, and showed two flavour volatiles clusters with loadings for PSD and fat content as influential factors. Fat content had polar influences on PC1 (65.2% variance) score while particle size had marginal influence on PC2 (25.6% variance) score. The PCA loading showed distinct relationships. Two components were extracted with eigenvalues ≥ 1, and volatiles segregated into two groups labeled A and B. Group A volatiles were: trimethypyrazine, 3-methylbutanal, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine,

tetramethylpyrazine, linalool-oxide and 2,3,5-triethyl-5-methylpyrazine, all characterised by *cocoa, chocolate, praline* and *roasted* notes possibly originating in cocoa.

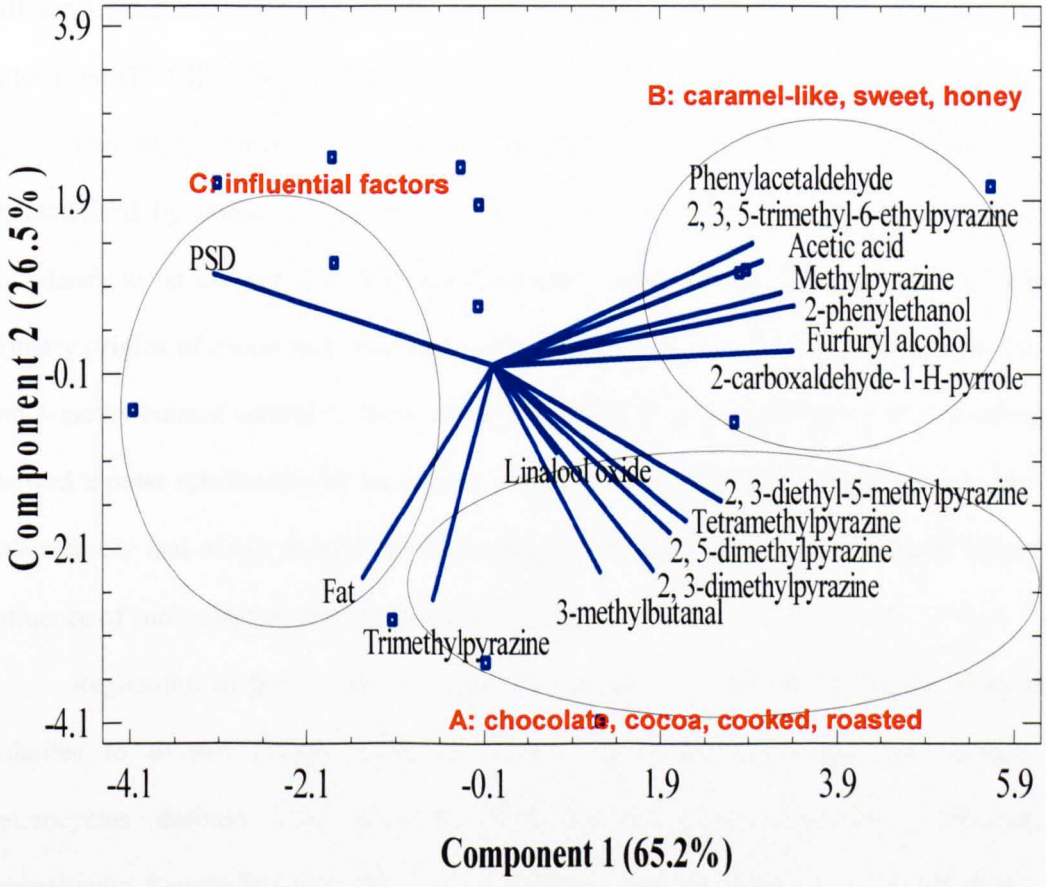

Figure 8.2. PCA biplots of dark chocolate flavour volatiles as influenced by PSD and fat content

Group B consisted of: 2-phenylethanol, furfuryl alcohol (furfurol), acetic acid, methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-H-pyrrole, characterised by *caramel-like, sweet, honey* and *candy* notes developed during chocolate manufacture. Both PC1 and to some extent PC2, differentiated chocolate samples with clearly groupings characterised by specific flavour notes from GC Olfactometry (Table 8.2).

The PCA loadings on PC1 showed that flavour volatiles within Group A characterised by *cocoa, chocolate, praline* and *roasted* notes were highly related in abundancy to fat content. The working hypothesis is that Group A flavour volatiles are primary origins of *cocoa* and *chocolate* notes in dark chocolates, with trimethylpyrazine and 3-methylbutanal central to these characters (Fig. 8.2). Contrariwise, the PCA loading showed a polar relationship of fat content with Group B observed to have *caramel-like, sweet, honey* and *candy* notes (Table 8.2), suggesting that increasing fat content reduce influence of such notes on flavour character in dark chocolates.

Regression models were developed to predict contribution of specific flavour volatiles to overall flavour character. One Strecker aldehyde and two nitrogen heterocycles derived from Maillard reactions had high regression coefficient, respectively: 3-methylbutanal, $R^2 = 0.843$, P=0.001; trimethylpyrazine, $R^2 = 0.819$, $p = 0.001$; 2,3-diethyl-5-methylpyrazines, $R^2 = 0.869$, $p = 0.001$ (Table 8.5). These emerged as probably the most interesting compounds in dark chocolates providing *cocoa, praline-chocolate* and *nutty* flavours. Three other heterocycles: 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and tetramethylpyrazine showed less but still significant effects with $R^2 = 0.516$, 0.614 and 0.510 ($p<0.05$), respectively, contributing *cocoa-chocolate* notes.

Others 2-methylbutanal, 5-ethenyltetrahydro-R,R,5-trimethyl-cis-2-furanmethanol (linalool-oxide) and 3,7-dimethyl-1,6-octadien-3-ol (linalool) had no significant influence ($P>0.05$) (Table 8.5), possibly due to their low contents in Central West African cocoa (Tables 8.3 & 8.4).

On the other hand, the regression models developed showed high and significant regression coefficients ($R^2 = 0.75 - 0.88$, $p = 0.001$) for Group B compounds. These predicted likely contributions of 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde and 2, 3, 5-trimethyl-6-ethylpyrazine of *caramel-like, sweet, honey* and *candy* notes. Acetic acid had high regression coefficient ($R^2 = 0.75$, $p = 0.001$), and likely contribute *astringent-sour* characters to dark chocolates. Others, 2-phenylacetate and 2-carboxaldehyde-1-H-pyrrole showed no significant effect ($R^2 = 0.095$ and 0.179, $p>0.05$ respectively) predicting their minimal impacts on flavour character in dark chocolate. This product space from PCA (Fig. 8.3), demonstrated the importance and relationships of the different flavour volatiles and likely effect on flavour character and furthermore the influence of solids PSD and continuous phase matrix fat content on the overall volatiles release into the headspace in dark chocolates.

8.5 CONCLUSION

Variations in flavour volatile release in dark chocolate matrices varying in particle size distribution and fat content were noted, suggesting the potential effects of matrix structure and lipophilic-flavour interactions. Increasing PS significantly reduced the release of 3-methylbutanal, 2-phenylethanol, furfuryl alcohol *(furfurol),* acetic acid, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine,

tetramethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine, 2-phenylethylacetate, 2-methylbutanal and 5-ethenyltetrahydro-R,R,5-trimethyl-*cis-2-furanmethanol (linalool oxide)*. Fat content was directly related to headspace concentrations of compounds characterised by *cocoa, chocolate, praline, fruity* and *roasted* notes: trimethypyrazine, 3-methylbutanal, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, linalool-oxide and 2,3,5-triethyl-5-methylpyrazine at all particle size distributions. Contrariwise, there was an inverse relationship between matrix fat content and headspace concentration of 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-H-pyrrole likely due to lipophilic matrix–flavour interactions.

The PCA and regression models predicted contribution of volatiles to overall flavour character. One Strecker aldehyde, 3-methylbutanal, and two nitrogen heterocycles derived from Maillard reactions, trimethylpyrazine and 2,3-diethyl-5-methylpyrazine, provided *cocoa, praline-chocolate* and *nutty* notes, with three others, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and tetramethylpyrazine likely making little contribution to showing only minimal effect to cocoa-chocolate flavour. Ethyl groups in pyrazine compounds suggest key role of alanine and its Strecker aldehyde and acetaldehyde in dark chocolate flavour formation. Others 2-methylbutanal, 5-ethenyltetrahydro-R,R,5-trimethyl-cis-2-furanmethanol (linalool-oxide) and 3,7-dimethyl-1,6-octadien-3-ol (linalool) had likely no effect on dark chocolate flavour character. Likewise, 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde and 2, 3, 5-trimethyl-6-ethylpyrazine emerged as compounds contributing *caramel-like, sweet, honey* and *candy* notes, with acetic acid contributing to

acid-sour sensations. Matrix effects on flavour release in dark chocolate merit attention as new product development and consumers demand a wider range of origins and defined products, and their influence on sensory effects with PSD and fat content remain unclear.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER STUDIES

9.1 Conclusions: Effects of PSD and composition on chocolate physical properties

Modifications in particle size distribution of suspended solids in dark chocolates can be employed to influence flow behaviour during industrial manufacture. This potential has not been fully realised due to a lack of pertinent information on the role of PSD in defining rheological behaviour. This study has enhanced understanding on effects of PSD and compositional variations on rheological (flow) properties and related quality characteristics. Research revealed that PSD parameters consist of many discrete components comprising specific surface area, largest PS D(v,0.9), Sauter mean diameter (D[3,2]), and mean particle diameter (D[4,3]), all of which exert significant effects on chocolate viscosity. This was confirmed by examination of crystalline network microstructures in molten chocolates varying in PSD and fat content. It was therefore concluded that PSD was a significant factor, but not the only determinant controlling dark chocolate rheology. Fat content exerted greatest effect on the variability in the rheological properties of molten dark chocolate, followed by lecithin content and then PSD.

Multivariate procedures were used to explain relationships between the two models (Casson's and the new ICA recommendations) used to determine dark chocolate viscosity. From this, it was found that the Casson reference parameters (yield value and plastic viscosity) and new ICA recommendations (yield stress and apparent viscosity) for evaluating chocolate viscosity are very closely related, and could be used independently. However, the ICA method proved to be relatively more efficient than the Casson model, which has limitations with chocolates with wide variations in viscosity. It was therefore recommended that for routine quality control purposes, the calculation of Casson's

reference parameters where the product history is known could be justified while the ICA would be better suited for research purposes with wide variations in component viscosity.

Textural properties of both molten and solid tempered dark chocolates were noted to decreases linearly with increasing particle size, fat and lecithin contents. At low (25%) fat contents, 5% and 2% increases in fat and lecithin respectively enhanced PSD effects on texture, with no significant effects at ≥30% fat. Effects on texture of changes in fat and lecithin depended on base fat content. Increasing PSD and fat inversely influenced appearance parameters (L*, C* and h°). Fat content exerted greatest effect on texture and appearance, followed by PSD and then lecithin content with the last having no significant effect on appearance. Textural parameters (firmness, consistency, cohesiveness, index of viscosity and hardness) and colour measurements (L*, C* and h°) were highly correlated suggesting prediction. The conclusion was that PSD, fat and lecithin content all interact to determine texture and appearance in dark chocolates, with significance for new product development and process improvements.

Microstructural analysis revealed that the smaller particles (D_{10}, D_{50}), largest particles (D_{90}) and specific surface area had direct influence on packing ability and inter-particle interactions. At low (25%) fat concentrations, inter-particle interaction of crystals led to flocculation, with an impact on microstructure and behaviour of molten and tempered products. Increasing fat reduced the crystalline network density, created more open and void spaces which were filled with fat, reducing resistance to flow, and enhancing spreadability and softening in products. Thus, application of PSD with fat and lecithin could be manipulated to control rheological and mechanical properties of molten

and tempered (solid) chocolates respectively, with importance for new product development and costs of manufacture.

Further work on melting properties using differential scanning calorimetry showed that variations in PSD, fat and lecithin content during dark chocolate manufacture influenced to varying extents, the degree of crystallinity and melting properties (T_{end}, T_{index} and ΔH_{melt}) of derived products. It was found that chocolates with finer particles, higher fat and lower lecithin contents, took longer and higher temperatures to complete melting than their corresponding products with larger PS, lower fat and higher lecithin content, suggesting that for chocolate of the same composition, processed under identical conditions, the PSD of the suspended non-fat solid, fat and lecithin contents play important roles in determining their melting behaviour. These findings have applications in defining chocolate quality in terms of nature of crystalline material, dimensions of crystals and polymorphic stability that dictate mechanical and rheological properties of chocolate products.

Rheological parameters (apparent viscosity and yield stress), textural parameters (firmness, index of viscosity and hardness) and melting index (duration or time) were highly positively correlated suggesting effective prediction. These explain that hardness (texture) could be effectively used to predict melting time (or duration) of finished dark chocolates during consumption. Other processing factors such as temper, polymorphism and cooling temperature controls could contribute to the variability in hardness and melting index of products. Principal component analysis revealed that with the exception of melting index which showed a moderate shift in space, the rheological properties (apparent viscosity and yield stress) and textural properties (firmness, index of viscosity

and hardness) were closely related. PSD, fat and lecithin contents all interact to determine rheological and textural properties, and melting index (duration) of dark chocolates, with significance for manufacturing improvements and quality control.

9.2 Conclusions: Tempering behaviour from response surface methodology

Tempering behaviours of dark chocolates varying in PSD and fat content were studied using models developed by response surface methodology. The models revealed that variations in PSD and fat content of products influenced optimal temperature settings of temperers during pre-crystallisation of products, causing wide variations in chocolate temper units. Differences in fat content exerted greatest variability in temperature settings of the different zones of multi-stage temperers used, for attaining optimal-tempered products. From this work, satisfactory and unsatisfactory temper regimes and corresponding temper slopes and chocolate temper units were generated to enhance understanding of the different temper regimes and boundaries of identification. Thus, different combinations of tempering temperatures could be employed to induce stable fat polymorph formation and are greatly dependent on fat content and partly PSD of the dark chocolate during manufacture.

9.3. Conclusions: Effects of tempering and fat crystallisation on microstructure and physical properties

Fat crystallisation behaviour during tempering of dark chocolate play vital roles in defining structure, mechanical properties and appearance of final products. Wide variations in mechanical properties and appearance were noted in products of differing

particle size (PS) and temper regime. Particle size was inversely related with texture and colour, with the greatest effects noted with hardness, stickiness and visual lightness at all temper regimes. Over-tempering caused increases in product hardness, stickiness with reduced gloss and darkening of product surfaces. Under-tempering induced fat bloom in products with consequential quality defects in texture, colour and surface gloss. Also, it was noted that variations in PS had no influence on crystallinity of dark chocolates whether optimally-, over- or under-tempered. Particle size had a limited but significant direct relationship with certain melting parameters - T_{onset}, T_{peak}, and ΔH_{melt} - independent of temper but significant inverse relationship with T_{end} and T_{index}. Contrariwise, varying temper influenced crystallinity and chocolate melting properties (T_{end}, T_{index} and ΔH_{melt}). Under-tempering of dark chocolate resulted in widened crystal size distribution with significant changes in T_{end}, T_{index} and ΔH_{melt}. Over-tempering caused moderate increases in crystal size distribution, with significant effects on T_{end}, T_{index} and ΔH_{melt} but no changes were noted in T_{onset}, or T_{peak}. Fat-sugar melting profiles were similar in all chocolates independent of PS and temper regime.

Examination using a stereoscopic binocular microscope revealed clear variations in surface and internal crystal network structures and inter-particle interactions among optimally-tempered, over-tempered and under-tempered (bloomed) samples. Blooming caused whitening of both surface and internal peripheries with consequential effects on texture and appearance. Scanning electron micrography showed an even spatial distribution of numerous small stable β-polymorph crystals in a network with well defined inter-particle connections in optimally-tempered chocolate. With over-tempered chocolate there were large numbers of very small crystals in network with similar well

defined particle-to-particle connections resulting from formation of stable β-polymorphs with early nucleation: the outcome was growth of seed crystals from melt into sub-micron primary crystallites and a fat crystal network stabilized by van der Waal forces. Under-tempering resulted in dissolution of a large number of small crystals, re-arrangement and re-crystallisation into a small number of larger (lumps) fat crystals (Ostwald ripening). In this process there was polymorphic transformation, nucleation and growth of new large crystals in a more stable polymorphic form with formation of solid bridges with weak and fewer inter-crystal connections within chocolate structures. Thus, attainment of optimal temper regime during tempering of dark chocolate is necessary for achievement of premium quality products and avoidance of defects in microstructure affecting mechanical properties, appearance and melting character.

9.4. Conclusions: Fat bloom formation and development with under-tempering

The rate of bloom development in under-tempered dark chocolate was dependent on solids particle size distribution and storage time. Blooming was initiated in chocolates within 24 h and essentially complete by 96 h. Changes during blooming were attributed primarily to growth of new fat crystals within the structural network with changes in light reflection yielding increases in surface whiteness and in hardness. From differential scanning calorimetry on melting properties, values for T_{onset}, T_{end}, T_{peak} and ΔH_{melt} suggested polymorphic transformation from βIV to βV within 24 h and further to βVI after 72 h. Micrographs showed similar crystal network structure and inter-particle interactions in chocolates of different PS immediately after tempering. Within 24 h, liquid and unstable re-crystallized fat had appeared on surfaces with initiation of bloom.

Unstable fat re-crystallised during storage into more stable polymorphs and crystal growth was promoted by Ostwald ripening with the appearance of white crystalline structure that had spread gradually throughout entire chocolate masses after 96 h. Chocolate of largest PS (50 μm) showed most rapid fat bloom, smallest PS (18 μm) slowest, attributed mainly to hydrodynamic forces of capillary action. It was concluded that bloom development was initiated by movement of liquid and unstable fat onto product surfaces through capillarity created by hydrodynamic forces within the inter-particle pores and crevices, followed by growth of new fat crystals promoted by diffusion gradients across the mass until the chocolate was fully bloomed. Understanding fat bloom formation and development in dark chocolate has potential applications in new product development.

9.5. Conclusions: Flavour volatiles and matrix effects related to variations in PSD and fat content

Variations in flavour volatile release in chocolate matrices varying in particle size distribution and fat content, suggested potential effects of matrix structure and lipophilic-flavour interactions. Increasing PS significantly reduced release of 3-methylbutanal, 2-phenylethanol, furfuryl alcohol *(furfurol)*, acetic acid, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine, 2-phenylethylacetate, 2-methylbutanal and 5-ethenyltetrahydro-R,R,5-trimethyl-*cis-2-furanmethanol (linalool oxide)*. Fat content was directly related to headspace concentrations of compounds characterised by *cocoa, chocolate, praline, fruity* and *roasted* notes: trimethypyrazine, 3-methylbutanal, 2,3-

dimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine, linalool-oxide and 2,3,5-triethyl-5-methylpyrazine at all particle size distributions. Contrariwise, there was an inverse relationship between matrix fat content and headspace concentration of 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde, 2, 3, 5-trimethyl-6-ethylpyrazine and 2-carboxaldehyde-1-H-pyrrole likely due to lipophilic matrix–flavour interactions.

The PCA and regression models predicted contribution of volatiles to overall flavour character. One Strecker aldehyde, 3-methylbutanal, and two nitrogen heterocycles derived from Maillard reactions, trimethylpyrazine and 2,3-diethyl-5-methylpyrazine, provided *cocoa, praline-chocolate* and *nutty* notes, with three others, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and tetramethylpyrazine likely making little contribution and showing only minimal effects on cocoa-chocolate flavours. Ethyl groups in pyrazine compounds suggest a key role of alanine and its Strecker aldehyde and acetaldehyde in dark chocolate flavour formation. Others 2-methylbutanal, 5-ethenyltetrahydro-R,R,5-trimethyl-cis-2-furanmethanol (linalool-oxide) and 3,7-dimethyl-1,6-octadien-3-ol (linalool) had likely no effect on dark chocolate flavour characters. Likewise, 2-phenylethanol, furfuryl alcohol (furfurol), methylpyrazine, phenylacetaldehyde and 2, 3, 5-trimethyl-6-ethylpyrazine emerged as compounds contributing *caramel-like, sweet, honey* and *candy* notes, with acetic acid contributing to acid-sour sensations. Matrix effects on flavour release in chocolate merit attention for new product development with consumer demand for a wider range of origins and defined products: sensory influences of PSD and fat content remain unclear.

9.6 APPLICATIONS OF THIS WORK

This work has brought greater understanding on the applicability of particle size distribution and ingredient composition to optimize flow behaviour and consequently textural and melting properties of finished dark chocolates. PSD could be manipulated with the combined action of fat and lecithin to control rheological properties of dark chocolates, with significance for quality control and reductions in production costs. This understanding would allow manufacturers to lower the viscosity of dark chocolates without changing the composition or cost, or to lower fat content without affecting viscosity and quality.

In addition, findings on crystalline network microstructure of the molten chocolate explained the defining role of PSD and fat content on the rheological, textural and melting properties of dark chocolates. These revealed that for chocolate of the same composition and processed under identical conditions, the PSD of the suspended non-fat solid, fat and lecithin contents play important roles in determining their melting behaviour. These findings would have application in defining chocolate quality during manufacture in terms of nature of crystalline material, dimensions of crystals and polymorphic stability that dictate mechanical and melting properties.

Results from models developed to study tempering behaviour revealed that PSD and fat content of products influenced crystallisation behaviour during tempering of products, causing wide variations in chocolate temper units. From these, satisfactory and unsatisfactory temper regimes and their corresponding temper slopes and chocolate temper units have been provided. These would limit the trial and errors currently used to identify appropriate temper regimes for dark chocolates, with industrial significance for

reducing processing (tempering) times with assurance in quality control and shelf characteristics.

Finally, findings from the tempering and fat crystallisation behavioural studies showed that attaining optimally-tempering during pre-crystallisation of dark chocolate is necessary and play vital roles in defining the structure, mechanical properties and appearance of finished products. As well, information from the flavour studies showed that differences in product matrices varying in particle size distribution and fat content resulted in variations in flavour volatile release in dark chocolates, suggesting potential effects of matrix structure and lipophilic-flavour interactions. These findings would help processors predict contribution of individual flavour volatiles and suggest how these can be regulated to attain defined flavour characters during manufacture. This knowledge is necessary for the achievement of premium quality products, manufacturing improvements, new product development and quality control.

9.7 RECOMMENDATIONS FOR FURTHER STUDIES

A number of points have been noted throughout this study that suggests the need for further in-depth investigations. These could include the following:

i. Further studies is required to integrate sensory and instrumental analyses of texture and flavour release from products varying in PSD and fat content, and establish relationships using multivariate analyses.

ii. Time intensity procedures could be employed to characterise the effects of optimal-temper and over-temper regimes on the melting behaviour of products during

consumption. It could also be deployed to study effects of varying PSD and fat content on reported variations in melting character of derived dark chocolates.

iii. Comparison of flavour characters in chocolate is complicated by variations caused by different genotypes, geographical origin, pod differences, fermentation and drying methods, and subsequent processing (roasting, alkalisation and conching). Although some major steps have been made to identify the causes of these variations, it is still premature to conclude that this is fully understood. To fully understand variations in dark chocolate character, further research is required to optimise post-harvest treatments (pod storage, pulp pre-conditioning, depulping, fermentation and drying) of cocoa beans differing in genotype, subsequent manufacturing processes (roasting, alkalisation and conching) during chocolate manufacture as well the sensory evaluation of final flavour character in chocolate.

iv. Milk chocolate solids comprise of particles from sugar, non-fat milk components and cocoa. This study investigated PSD of solids from only sugar and cocoa, which cannot be related directly to milk chocolate products. Changes in the sizes of particles from the other two particulate ingredients should also be investigated for effects on the physical and sensory character in milk chocolates.

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3. **Afoakwa, E. O.,** Paterson, A. & Fowler, M. (2008). Effects of particle size distribution and composition on rheological properties of dark chocolate. *European Food Research & Technology, 226,* 1259-1268. doi.10.1007/s00217-007-0652-6.

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10.3 CONFERENCE PRESENTATIONS

1. **Afoakwa, E. O.,** Paterson, A., Fowler, M. & Vieira, J. (2007). Comparative evaluation of rheological models used for evaluating dark chocolate viscosity. Paper presented at the First International Chester Conference of Food Science and Technology, Chester, UK. April 10-13, 2007.

2. **Afoakwa, E. O.,** Paterson, A., Fowler, M. & Vieira, J. (2007). Relationship between rheological and textural properties of dark chocolates as influenced by particle size distribution and composition. Paper Presented at the Annual Meeting of the Institute of Food Technologists (IFT), McCormick Place Convention Center, Chicago, Illinois, USA. July 28 – August 1, 2007.

3. **Afoakwa, E. O.,** Paterson, A., Fowler, M. & Vieira, J. (2007). Crystallization behaviour and quality characteristics in dark chocolates from varying particle size distribution and temper regimes. Presented at the Nestle UK Professional Development Seminar, York, U.K. September 10 – 11, 2007.

4. **Afoakwa, E. O.,** Paterson, A., Fowler, M. & Vieira, J. (2008). Factors influencing quality characteristics of chocolates systems during industrial manufacture. Presented at the Ghanaian Scholars UK (AGGOSS) Seminar, Ghana High Commission, Highgate Hill, London, U.K., June 21, 2008.

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6. **Afoakwa, E. O.,** Paterson, A., Fowler, M. & Vieira, J. (2008). Tempering and fat crystallisation effects on microstructural and melting properties in dark chocolate systems. Accepted for oral presentation at the 14th World Food Congress of the *International Union of Food Science and Technology (IUFoST)*, Shanghai, China, October 19 – 23, 2008.

7. **Afoakwa, E. O.** (2008). Particle size distribution and compositional effects on the rheological and microstructural properties of dark chocolate systems. Accepted for oral presentation at the *ZDS Chocolate Technology* Congress, Cologne, Germany. December 9 – 12, 2008.