Oral Processing of Dark and Milk Chocolate

by

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Abstract

The thesis reports novel scientific understanding and findings generated on the subject of chocolate oral processing. Research was carried out with a view to unravel the role of food (chocolate) - and human-related factors in governing structural and physical transformation of chocolate matrices during human oral processing. Dark and milk chocolate were studied as contrasting model matrices to investigate the influence of composition and physical properties of chocolates on microstructure and physical properties of ready-to-swallow chocolate boluses formed as a consequence of distinct eating and saliva incorporation strategies. Microstructure, and physical/material properties, in particular, particle size distribution, hardness, mechanical and rheological properties of melts, and thermal behaviour and solid fat content (SFC) of the chocolate models were characterised and compared. Differences in particle size distribution between the chocolates, and presence of milk ingredients (milkfat, milk powder, lactose) and surface-active agents (soy lecithin) in the milk chocolate, as opposed to their absence in the dark chocolate, were recognised and discussed as prominent factors contributing to underlying differences in microstructure and physical properties between the chocolate models. The dark chocolate was significantly harder as compared to milk chocolate, and in addition demonstrated greater firmness, consistency, cohesiveness, index of viscosity, yield stress and plastic viscosity of melt. Analysis of melting behaviour suggested that in comparison to milk chocolate, the dark chocolate had a slower melting-rate and greater SFC, and hence demonstrated greater energy requirement for complete liquefaction. This was reflected through the thermal parameters of solid fat index, melting onset, end and peak maximum, and enthalpy of melting assessed using differential scanning calorimetry.

A 24 subject human panel study undertaken to investigate eating (mastication and swallowing) strategies of consumers suggested that chocolate eating behaviour varied considerably across consumers. Findings highlighted that chocolate eaters adapted their overall eating strategies in response to differences in physical and related-textural properties of chocolates. In particular, total number of chews and oral processing time for the complete masticatory sequence and until the first perception to swallow, significantly differed between the two chocolates. These eating parameters were greater in the case of dark chocolate as compared to milk chocolate. Furthermore, subjects also conserved their general eating patterns and maintained similar masticatory frequencies between chocolates. Taken together, it was postulated that chocolate composition and physical properties, as well as human-related physiological and behavioural factors influenced dynamics of chocolate oral transformation, and were consequently involved in modulation of mastication and swallowing strategies. Hierarchical cluster analysis and analysis of variance were successfully implemented for segregation of population into three clusters with significant differences in eating parameters. This was followed by principal component analysis which facilitated the selection of 3 test subjects who exercised distinct overall

chocolate eating strategies significantly different from each other, and moreover were from a related parent cluster.

Regardless of eating strategy, occurrence of several voluntary swallowing events before complete oral clearance of chocolates indicated that only a part of the bolus was ready-to-swallow at the first perception to swallow. Observation of expectorates confirmed that at this point, chocolate boluses constituted a pool of liquid bolus phase (molten chocolate + saliva) as well as cohesive bolus lumps (solid/partially-melted chocolate particles aggregated together by the action of saliva and molten fat). While the liquid phase was swallowed by subjects, cohesive lumps underwent further oral processing to be transformed into a swallowable consistency. Microstructure analysis of bolus liquid phase by optical microscopy and confocal laser scanning microscopy revealed a coarse oil-in-water emulsion microstructure in the case of either chocolate wherein, a relatively denser bolus structure resulting from extensive ingredient and fat globule flocculation was witnessed for dark chocolate boluses.

Results further suggested that solid fat content-related physical properties and melting behaviour were related to saliva incorporation. Greater hardness and energy requirements for liquefaction, and slower rate of melting in dark chocolate resulted in relatively longer oral processing time invested by subjects in bolus preparation. This in turn resulted in higher moisture content in ready-to-swallow boluses of dark chocolate (40.25 wt%) as compared to milk chocolate (32.20 wt%). Furthermore, these properties also resulted in cohesive-lumps of dark chocolate boluses being significantly firmer and requiring greater work for compression. In contrast, adhesiveness of milk chocolate boluses was greater in comparison with dark chocolate boluses, and was explained through the presence of milk ingredients in its chocolate matrix. Subjects processed both chocolates to similar cohesiveness of bolus lumps, interestingly indicating that this property may not be chocolate-dependent. Nevertheless, bolus saliva contents at the first point of swallow, and all mechanical properties accept adhesiveness of bolus lumps, were subject-dependent. Results indicated that this effect could be largely related to variation in physiological parameters, in particular oral processing time and salivary flow rates. Interestingly, liquid phase viscosities of milk chocolate boluses were similar to that of dark chocolate within-subjects, while this property was also subject-dependent. Adaptation of eating strategies and saliva incorporation demonstrated by subjects in response to differences in chocolate texture, and the presence of a relatively greater percentage of water-soluble solids in milk chocolate were factors which supported the fact that ready-to-swallow boluses of both chocolates had similar viscosities. Subject-dependency of chocolate bolus viscosity was explained through physiological parameters of eating behaviour and saliva flow rate which influence final moisture content in the bolus liquid phase.

Considering the importance of the continuous fat-phase in influencing oral processing and bolus formation of chocolates, effect of storage temperature (0°C, 20°C, 30°C)-induced physical changes in dark and milk chocolate on physical properties of ready-to-swallow boluses, and eating and

saliva incorporation strategies of selected subjects was investigated. Thermal analysis revealed mainly SFC-related changes in the physical properties of hardness and enthalpy of melting (ΔH_{melt}). Relative to 20°C, storage at 0°C resulted in increased hardness and ΔH_{melt} for both chocolates, while an inverse effect resulted from storage at 30°C. In the case of both chocolates, all subjects adapted their oral processing time, number of chews and saliva incorporation strategies in positive relation to increase/decrease in hardness and ΔH_{melt}. Again, they conserved their general eating patterns, and maintained similar masticatory frequencies to form boluses suitable for swallowing. In the case of both chocolates, significant softening and relatively greater reduction in ΔH_{melt} of chocolate stored at 30°C resulted in significantly low firmness and work of spreading of bolus lumps obtained at the point of swallow. Once again, in the case of all subjects, adhesiveness of bolus lumps was independent of these changes in physical properties for either chocolate-type. Lastly, results suggested storage treatments resulted in each subject processing a similar chocolate-type to different endpoints in terms of bolus liquid phase viscosity. Different SFC which governed the relative extent of melting that a chocolate underwent until the point of swallow, may have influenced the degree of bolus dilution, and hence its viscosity. Throughout this study, the excellent within-subject repeatability in eating strategies, saliva incorporation, and rheological properties of ready-to-swallow bolus for a particular chocolate- and/or texture-type was noteworthy.

Aside

Tavern upon the Woodhouse hill

A Yorkshire mist and its simmering prowl within

Rosewood decks and an enfeebled liqueur creel

Amidst the tranquil night, it had an enchanted feel.

Vish Gaikwad

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Contents

List of Illustrations

Chapter - 1 Introduction

Chapter – 2 Review of Literature

Chapter – 3 Characterisation of Physical Properties and Microstructure of Dark and Milk Chocolate Models

Chapter – 4 Chocolate Bolus Formation and Characterisation of Chocolate Eating Behaviour

Chapter – 5 Physical Properties and Microstructure of Ready-to-Swallow Dark and Milk Chocolate Bolus

List of Tables

Chapter – 2 Review of Literature

Chapter – 3 Characterisation of Physical Properties and Microstructure of Dark and Milk Chocolate Models

Chapter – 4 Chocolate Bolus Formation and Characterisation of Chocolate Eating Behaviour

Chapter – 5 Physical Properties and Microstructure of Ready-to-Swallow Dark and Milk Chocolate Bolus

CHAPTER 1

Introduction

The significance of oral processing in the sensory appreciation and digestion efficacy of foods has led to major advances in the understanding of food-related and human-related factors influencing physico-chemical transformation of food in the mouth [1-4]. With these advances has come the fundamental realisation that "composition-structure-property relationships" of foods influence the mastication process and dynamics of bolus formation, and hence warrant further investigations taking into account behavioural and physiological factors involved in food oral processing [5-8]. Moreover, due to the appreciable interest in investigating potential physical markers (e.g. particle size, lubrication, flow behaviour, etc) involved in swallowing, there have been growing investigations on the extent of oral transformation different foods may undergo to be deemed as ready-to-swallow or safe-to-swallow [9-12].

Oral processing is the first stage of food digestion wherein mechanical action of teeth and tongue, and biochemical action of saliva act concurrently to transform the ingested food into a bolus suitable for swallowing [13]. It comprises of two main interrelated functions of mastication and swallowing [14], and overall, these are complex as food-related (composition, structure, physico-chemical and related-sensory properties), human-related (age, gender, dentition, salivation, etc.) and behavioural factors interact simultaneously during the complete process [15-21]. Extensive sensory information from the food and the continuously evolving bolus properties is utilised for the adaptive regulation of mastication and swallowing to the dynamic physical changes occurring in the mouth, and swallowing obviously relies on these tactile stimuli as sensory inputs governing the "swallowing threshold" [22-24]. The readiness and suitability of a food bolus for swallowing is largely governed by the criteria of degree of lubrication, particle size reduction, cohesion and consistency after it undergoes progressive restructuring as it is orally processed over a certain period of time [9-11, 25, 26].

A large subset of the existing research in this area has indicated the importance of physical and related-textural properties of various foods in influencing mastication [27-31], the physical transformation of food during oral processing [10, 32, 33], and the properties of a swallowable food bolus [12, 34-36]. For instance, a wide range of natural and processed solid

foods have been studied, and differences in their mechanical/rheological properties (and in some cases, their related breakage and deformation mechanisms) have been shown to influence masticatory and swallowing parameters, lubrication, particle size distribution and rheological properties of the bolus. These foods have ranged from fruits, vegetables, nuts, cereals, meats, cheeses, rice, biscuits, chewy confectionery and breads, to homogenous and heterogeneous model food matrices [12, 28, 34-41]. Similarly, research is also present on physiological and food-related factors involved in microstructural and physico-chemical restructuring of food hydrocolloids and emulsion-based foods and models during oral processing [42-43], while the bolus flow properties of a wide range of semi-solid and fluid foods demonstrating various rheological behaviours has also been investigated in relation to the oral processing and swallowing [11, 44, 45].

In contrast, similar knowledge pertaining to one of the most widely consumed foods – *Chocolate*, is very limited. Obviously, the multi-billion pound chocolate industry has for long controlled processing, composition and structuring of formulations for quality, stability, and acquiring desired sensory functionalities. Although, avenues attempting a fundamental characterisation of the influence of food-related (compositional, structural, and physical properties) factors on oral processing of chocolate, in particular, on mastication, physical transformation (bolus formation) and the properties defining a ready-to-swallow bolus ("swallowing threshold") are relatively nascent or unventured.

As for most processed composite foods, chocolate structure and physical properties are related to its underlying composition, ingredient interactions, and processing operations, and in turn influence its physical transformation during oral processing (Figure 1-1) [46, 47]. Chocolate structure can be defined as a concentrated suspension of solid particles (cocoa particles and sugar, and in the case of milk chocolate - cocoa particles, sugar, and milk solids) in a continuous fat phase (cocoa butter, milkfat) [48, 49]. Firstly, oral behaviour of chocolate is predominantly dependent on the character of its continuous fat phase. Lipid composition [50-52], processing operations (refining, conching, tempering and cooling) [53, 54], continuous phase volume fraction [55, 56], and storage temperature [57] are the main factors governing crystal morphology and distribution in the continuous phase of chocolate. Hence, these in turn relate to physical attributes like melting behaviour, hardness, and solid fat content, and together with the particle and emulsifier phase, influence mechanical and rheological properties of the melt [58-61]. As the continuous phase character constrains the characteristic phase transition of chocolate to take place just below the oral temperature, chocolate evolves

from a solid to a shear-thinning liquid during oral processing, consequently changing its physical properties rapidly over time [62, 63]. This inherently makes its oral transformation and bolus properties difficult to characterise, as approaches undertaken for similar studies of solid and fluid foods may not be independently applicable and/or sufficient.

Secondly, grinding and refining operations control the particle size distribution of chocolate. Particle composition, shape, size distribution and concentration (phase volume fraction) influence the mechanical properties and melt rheology of chocolate [61, 64-66], which are in turn related to crucial factors like sensory melt viscosity and product hardness [48, 67]. Furthermore, particle size also governs the sensory perception of 'smoothness' or 'grittiness' [46]. Hence, these are important structural contributors to chocolate mastication and flow behaviour in the mouth, consistency of the ready-to-swallow bolus, and oral texture perception. Finally, emulsifiers are used for modulation of chocolate flow behaviour as they facilitate the distribution of particles in the continuous fat phase and coating of particle surfaces efficiently with fat [48, 68]. Therefore, just as the particle phase, the composition and concentration of the emulsifier-phase also influences rheological behaviour and consequent physical and sensory phenomena associated with oral processing.

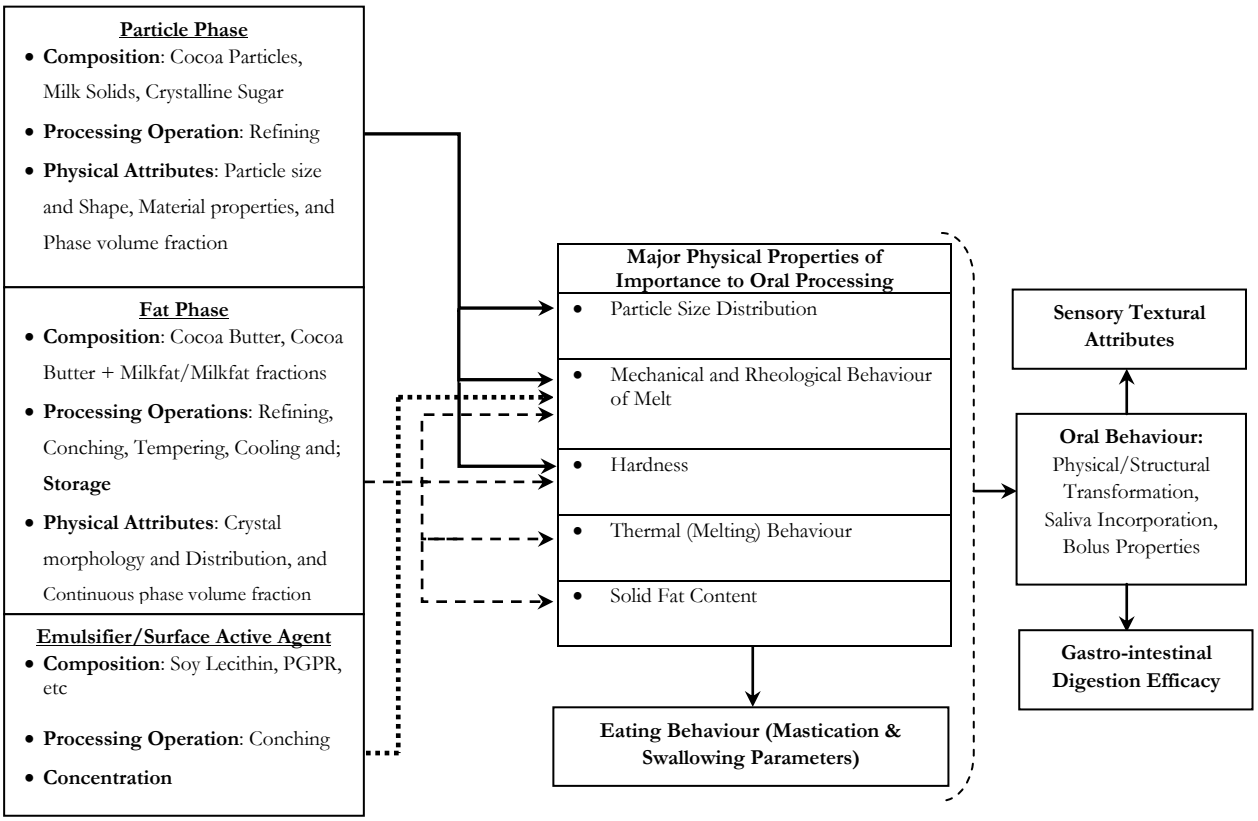


Figure 1-1 Diagrammatic representation of relationship between factors influenced by oral processing (sensory textural perception and digestion), and food-related (composition, process, physical properties) factors, and eating (mastication and swallowing) parameters for chocolate.

It is clear that chocolate has complex structure-physical property relationships largely governed by its composition, processing operations and storage. Moreover, their possibility of interaction with physiological and behavioural factors will surely make it challenging to understand how these relate to mastication, bolus formation, and properties of a ready-to-swallow bolus formed as a result of oral processing. This knowledge is of particular importance towards sensory appreciation and health implications associated with chocolate. Mastication and bolus properties have been previously related to texture perception [28, 69, 10]. Food-related factors and variations in oral processing have been shown to affect food intake levels [70, 71], and by extension, impact satiety [72, 73]; particularly, this is of high importance to consumption levels of fat- and sugar-rich foods like chocolate. Importantly, chocolate is a very rich source of health-promoting antioxidants – cocoa flavonoids [74, 75]. Research on gastro-intestinal bioaccessibility and bioavailability of cocoa polyphenols has highlighted the need for unravelling the physico-chemical and microstructural restructuring of chocolate matrices during oral processing [76]. This is because factors influencing oral processing, and by extension, properties of a ready-to-swallow bolus (extent of physical and microstructural transformation) are potentially important in influencing postmasticatory digestion of chocolate, and bioaccessibility and absorption efficacy of chocolate polyphenols.

Chocolate is estimated to account for nearly 55-60% of global confectionery sales. Globally, the chocolate market is estimated to reach £61.3 billion in 2016 from £51.9 billion in 2010, at a CAGR of 2.7% from 2011 to 2016. As of 2010, milk and dark chocolate have together constituted nearly 82% of the global market segment of eating chocolate, valued at around £42.5 billion [77]. Sensory pleasure derived from eating experience i.e. flavour and mouthfeel, and more recently, the growing awareness of proven health benefits derived from its cocoa content are the primary drivers of the large chocolate market around the world. Scientific research aimed at generating a knowledge base contributing to these primary factors would most certainly be one of the critical focal points of industry investments directed towards developing novel products and ingredient formulations for the future.

This thesis describes the work aimed at gaining a fundamental understanding of structural and physical transformation of chocolate resulting from oral processing. The research project aimed at investigating if differences in chocolate composition- and structure-related physical properties, along with inter-individual mastication and saliva incorporation strategies, influence the properties of a ready-to-swallow bolus. As the oral transformation behaviour and major sensory textural attributes of chocolate are chiefly governed by the

character of the underlying continuous fat phase, a strategy to induce physical changes in the fat matrix by subjecting the chocolates to a wide range of controlled storage-temperature treatments was implemented. These temperature-induced physical changes were characterised, and their effect on bolus formation, properties of a ready-to-swallow bolus, and mastication strategies exercised by individuals during chocolate oral processing were investigated.

Amongst the different forms in which chocolate is consumed, dark and milk chocolate are the most widely consumed globally, and were utilised as models to address the objectives of this research. Moreover, selection of dark and milk chocolates as models facilitated comparative understanding of differences in bolus formation and its physical properties related to differences in chocolate composition and material properties, and mastication strategies exercised during their consumption.

The main objectives of this research were:

1. Characterisation of physical properties and microstructure of selected dark and milk chocolate models varying in composition. Furthermore, to establish how differences in physical properties and microstructure relate to differences in composition of selected chocolates.
2. Designing and implementing a unique human panel study for characterisation of chocolate eating strategies (mastication and swallowing parameters). Furthermore, highlighting how differences in eating parameters may relate to differences in physical properties of chocolate models.
3. Development of a strategy to segregate individuals based on chocolate eating parameters, and to screen-out specific test subjects differing in their chocolate eating strategies.
4. Utilising selected test subjects, investigating –
    a. Characteristics of chocolate bolus formation during oral processing.

    b. Effect of chocolate-type (differences in composition-related physical properties) and eating strategies on physical properties of ready-to-swallow bolus.

    c. Effect of storage temperature-induced physical changes in dark and milk chocolate on eating strategies and physical properties of ready-to-swallow chocolate bolus.

CHAPTER 2

Review of Literature

2.1: Mastication in Humans, Bolus Formation and Swallowing – an Overview

Mastication is a complex process involving rhythmic jaw movements wherein the physico-chemical structure of the food is restructured by trituration and lubrication/dilution by the actions of teeth and saliva to a safe-to-swallow state [1]. A related concept of oral processing which may vary amongst individuals is the "objective masticatory function"; an individuals' ability to break-down food during chewing [2]. Starting with the first-bite to complete clearance, sensory inputs taken from the food bolus lead to continuous adaption/modification of masticatory patterns in response to the progressive restructuring of the food [3]. Figure 2-1 shows the anatomic diagram of human oral organs. Oral processing mostly involves the upper (maxilla) and lower (mandible) jaw, the tongue and to a lesser extent the cheeks and lips [4]. The Central Pattern Generator (CPG), a nervous system controlling rhythmic behaviours, activates the motor programme which coordinates the facial muscles, jaws and tongue [5]. This motor programme adapts consistently throughout the masticatory sequence to the evolving properties of the food bolus through sensory feedback from different types of receptors (for example, muscle spindles of the elevator muscles, periodontal receptors, skin and taste receptors, etc.).

2.1.1 Transport in the Mouth and Role of Saliva

Food transport in the mouth can be divided into three stages. Stage I – the preparatory stage – represents transport of food from front of the mouth to the premolars, and is governed by low amplitude simple jaw movements in which the teeth do not come into occlusion.

Stage II – reduction phase – represents the size reduction of food particles by the mechanical action of teeth (chewing cycles). The cycles include a closing phase, a phase when the teeth are close to full occlusion, and an opening phase. In some cycles, food is only

transported by the action of opening and closing strokes without occlusion. The number of cycles is related to the bite volume and consistency of food [6] [7] [8] [9].

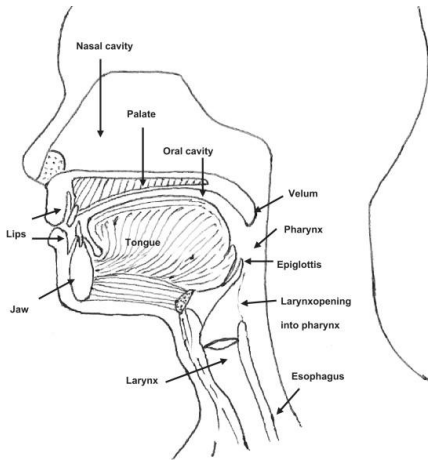


Figure 2-1 Human oral organ anatomy [1]

The tongue plays an important role at this stage in deciding whether the food particles are sufficiently small and lubricated, or require further processing. Particle size reduction during mastication can be considered a result of two distinct processes; selection: the chance of a particle being contacted by the teeth (termed as the *selection function*), and breakage: the degree of size reduction when selected particles break (termed as the *breakage function*) [10]. The selection function largely depends on physiological factors like tooth morphology, movement of jaws, total occlusal area of post-canine teeth, action of tongue and cheeks, as well as on particle size and number. Breakage on the other hand depends on degree of coordination of jaw-muscle activity, tooth morphology, mechanical/rheological characteristics of food, particle size and shape [11].

Stage III – the preswallowing stage – is the transport stage of the food to the back of the tongue for preparation of swallowing [12]. This stage is mediated by tongue-palate interactions without the involvement of any distinct jaw movements [13].

The oral propulsion phase is often considered as the beginning of a swallow [14]. Swallowing or clearance involves a pattern of tongue and irregular jaw movements to remove the safe-to-swallow bolus formed after a masticatory sequence [9] [12]. It is a sequential process wherein aggregation of particles to form a ready-to-swallow bolus takes place, and likely depends on factors like adhesion, friction, salivary viscosity and surface tension [15]. Once the food bolus enters the pharynx by the action of the tongue, swallowing occurs as an involuntary reflex action [16]. Before swallowing is initiated, a level of physical restructuring

("ready-to-swallow consistency") must be reached through bolus lubrication and particle-size reduction in order to prevent discomfort from distension of soft-tissue of the pharynx and oesophagus [17] [18]. Although in the past few years there has been progress on the front of understanding properties of a ready-to-swallow bolus for the optimal state, in terms of degree of lubrication, bolus consistency, particle size reduction and cohesiveness as factors representing the bolus-state at the point of swallowing, related physical markers in food and conditions of the swallowing threshold are not fully understood [19] [20] [21].

There is considerable inter-individual variability in oral motor functions, motility and mastication behaviours related to food-type [9] [22] [23]. The chewing response to different textures (hardness, crispiness, elasticity and plasticity, viscosity) varies widely from person-to-person, although studies do suggest intra-individual reproducibility and adaptability [1, 12, 24]. Mioche [25] highlighted how the rhythmic activity of jaw opening and closing during mastication is adaptively modulated through sensory information in accordance with physical and textural characteristics of the bolus. The sensory inputs influence mandibular movements and forces, and the duration and number of masticatory cycles [26]. Lucas [10] explained that this requires information on the position and velocity of the jaw, activity of the masticatory muscles, and the forces acting on the teeth and jaws. The time taken from intake until clearance is influenced by food properties and will vary among individuals exhibiting different chewing and swallowing strategies [27] [28] and furthermore, will depend on the type of sensory judgement being derived [29].

Lastly, not only is saliva important in softening and changing the mechanical and rheological properties of food [30], it also provides lubrication to aid particle flocculation for bolus formation, bolus motility in the oral cavity and swallowing [31] [32]. It also plays an important role in facilitating taste and texture perception. Saliva contains up to 99.5 wt% water and is secreted by sublingual- and submandibular-salivary glands. It acts as a medium for dissolution and carrier of food tastants, and plays an important role in aroma release and enzymatic digestion via α-amylase and lingual lipase bio-activity [33]. Salivary mucins are important in facilitating particle lubrication and modulating saliva viscosity [34], which in turn influences rheological properties of the bolus. Moreover, the autonomic nervous system controls both the volume and type of saliva secreted [9], while its flow rate and composition is influenced by food-type and shows large inter-individual variability [32] [35].

2.1.2 Swallowing

2.1.2.1 Stages of Swallowing

As introduced earlier, bolus formation and swallowing are integrated oral actions of the eating process. Bolus formation is essentially a process wherein food is triturated and lubricated, and is converted into a ready-to-swallow status; whilst swallowing transports the bolus from the oral cavity through pharynx and oesophagus to the stomach. An eating process may involve few swallowing actions, atleast one initial, and one final swallow (oral clearance) which marks the completion of the process [14]. Swallowing is a highly complicated oral action in that it involves a series of simultaneous and coordinated contractions and inhibitions of muscles located around the mouth, larynx, pharynx, oesophagus, and at the tongue [36].

The physiological action of swallowing has been described in three phases representing the anatomic regions transversed by the bolus – *oral phase*; which is normally seen as voluntary, and the *pharyngeal* and *oesophageal phase*; which last for a very short time, and are reflex responses [1] [11].

Oral Phase

This phase is usually divided into two sub-phases - oral preparatory and oral propulsion phase. The preparatory phase involves moulding of food (particles) and saliva into a swallowable status. The mechanisms involved in this phase are highly dependent on the microstructure and rheological nature of food. For liquid foods, chewing is not required and oral preparation is relatively simple. Liquids are very shortly held anterior to the floor of the mouth and buffered with saliva, while the oral cavity is sealed posterior by contact of the soft palate and the tongue. For solids and semi-solids, mastication has to be involved in the preparatory phase. The tongue moves cyclically in association with the lower jaw, and more importantly, there is no posterior sealing of the oral cavity, permitting open passage between the oral cavity and pharynx [1].

After oral preparation, in the propulsion phase, the bolus is forced backwards in the oral cavity towards the pharynx for swallow initiation. Here, the teeth are in centric occlusion and the lips are closed. The lingual propulsive force is believed to be the main driving force for bolus flow [37], but the pressure gradient in the pharyngeal region has also been reported possible [38].

Pharyngeal Phase

This phase is defined from a triggering of swallow reflex to the closure of the upper oesophageal sphincter, and sees the transport of the ready-to-swallow part of the bolus to the distal oesophagus [39]. An important food-related feature of this phase is that the bolus should have attained specific flow-ability and stretch-ability to have smooth flow during its motility. At its characteristic consistency in the pharyngeal region, the accumulated bolus is subjected to significant shear and extensional deformation as it transits to the oesophagus [14]. Recently, Kumagai [40] reported linear correlation between maximum velocity of the bolus and its viscosity during swallowing. They observed maximum velocity of 0.6m/s for water, which decreased to around 0.1m/s for viscosity of boluses of cellulose gum, xanthan gum, guar gum and pregelatinised starch. Previously, maximum swallowing velocities of 0.5m/s for water, 0.2m/s for yogurt boluses, and around 0.1m/s for semi-solid food bolus have been observed using ultrasonic pulse Doppler [41].

Oesophageal Phase

The oesophageal phase constitutes of further propulsion of bolus towards the stomach by primary and secondary peristalsis produced in the oesophagus as sequential low-amplitude contractions. The whole swallowing could last for few seconds from initiation to completion [42].

2.1.3 Dynamics of Bolus Formation and the Criteria of a Ready-to-Swallow Bolus

2.1.3.1 Dynamics of Bolus Formation

As for the involvement of several oral actions and its dynamic nature, transformation of food during bolus formation has never been treated as an independent step of the eating sequence in various models of food oral management [43]. The dynamics of formation of a bolus suitable for swallowing involve concurrent actions of size reduction of food and lubrication and buffering with saliva to form a cohesive bolus which is then transferred back into the oral cavity for deglutition (Figure 2-2). The readiness and suitability of a bolus for swallowing may depend on several influencing factors like consistency, degree of cohesiveness and particle size reduction, mechanical characteristics and geometry of food particles, surface lubrication, surface tension of oral fluid and so on [14] [10] [30].

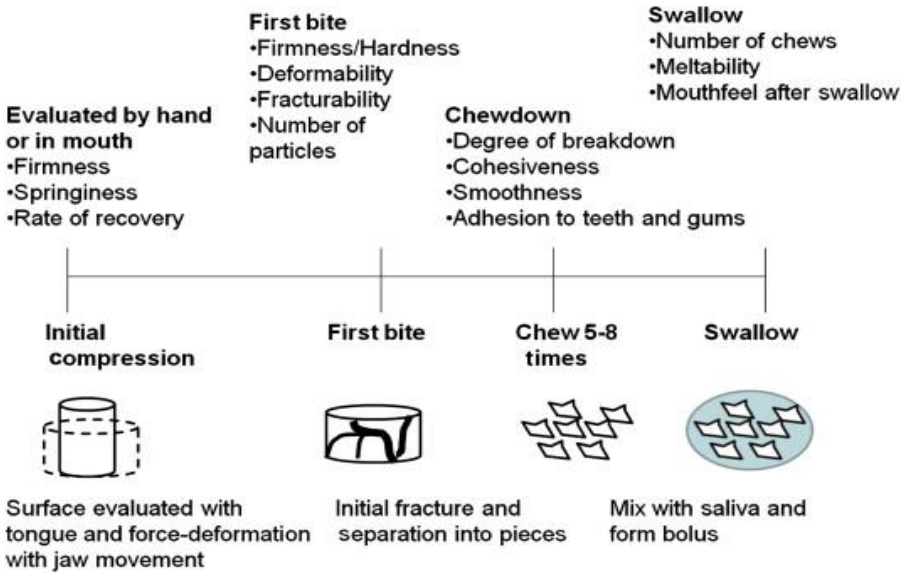


Figure 2-2 A model showing the oral processing of a soft solid material and the associated sensory texture terms [3]

Among the tactile stimuli during bolus formation which serve as major sources of information about the bolus-state, optimally reduced particle size has long been recognised critical in producing the stimulus marking both end-of-mastication and starting-point of swallowing [21]. The particle size distribution of a ready-to-swallow bolus was first named as the "swallowing threshold" [44] [45]. Later, the role of saliva incorporation and fluids from foods in providing a certain level of lubrication was considered a further source of sensory information from the bolus [46]. Some researchers using a modelling approach have considered the evolution of bolus cohesiveness during mastication, and proposed the peak in cohesive forces to coincide with the optimum time for swallowing [47]. The role of bolus flow behaviour has also been recently highlighted for some foods in investigations relating rheological behaviour of food with the ease of swallowing [48]. Recently, Peyron [21] investigated the change occurring in bolus physical (mechanical/rheological) properties during the masticatory sequence and at the point of swallow. They aimed to test the hypothesis that measuring the dynamic changes in these bolus physical properties over mastication would potentially reveal information necessary to identify physical markers involved in swallow initiation. Nevertheless, they also reported that, the concept of the swallowing threshold still remains largely theoretical, since no experiments have investigated the multiple dimensions of a swallowable bolus, their relation to simultaneous physiological and sensory events, and that to the physico-chemical properties of foods.

Hutchings and Lillford [46] were probably the first to propose a simple model to explain the dynamic nature of bolus formation and highlight the essential characteristics of a ready-to-swallow bolus. Their model proposed three dimensions to be considered for the dynamic nature of food oral transformation leading to the formation of a ready-to-swallow bolus. These were – degree of structure, degree of lubrication and time (Figure 2-3).

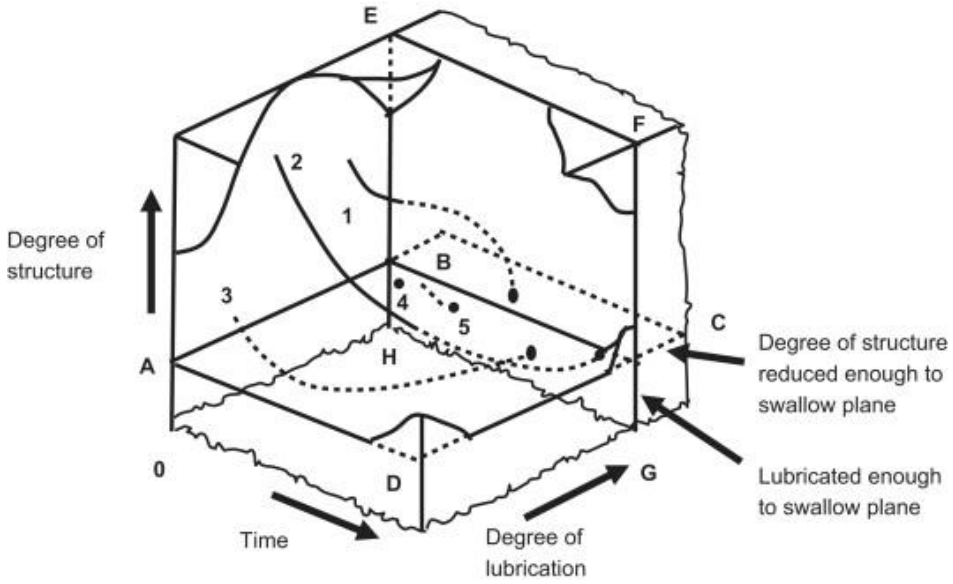


Figure 2-3 The 'mouth process model': (1) tender juicy steak; (2) tough dry meat; (3) dry sponge cake; (4) oyster; (5) liquids. Before a food may be swallowed, its 'degree of structure' must have been reduced below the level of plane ABCD, and its 'degree of lubrication' must have crossed plane EFGH (adapted from [46]).

The model proposes that a suitable food bolus should contain sufficiently size-reduced food particles, appropriate level of saliva incorporation and lubrication, and both of these criteria require a certain amount of oral processing time to be attained. Based on this proposal, the dynamics of bolus formation and the readiness of the bolus for swallowing could be quantified as a function of -

$$S = f(d, \varphi, t) \qquad 2.1$$

where, d represents the particle size, φ is the volume fraction of saliva, and *t* is time. Since both particle size and the amount of saliva is a function of oral processing time, therefore, the readiness of a bolus for swallowing can be simplified as a function of particle size, *d*, and saliva volume fraction, φ, or simply a function of oral processing time, *t*. Although this model has been widely referred in literature to describe the mechanisms of bolus formation and the underlying criteria of swallowing, little experimental evidence has so far been generated in its support [14].

Prinz and Lucas [47] believed that the characteristics of formation of a suitable bolus were dependent on the combined effect of particle-size, oral lubrication and saliva incorporation. They proposed a different model which has also been generally well accepted because of its simplicity and incorporation of multiple variables. Their model proposed the action of colloidal forces acting on food particles under oral processing. They treated the food bolus as a cluster of particles held together by oral fluid and argued that these particles are subjected to two forces during an eating process: the adhesion force (F_A) and the viscous force (F_V). Depending on the balance of these two counter-acting forces, food particles (assumed spherical) either tend to attach to the oral surface or flocculate together to form a cluster.

$$F_A = 4\pi \cdot r \cdot \sigma \qquad 2.2$$

$$F_V = \frac{3 \cdot \pi \cdot \eta \cdot R^4}{64 \cdot h^2 \cdot t} \qquad 2.3$$

where, r is the radius of the food particle, σ is the surface tension of the oral fluid, η is the viscosity of oral fluid, h is the distance between particles, R is the radius of the bolus and t is the time-span of separation. The adhesion of the food particles to the oral surfaces was accredited to the surface tension of the oral fluid, while the viscous forces tend to drag particles to flow together (Figure 2-4). It was further recommended that the combination of the two forces gave an indication of the consistency or the cohesiveness of the bolus, F_C, and could play the determining role in triggering a swallow:

$$F_C = F_V - F_A$$

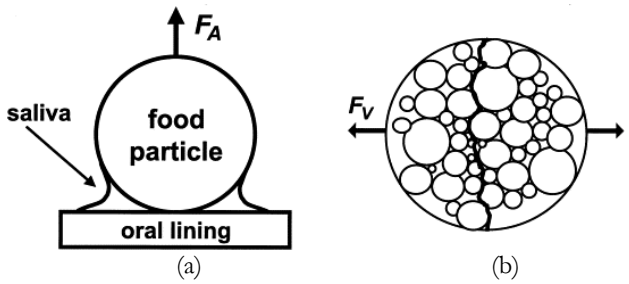


Figure 2-4 (a) The geometry assumed for a surface tensional force, F_A, which could attract a spherical food particle to the relatively flat lining of the oral cavity. This force depends on particle size but is independent of the distance between the particle and lining. (b) An idealised ball of spherical food particles, after being packed by the tongue against the hard palate, with the spaces between particles being filled by saliva. There is a highly distant-dependent viscous force that tends to hold the particles together to form a bolus [10].

The above model was tested on boluses of carrots and Brazil nuts using a numerical calculation method, and it was suggested that the boluses should be swallowed at their maximum consistency [10]. However, Chen [14] recently reported that there has been no

experimental confirmation from an independent study to validate the above model taking into consideration material properties which various foods could exhibit. Chen and Lolivret [30] questioned the criteria of maximum consistency as a marker for triggering a swallow. They argued that at maximum consistency the food bolus would be most difficult to deform and swallow, and consequently the oral muscles would have to work harder to create a high enough pressure to push the bolus through the oropharyngeal region. They further proposed that the ideal state of a bolus at the swallowing threshold would be somewhere beyond its maximum consistency, and that the criteria would be of rheological "flow-ability" instead of consistency. Their findings are discussed in the next section.

Although well accepted across related literature, the Prinz and Lucas model may have limited relevance in the early stages of mastication [14]. This may be due to the fact that there may be insufficient surface-wetting and particle size reduction in the early stages of the eating process. During the first few chewing strokes, there may be a fast increase in the surface area of food particles depending on their breakage properties, although there may be limited saliva incorporation. Triturated food particles remain relatively dry and only become increasingly lubricated after certain time into the masticatory sequence. This surface-wetting leads to flocculation of particles and therefore, the formation of a suitably compact bolus.

2.1.3.2 Minimum Oral-Effort as a Criterion in Triggering a Swallow

Recently, Chen and Lolivret [30] investigated the correlation between oral residence time of fluid foods, with their perceived difficulty of swallowing and intrinsic material properties of the foods (shear-viscosity and tensile stretch-ability). In particular, their selected foods covered a broad range of rheological behaviours like Newtonian liquids - orange juice, milk and yoghurt drinks; semi-solid and shear-thinning foods with varied yield stresses and shear viscosities – tomato ketchup, yogurts, custards, jams, mayonnaise, chocolate spread, etc.; and viscoelastic foods like jellies with different water contents and ricotta cheese.

They observed that the higher the viscosity of a fluid food, the longer was its oral residence time before being swallowed. They also observed that oral residence time was positively correlated to perceived sensory difficulty in swallowing (Figure 2-5) and shear-viscosity, with highest correlation observed for tensile stretch-ability of foods. They explained that the longer oral-stay helped in producing optimal levels of saliva and saliva incorporation/lubrication of foods. This dilution effect inevitably increased the flow-ability of the boluses, making them easier to swallow. Their investigation was carried-out to test the

hypothesis that depending on food-type, the bolus reaches a state which is associated with minimum oral effort in swallowing it i.e. optimum energy consumption. The authors argued that the energy required for bolus transport must be matched by the work of the oral apparatus through combined contraction efforts of swallowing.

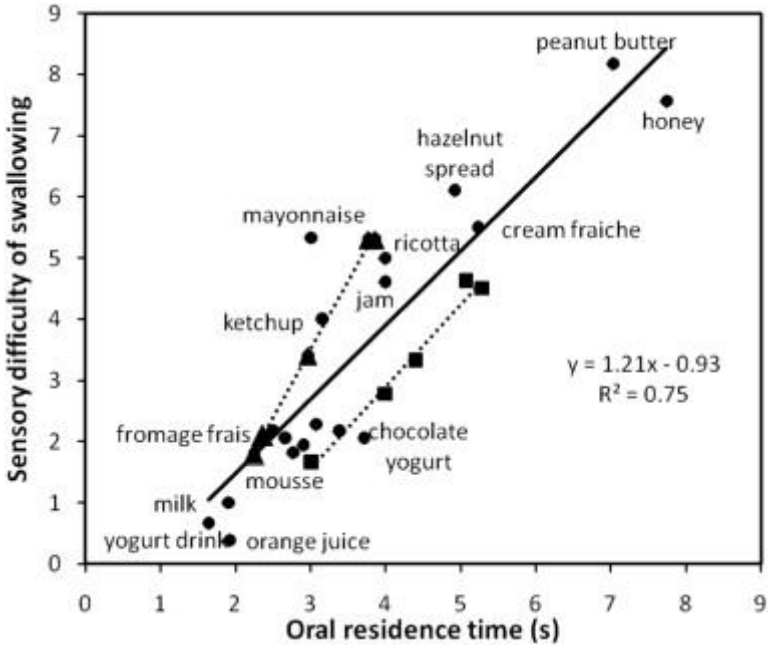


Figure 2-5 Correlations between the sensory difficulty of swallowing and the measured oral residence time. The solid line gives a regression of all tested food with a R^2 of 0.75. The two dashed lines are regression lines for the two sets of lab-constituted jelly (triangles) and custards (squares), with R^2 of 0.99 and 0.98 respectively. For the reason of legibility, only some of foods are marked in the graph [30].

Considering the concept of objective masticatory function, since humans have limited muscle strength and ability of creating oral and pharyngeal pressure, their hypothesis is quite obvious and logical. The food bolus should be processed to a physical state at which it is sufficiently deformable and flow-able so that it requires minimal oral effort (or a minimal amount of energy) for swallowing. They accredited fulfilment of such a physical criteria to particle size reduction and saliva incorporation, although it should be noted that in the case of most semi-solid and viscous liquid foods, interaction with oral surfaces (degree of mouth-coating), frictional and depositional phenomena, and shear-induced emulsification and flocculation may also play an important role [49].

Taniguchi [50] investigated the swallowing profiles of some highly consistent foods varying in viscosities and liquid contents using videofluorographic examination. Their observations indicated that foods with increasing agar powder contents (increasing viscosities) resulted in significantly increased swallowing times and oral ejection times, which was also the case with pharyngeal transit times. Prolonged oropharyngeal transit times for increased

consistency has also been confirmed by other researchers [51] [52]. In investigating whether altering bolus consistency helped swallowing, Raut [53] observed that increasing bolus viscosity led to prolonged clearing contraction of pharynx and increased amplitude of bolus-wave with reduced propagation velocity in the oesophagus and longer transit times, which are related aspects to increased oral energy expenditure.

All this evidence suggests that flow-ability of bolus influences oral actions related to swallowing, and moreover, the degree of flow-ability and its alteration by physical manipulation during oral processing could be factors related to the swallowing threshold. However, minimal flow-ability for bolus swallowing is an attribute which is dependent on food-type and its properties, and may vary with the objective masticatory function of individuals.

2.2: Physical Properties of Food and Related Phenomena during Oral Processing

Figure 2-6 shows the interactions among some food properties, different stages and functions of oral processing, mastication behaviour and food-related sensory perceptions. Food properties such as composition, microstructure, appearance, size and shape influence masticatory functions [55] [56] [57] [58].

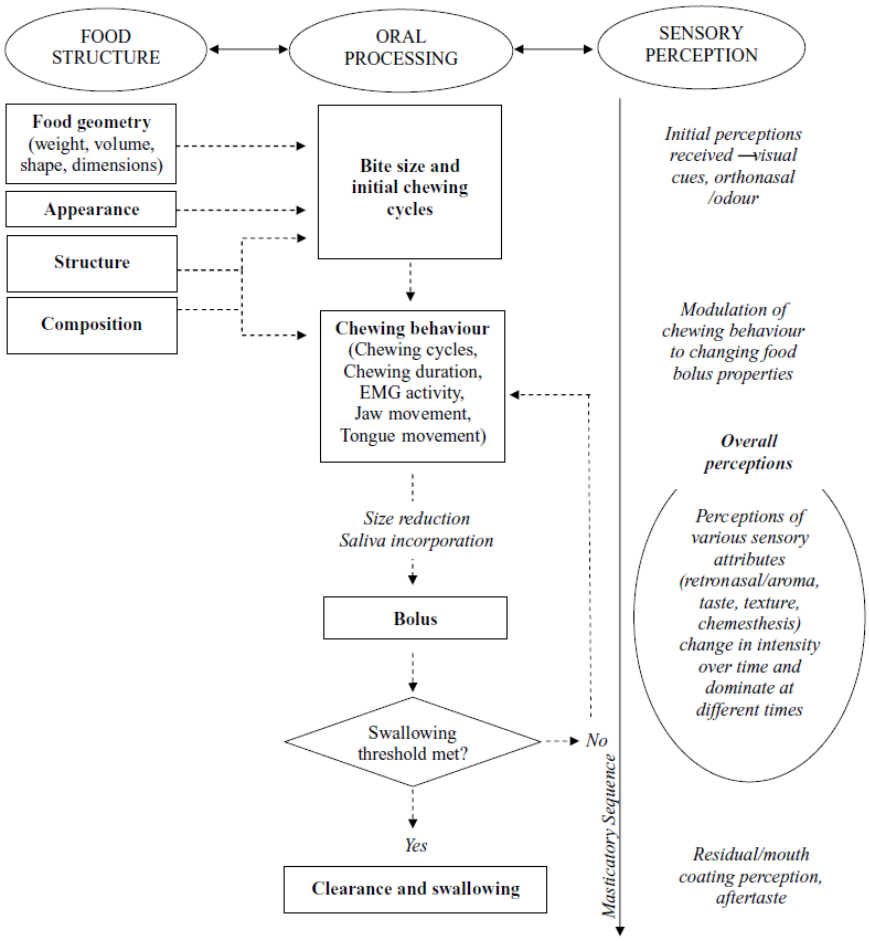


Figure 2-6 Overview of interactions among food properties, oral processing, and sensory perception [11].

At this stage, it is important to briefly consider the concept of structure-function relationship in foods in relation to oral processing. Food structure results from ingredients (composition), their characteristic interactions, and the processing they may undergo to form the 'desired' food product. Hence, these three factors also govern the physico-chemical properties (for example, mechanical, rheological and thermal behaviour) of the final food product. During oral processing of a specific food, its structure and related-properties not only manifest themselves as textural characteristics of that particular food, but also relate to a characteristic oral-transformation behaviour which the food will undergo until it is swallowed.

Hence, from a structure-function relationship point-of-view, a major consideration in controlling the functionalities related to texture and oral transformation behaviour of a particular food is that - one must control and/or acquire the desired structure, which in-turn is only possible by controlling the three factors - ingredients (composition), their interactions, and processing.

Also, as highlighted previously, apart from the perception of food texture, oral processing serves as an important step in efficient food-breakdown and lubrication related to safe-swallowing, and influences further digestion efficacy and nutrient release. Hence, quantifying physical properties of the food and related physical properties of the bolus during its oral transformation and at the point of swallowing would help mechanistically understand the structure-related sensory cues involved in texture perception and swallow initiation, and will also help better understand the physical-state of the swallowable bolus.

The following section discusses certain phenomena that occur during oral processing in their contextual relation with food properties of importance to this study.

2.2.1 The First Bite

The first-bite is normally seen as the beginning of the oral masticatory process. It is usually a one-biting process, and results in the sensory perception of a wide range of textural features [1]. The first bite includes partial or complete acquisition of a sample from a food product or may include the first chewing cycle where the subject is given a constant sized sample in an experimental situation [11]. This acquisition is facilitated by external assessment by sensory organs. Bite sizes and bite volumes vary among individuals and among foods; for example, females are known to have smaller bite sizes than males [59]. Size of first bite in semi-solid or solid foods is relatively larger compared with that of liquid foods. However, successive bites in liquids become gradually bigger, while for semi-solid and solid foods, the opposite has been reported [60]. All this seems mainly indicative towards the criteria of "comfort in oral-accommodation and mastication" which is subject- as well as food-dependent.

The amount of food accommodated in the mouth at first bite (bolus size) has a strong effect on how a bolus is dealt with inside the oral cavity [11]. Accommodation of larger amount of food or a larger bolus has been shown to be associated with a significant increase in the distance and range of chin movement during the masticatory sequence [39]. Examination of the effect of bolus size (1, 2, 4 and 8g) of soft and hard gums on kinematics of chewing, showed that 2g bolus gave the least within-subject variability. Increase in

thickness of the food and the volume acquired at first bite has also been shown to increase most masticatory parameters [55]. In a 45 subjects study to compare the parameters of natural bite sizes (bite-weight, bite-volume and bite-length) for manufactured heterogeneous food bars, Hutchings *et. al.* [61] reported that bite length was more consistent amongst food bars as compared to bite volume. Their work further suggested that constant bite volume might represent normal feeding behaviour better and be more appropriate than offering constant mass samples. Most of the studies highlighted above suggest that normal individuals are probably used to accommodate a specific quantity of food for the eating process, with too-large or too-small bolus sizes leading to non-habitual or irregular oral behaviour.

2.2.1.1 Hardness at First Bite

At the first bite, one of the most important physical properties of food which is assessed is hardness. Hardness is defined as a measure of resistance of a material to permanent (plastic) deformation [62]. In sensory terms, it can be defined as the resistance elicited by a food structure to the compression force applied on it between the molar teeth [63]. It is an intrinsic material property of a food structure which depends on the organisation and interaction of its structural elements, and may vary with extrinsic factors such as temperature and humidity of the surrounding [64].

For example, the hardness of solid chocolate is an important quality which is gauged by the consumer at "first-snap", either in the hand or at first bite. Chocolate hardness is largely governed by the crystalline morphology of its continuous fat phase and its degree of crystallinity (solid fat content). Cocoa butter is highly polymorphic, and to acquire desired qualities of sharp melting at oral temperature (37°C), hardness, surface gloss, contraction and stability, it is tempered and set in the β Form-V crystal morphology. Furthermore, a minimum 45% solid fat is necessary to produce acceptable, good quality and stable products. Alteration in either of these two components affects hardness of chocolate drastically. In milk chocolate, the fat phase may be a blend of cocoa butter and free-milkfat. The thermodynamic incompatibility of milkfat and cocoa butter, along with the presence of other milk components like milk powders, influences structural arrangement of the particulate and crystalline phase, and kinetics of blend co-crystallisation, in turn leading to softening and changes in melting behaviour [65]. As a result, milk chocolates are usually softer at first bite as compared to dark chocolates. Also for example, if stored at a relatively elevated or depressed temperature as compared to those recommended (e.g. 16-20°C), the solid fat content of the chocolate will change accordingly due to crystallisation or melting, leading to either too soft or

too hard products. These phenomena and their underlying compositional and structural basis have been reviewed in Section 2.3 and 2.4.

It has been found that perception of hardness at first bite is also dependent on the geometry of the food serving. Peyron *et. al.* [66] demonstrated that the perception of food hardness increased with an increase in sample thickness. Kohyama *et. al.* [67] studied the biting force and contact area at first bite using a multiple-point sheet sensor. They found that the peak force, contact area and peak stress for hard and brittle foods were greater in thicker samples. For soft and tough foods, the peak force and contact area increased as thickness increased, while the maximum stress remained similar. However, Agrawal and Lucas [68] argued that the effect of food geometry on hardness at first bite was work-related rather than magnitude of force. They proposed that thicker samples were associated with larger cross-sectional area, and therefore required higher fracture energy. Chen [1] explained that, since force and work are two parameters with different physical meanings, the above conclusions seem contradictory to each other. Furthermore, it was stated that it is not yet clear whether the human perception of hardness at first bite is based on applied force or amount of work, or even on the power (amount of work per unit of time).

2.2.2 Masticatory Sequence

2.2.2.1 Physical Properties of Food and Eating Behaviour

Masticatory muscles and jaw movements during the masticatory sequence are continually modulated as a response to changing physico-chemical properties of foods [69]. Eating behaviour is complex, and is the result of various physiological, anatomical and psychological factors [70]. Changes in physico-chemical properties of food will also alter chewing behaviour, processing and transformation of food in the mouth, and the occurrence of swallowing events [23].

With progress in mastication, it has been observed that the amplitude of vertical jaw movements tend to decline gradually [71], while the horizontal jaw movements also reduce with an increase in chewing cycles [72]. It has been reported that the length of chewing and number of chewing cycles vary hugely as per food-type (as well as among individuals) [26]. Brown *et. al.* [73] reported that apples and carrots are principally broken-down using vertical compression, whereas this is true for biscuits in the initial stage but transfers to shearing action over the later course. They also reported that as opposed to other foods, biscuits required larger oral muscle work after 6 to 10 cycles than they required initially. In general,

foods with firm-texture have been reported to be chewed between molars with slower jaw-closing velocities [74] and more lateral movement of trajectory compared with those of soft texture, which are ruptured between the tongue and palate [11].

An increase in hardness in foods of many different types has been shown to result in increased electromyographic (EMG) activity of the masseter and temporalis (per sequence and per cycle), number of chewing cycles, and duration of masticatory sequence [13] [55] [75] [76]. Dry and hard products have been found to require more chewing cycles before swallowing. More time is needed to fragment the food and to add adequate saliva to form a cohesive bolus suitable for swallowing [77]. Engelen *et. al.* [78] measured chewing cycles for a wide range of foods (peanuts, cheese, cake, bread, toast, carrots) for which they also determined the mechanical properties. They concluded with the finding that harder and drier foods required more chewing cycles to process into a ready-to-swallow bolus. This also agrees with observations of Fontijn-Tekamp *et. al.* [79] who studied chewing behaviour of 87 subjects eating cheese, carrot and peanuts. Similar conclusions have also been made by Wilson and Brown [80] who investigated chewing of model gelatine gels. Engelen *et. al.* [78] also explained that physiological parameters (saliva amylase content, saliva flow rate, maximum bite force and masticatory performance) explained less than 10% of the variance in swallowing threshold, whereas the dominant factor resulting in variation of oral processing was the rheology of food.

Recently, Carvalho-da-Silva *et. al.* [28] investigated the eating behaviour of chocolate in a first of its kind study. They investigated chewing and swallowing activity for 2 milk chocolates which were formulated to have similar composition, comparable melt viscosities, but different textural attributes, using electromyography and electroglottography. Unfortunately, they did not disclose any details of composition, processing or the above mentioned textural functionalities of the chocolate due to confidentiality reasons. They found wide inter-subject variability in chocolate eating behaviour. However when analysing if eating behaviour changed between chocolates, they found significant differences in parameters of total number of chews, total chewing time, time of last swallow and total number of swallows, wherein values of these parameters were larger for the more mouth-coating and harder of the two chocolates. Their results also suggested that the behavioural traits of chewing and swallowing for subjects showed no change between chocolates i.e. subjects retained their 'general pattern' of eating across the chocolate samples.

2.2.2.2 Fracturing and Breakage

Apart from its influence on the first bite, fracturing and breakage behaviour of foods may also influence the bolus formation process during the subsequent masticatory sequence [54]. These phenomena are of particular importance in solid foods, including complex foods like chocolates which are solid at oral acquisition, break into smaller particles during the initial few chewing cycles, and subsequently melt into a viscous liquid bolus. The rate of particle size reduction firstly depends on the probability of a food particle being contacted with the teeth (oral selection/selection function), and the extent of fragmentation upon application of stress (breakage function) largely dependent on the mechanical properties of the food and dental performance of the subject [10] [58].

A food of high breakage function would imply that it is easy to masticate and requires less chewing [1]. Agrawal *et. al.* [86] tested 28 foods from 3 product groups (cheese, nuts and raw vegetables) for their fragmentation behaviour and reported linear relationship between breakage function and mechanical properties of foods, in particular Young's modulus (R) and toughness (E). Lucas *et. al.* [10] furthered this study to 38 foods of different mechanical properties and constructed a master curve for correlation between breakage function and $\sqrt{R}/\sqrt{E}$ (Figure 2-7). Hard and brittle foods like sugar crystals and nuts were found to have a high breakage function but very small values of $\sqrt{R}/\sqrt{E}$. Soft foods like most cheeses, cakes, and bread had the highest values of $\sqrt{R}/\sqrt{E}$ but the smallest breakage functions. Foods with values of $\sqrt{R}/\sqrt{E}$ above 25 $mm^{1/2}$ were found to be plastically distorted rather than broken into discrete fragments. For such soft and easily deformable foods, mechanical properties seem to have very limited influence on fragmentation behaviour.

While these studies show that the mechanical properties of the selected foods correlate with fragmentation behaviour, there is currently no way of predicting the fragment size distributions. Chen [1] highlighted some drawbacks in the above discussed approach. The researchers have excluded the influence of saliva in determination of breakage function and rheological properties which, if allowed, may have changed the food properties due to moisture absorption, dilution of liquid phase, fat melting or dissolution of solids.

Saliva affects the rheological properties of foods, meaning the fragmentation behaviour of the food will change during mastication. Furthermore, temperature could not be excluded from the above experiments. High fat foods warm-up, soften, and then melt as chewing proceeds (which will occur faster if mixing with saliva occurs). Chocolate is a good

example, where the breakage function progresses from fracture to fragmentation to melting and mixing with saliva (viscous liquid flow).

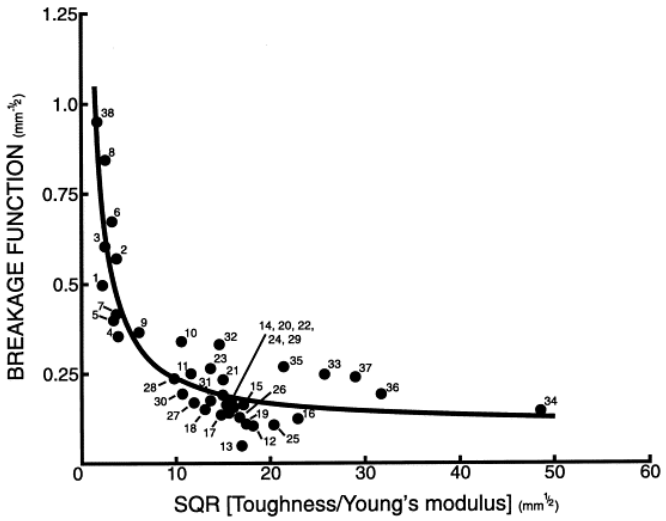


Figure 2-7 The correlation between the breakage function and the mechanical food property group $\sqrt{\boldsymbol{R}}/\sqrt{\boldsymbol{E}}$ for 38 foods; 1-9 are nuts; 10-27, cheeses; 28-32, fruits and vegetables; 33-36, breads; 37 – a type of soybean curd; and 38, monocrystal sugar [10].

Theories relating mechanical properties to fragmentation are best applied to homogeneous solids with linear stress-strain relationships. For this reason Agrawal *et. al.* [86] may have limited their study to homogeneous food structures. Finally, for foods like chocolates, effect of temperature is very important as phase change occurs in the temperature range between product temperature before consumption and the temperature in the mouth. For such foods, the investigation of breakage function may be valid only over the first few chewing cycles before the fat melting is initiated. This approach requires separation of bolus particles and quantification of particle size, which may be very difficult, if not impossible for foods like chocolate. Nevertheless, the approach of investigating the link between mechanical properties and breakage behaviour may prove very useful. The breakage function over the initial few cycles may provide an understanding of how chocolate mechanical properties influence its oral breakage behaviour (particle size distribution and particle surface area) which in turn influences rate of melting and bolus formation.

2.2.2.3 Mixing and Shearing by Oral-Flow

Information on mixing and shearing conditions generated by flow in the mouth is essential for understanding the oral mechanisms of bolus formation and sensory perception. However, due to the complex geometry of the mouth and the complexity of the motions of the oral surfaces, the mixing and flow conditions in the mouth are difficult to quantify [87]. Moreover, the movements and forces of the tongue applied during mastication are highly variable, and are

adjusted by consumers according to food-type. Since many foods have a complex rheological behaviour (shear thinning or display yielding behaviour), flow itself modulates the product while it is processed in the mouth. Also, food materials are mixed and diluted with saliva in a rather inhomogeneous manner [87].

For Newtonian liquids perceived thickness (T) can be directly related to viscosity (η) by the Stevens power law: $T=k\eta^n$, with n=0.2 [88]. Most foods however demonstrate non-Newtonian behaviour i.e. apparent viscosity is shear-rate dependent. Shama and Sherman [89] compared the rated thickness of a range of foods with a wide variation in viscosity and shear-thinning behaviours. They showed that a certain thickness rating corresponded to a window of shear stresses and shear rates. When shear rates are plotted against shear stresses, the ensemble of all windows forms a curved region, with one branch corresponding to low viscosity (<100 mPa) systems relating to low shear stresses (typically 10 Pa) and higher shear rates (> 100 s^{-1}), and the other branch corresponding to high viscosity systems and shear rates between 10 - 100 s^{-1}. It was thought that the regime in the former branch related to systems that would spread in the mouth by gravitational/inertial forces, while for that in the latter, force applied by tongue is required to spread/displace the food in the mouth. The effective shear rate by which the food consistency is judged in the mouth in this way depends on the rheological behaviour of the food itself.

Subsequently, van Vliet [90] highlighted the implicit assumption of shear flow in the mouth during mastication as one of the main drawbacks to the approach by Shama and Sherman [89]. He explained that the flow pattern during mastication will vary in different regions of the oral cavity, and in many cases extensional and/or turbulent flow patterns may be more valid as compared to steady flow. Furthermore he also hinted towards possible effects due to mixing with saliva which could be factors like dilution effect, solid loss due to dissolution of sugars and starch hydrolysis by action of salivary amylase. In a study involving analysis of spit-outs of oil-water mixtures, De Bruijne *et. al.* [91] reported that mm-sized droplets could be broken down to droplets with diameters of 20 – 30 μm, corresponding to shear stress exerted on the droplets of approximately 50 Pa.

2.2.2.4 Interaction with Oral Surfaces and Frictional Phenomena

The interaction of food components with oral surfaces is not only important for understanding sensory perceptions, but is important in governing the clearance behaviour of food during and after the masticatory sequence [81] [87]. Malone *et. al.* [49] highlighted the

importance of considering properties such as colloidal, bulk-rheological and thin-film rheological behaviour towards this aspect.

Oral viscosity not only plays an important role in texture perception of fluid and semi-solid foods, but also influences retention and work required in clearance and swallowing. Although studies have been performed to investigate the correlation of sensory perception of thickness with the rheological (small and large deformation) measurements of foods [49], there are very limited studies which have attempted to investigate such relationships for fluid or semi-solid foods by measurements of the bolus per se. Although, such studies would provide very useful quantitative information on transient bolus properties during oral processing, high correlation between thickness perception and rheological properties of foods demonstrated by many authors, nevertheless is indicative of oral behaviour of these foods [88] [92].

A number of studies have attempted to investigate the shear rates acting in the mouth so as to account for non-Newtonian shear-thinning behaviour of many foods [88] [89] [93] [94], nevertheless authors have noted that there is still much speculation as to what shear conditions actually occur in the mouth, and how they vary with the physical properties of foods [54] [49]. As mentioned earlier, Shama and Sherman [89] found correlation between thickness perception and instrumental parameter of viscous force and reported that a range of shear rates between 10-1000 s^{-1} depending on the shear-thinning behaviour of particular foods, have to be considered. For example, the shear rates occurring in the mouth ranged from 5 s^{-1} for products such as hard margarine to 37 s^{-1} for more fluid foods like tomato ketchup [89] [94]. Furthermore, measurements at oscillatory frequency of 50 rad s^{-1} in dynamic small deformation experiments have also shown good correlation with thickness of a wide range of Newtonian foods, flocculated solutions, weak gels, lemon-pie fillings, and stickiness and sliminess for fluid, semi solid and shear-thinning foods [92] [95].

Carvalho-da-Silva [96] investigated bolus microstructure and frictional behaviour of two milk chocolates which had identical composition and melt viscosities, although differed significantly in mouth-coating and clearance-time from the oral cavity. Their study revealed that regardless of no significant differences in saliva incorporation, the more mouth-coating and less flocculated (bolus) chocolate melted faster, as they concluded that flocculation hindered heat transfer in the mouth. They also demonstrated that the more mouth-coating chocolate had a higher friction coefficient (in mixed regime of the Stibeck curves) indicating

pronounced interaction of "interfacially active" chocolate ingredients in this chocolate with instrumental/oral surfaces.

Adams *et. al.* [81] studied the distribution of food material in the oral cavity and clearance behaviour using video rate endoscopy. They showed that the amount of residue on the oral cavity after processing of polysaccharide solutions was dependent on their viscosity. They also reported that the clearance behaviour was highly dependent on saliva flow rates. De Jongh and Janssen [97] analysed oral surface residues of mayonnaise dressings containing 40% fat, 10-14% protein and starch and/or xanthan thickeners. Their results indicated gradual clearance of various components from the mouth; oil within 2 - 4 min irrespective of dressing-type, oral location or subject, and protein and carbohydrate following the same clearance pattern as fat, although with some exceptions. Prinz *et. al.* [98] implemented an approach of analysing the turbidity of rinses with water after swallowing custard desserts varying in fat contents and thickener concentration. Their results demonstrated high correlation between turbidity (of first rinse), food viscosity and perceived thickness, creaminess and fattiness.

Studies have also been performed to understand how food properties influence lubrication behaviour using in mouth models. Malone *et. al.* [49] demonstrated that the oil content and viscosity strongly influenced friction between rubber surfaces lubricated with oil in water mixtures not stabilised by emulsifiers. Dresselhuis *et. al.* [99] showed that protein-stabilised oil-in-water emulsions which had low stability towards shear induced coalescence (e.g. due to low protein emulsifier content or due to presence of crystallised fat within the droplets) had a large tendency to coalesce and form bigger droplets during oral processing, as observed from expectorated emulsions. Moreover these emulsions also showed increased perception of creaminess and fattiness, decreased orally perceived and instrumentally measured friction, attributable to increased interaction of lipids with oral or instrumental surfaces.

2.2.2.5 Oral Processing of Non-Emulsified Lipids

Examining the manner in which the physical form of non-emulsified "free" fat changes during oral processing would be essential in gaining insights about not only fat continuous food matrices like chocolate, but also into changes associated with non-emulsified oil contents of many foods. While there are extremely few studies on oral processing of unemulsified fat systems, some research is present which examines changes in physical form of bulk-oils differing in viscosities (corn and castor oil) during oral processing using video-endoscopy [81].

Here, the authors reported coarse oil-in-water emulsion formation in the remaining intra-oral fluid, those from lower viscosity oil having slightly smaller droplets. In situations where the oil volume is in excess of that of saliva (typically > 10 ml), a coarse water-in-oil emulsion is formed. This may persist even after 10 min of rinsing the mouth, before inverting to an oil-in-water form with droplets sized between 10 - 40 μm. Correspondingly, the initial formation of a water-in-oil emulsion may result either from low initial volume of aqueous phase or from some limitation of the emulsifying action of saliva [82].

Along with the shearing induced by the tongue and palate, the mouth can also promote biochemical emulsification for free fats. Studies have shown that a number of salivary proteins exhibit surface activity, and hence can form interfacial films with high degree of elasticity [83] [84]. Furthermore, amphiphilic ingredients in many foods, such as emulsifiers or surface active biopolymers, can also augment formation of emulsions. These mechanisms may also operate when foods that contain proteins and free-fat, such as butter and chocolate have been chewed or sufficiently melted to cause the oil to separate from the matrix.

Such mechanisms have been demonstrated for margarine, which may contain up to 80% fat as a crystalline network stabilising dispersed water-droplets. Oral processing at 37°C melts the fat network allowing mixing of water and saliva. Shear forces in the mouth initially promote rapid coalescence of the dispersed phase, and upon sufficient coalescence, the contained milk proteins are able to facilitate inversion to an oil-in-water form [85]

2.2.3 Properties of a Ready-to-Swallow Bolus

Particle size distribution [100] [101] [102], moisture content [78] [103], and recently, bolus mechanical/bulk-rheological properties [30] [21] [104] have been the commonly investigated characteristics of a ready-to-swallow food bolus. These characteristics give an overall understanding of bulk-properties of the food bolus which, individually or in coalition, are involved in defining the point of swallow.

The particle size distribution (PSD) of a ready-to-swallow bolus is highly food-dependent [11] [19]. Peyron *et. al.* [19] and Mischellany *et. al.* [101] investigated the particle size distribution of food boluses after mastication of different types of raw vegetables and nuts. They showed that the PSD in the ready-to-swallow boluses was significantly different from one food-type to another, but similar for a given food-type across all subjects. These end-point similarities were contrasted by the large variability in intermediate measurements of

masticatory parameters like amplitude of mandibular movements, chewing strokes and degree of EMG activity during cycles [55].

These studies were furthered by Jalabert-Malbos *et. al.* [102] who included gherkins, coconut, cheese, chicken breast, egg white, ham, green olives and mushrooms in their investigation. Again, major particle size variations were observed only among foods, as opposed to among subjects. Hardness of the food appeared to be correlated with the average particle size in the ready-to-swallow bolus. Hard and brittle food bolus in general had smaller particles as opposed to boluses of soft and deformable foods which had much larger particles. Studies highlighted above suggest that food properties play a vital role in bolus formation and swallowing. Moreover, their results suggested that individuals may employ different mastication strategies depending on food-related and/or psychological, physiological and anatomical factors to reach a common end-point, i.e. a safe-to-swallow bolus. No significant intra-individual variability and only a very small inter-individual variability encountered in these extensive studies suggest that food boluses have to comply to specific conditions before swallowing is triggered.

Although recognised critical in producing the stimulus marking both end-of-mastication and starting-point of swallowing, particle size of the food bolus does not account for other determinants of the swallowing threshold like lubrication, flow-ability and stretch-ability, and cohesiveness [30] [75] [46] [18] [47]. Nevertheless, it obliviously acts as a structural factor governing the mechanical/rheological state of the ready-to-swallow bolus [101].

The ability of lubrication and plasticity of saliva due to the presence of salivary mucins, proline-rich proteins and staterins, and the presence of high quantity of water (on average 98%) has been reported previously [105]. Moisture incorporation in foods after mastication has been investigated in several studies and seems to be food-dependent [14] [100] [106] and influenced by the dry matter content of food [103]. It is reasonable to say, after keeping the principal roles of saliva during oral processing in mind, that mastication continues until the moisture content of a mouthful leads to a specific physical state in a food bolus which becomes suitable for deglutition. Loret *et. al.* [104] studied the moisture incorporation after mastication of cereal boluses. The test cereals were prepared with different toasting and rolling treatments and differed in hardness, thickness, moisture content and textural properties. Their results suggested that regardless of the differences in initial moisture levels and physical properties of the cereals and the use of distinct mastication strategies by test subjects, the water content in the swallowable boluses were very similar at around 50 wt%.

Chen [14] reported the differences in saliva incorporation in swallowable vegetable and nut boluses. On an average, boluses of nuts (roasted peanuts and macademia) contained 36 ± 10%wt to 44 ± 9%wt saliva, as compared to 17 ± 8%wt in raw carrot boluses. He suggested that because the hard and brittle nut particles are more difficult to swallow, they require more lubrication to wet and cluster particles, while the differences of saliva incorporation within different nut may depend on their initial oil content. Engelen *et. al.* [78] studied the moisture incorporation in boluses of buttered/unbuttered – bread, toast, melba toast, breakfast, and in boluses of peanuts and cheese. They reported significant differences in number of chewing cycles (ranging from 17 for cake to 63 for carrots), with hard and brittle foods needing more chewing cycles before swallowing. Saliva incorporation was significantly and negatively correlated with the number of chewing cycles for melba toast and cake. Hence subjects with more saliva needed less chewing cycles for dry foods. They concluded that hard and dry foods required higher number of chewing cycles and longer residence times in mouth for sufficient breakdown to occur and for enough saliva to be added to form a cohesive bolus.

Similar findings have been demonstrated by Mioche *et. al.* [107] who reported higher saliva incorporation (mean weight increase: 36%) in tough and dry meats which required higher masticatory muscle activity, chewing cycles and chewing duration as compared to tender and juicy meats (mean weight increase: 30%). In a study to investigate the relationship between saliva and food bolus properties from model dairy products (model chesses, with or without rennet, varying in ultrafiltered skim-milk powder and anhydrous milkfat), Drago *et. al.* [103] reported that the saliva incorporation in boluses followed the same order as the water content of initial products, i.e. the higher the dry matter content of the food was, the higher dry matter content the bolus was. Non-renneted samples had lower saliva incorporation in boluses, probably due to less or no requirement of mastication and very low oral-residence times. When related to dry matter sample content, they observed higher saliva incorporation in boluses for non-fat samples than fat samples.

Research in to understanding bolus rheological properties at the point of swallowing is relatively new, and hence, investigations seem to be limited. Nevertheless, rheological properties of a bolus at any stage of the masticatory sequence are not independent of the other two components i.e. particle size distribution and saliva incorporation, and moreover are dependent on the food-type [1]. Recently, Loret *et al.* [104] reported that as the optimal rheological profiles of boluses of different foods is not known, inherently there is lack of understanding of the physical mechanisms underlying their swallowing. There appears to be

some consensus among authors that the food bolus should be viscous, plastic, cohesive and sufficiently deformable to be safely swallowed [18] [47] [108] [109] [110], although only a handful of studies have attempted to investigate rheological characteristics of a ready-to-swallow bolus due to the difficulties associated with making these measurements.

Approaches present in literature pertain to: numerical simulation of swallowing [110] [111], characterisation of slipperiness and compliance of food bolus [112], texture profile analysis (TPA) to extract bulk-rheological properties of boluses, small and large deformation characteristics of boluses [103] and recently, controlled rheological methods employing either viscosity or dynamic-viscoelasticity measurements of "simulated" boluses of semi-solid and liquid foods [30] [48], or oscillation and rotational measurements of cereal boluses to evaluate storage (G') and loss (G") moduli of expectorated cereal boluses [104].

Drago *et. al.* [103] demonstrated that saliva incorporation in model dairy products is influenced by initial food properties and in turn impacts rheological properties of a ready-to-swallow bolus. They demonstrated that both subject- and food-type had significant effect on saliva incorporation and almost all bolus rheological properties. As mentioned earlier, their model dairy products differed in composition which influenced their rheological properties (firmness, adhesivity, cohesiveness, G' and G"). They tested bolus rheological properties of spreading ability, work of adhesion, work of spreading, and rigidity using compression analysis by a texturometer and observed direct relationship between G', firmness and adhesivity: bolus self-standing (initial height) (r^2 =0.891 for G', r^2=0.797 for firmness and r^2=0.795 for adhesivity); work of spreading (r^2 =0.835 for G', r^2=0.693 for firmness and r^2=0.807 for adhesivity); bolus rigidity (r^2=0.830 for G', r^2=0.666 for firmness and r^2=0.930 for adhesivity).

Peyron *et. al.* [21] studied the rheological properties of wheat-flake breakfast cereal bolus to investigate whether change in these properties during the initial masticatory sequence, or those at the point of swallow were involved as sensory inputs governing swallowing. Subjects chewed the cereals and expectorated the bolus at different stages of the masticatory sequence corresponding to fractions of the complete sequence and at the first natural swallow-point. During the course of mastication, the hardness decreased whilst the cohesiveness, adhesiveness and springiness all increased. Two compression tests were performed, either at 25% or 65% deformation. For both deformation tests, there were significant differences between boluses for the four rheological characteristics but differences in hardness and adhesiveness were more distinct at 65% deformation. Differences in springiness and cohesiveness were more profound at 25% deformation.

In their study it was observed that the modification of boluses even occurred just before the point of swallowing, and in fact the changes in rheological properties were more pronounced towards the end of the masticatory sequence. This led them to conclude that these changes could be involved as strong candidates in swallow initiation. As D_{50} and hardness changed little from the middle of the masticatory sequence, they also hinted that particle size and hardness were not the only decisive factors in the swallowing threshold. It was suggested that particle size and saliva incorporation and lubrication are important as initial contributing factors by which the final rheological state at the swallowing threshold is reached. Although, multiple thresholds critical in swallowing may not be achieved simultaneously for a food bolus; swallowing threshold is probably an integrative process which combines various bolus properties enabling safe swallowing of the food bolus.

2.3: Chocolate – Composition, Processing and Structure

Chocolate making is over a century-old process which relies heavily on the initial quality of the cocoa beans and their processing, ingredient mixing, refining, conching as well as meticulous control of cocoa butter (fat phase) crystallisation [113] [114]. From a colloidal perspective, chocolate can be defined as a concentrated suspension of fine solid particles of sugar, cocoa, and milk powder (in the case of milk chocolate), about 70% total, in a continuous fat phase [115]. Cocoa solids i.e. cocoa particles and cocoa butter are derived from beans obtained from pods of *Theobroma Cacao*. There are three main types of cocoa obtained from cocoa growing countries with suitable climatic conditions, located 20° north and 20° south in the equatorial region [116]. The world production is dominated by the *Forastero*-type, made up of small, flattish and purple beans. Another type, *Criollo*, is rare in production; *Trinitario*, a disease-resistant hybrid of Criollo and Forastero, regarded as a flavour bean [117], is about 3% of world production. Growth of Forastero in the trade-name basic or bulk-cocoa occurs mainly in the countries of Côte d'Ivoire and Ghana in Western Africa, and Brazil in South America. The Caribbean Islands, Nigeria, and some South East Asian countries namely, Indonesia, Malaysia and Papua New Guinea make-up for the rest of cocoa cultivation around the world. Rich, deep, well-drained, equatorial soil resulting from plentiful rainfall (1000 – 2000 mm), high average temperature (> 27 °C) and constantly high humidity are essential for cocoa plantation, and are factors unique to these equatorial countries [113]. In 2009-10, Western Africa accounted for over 70% of the 3.6 million tonnes of cocoa produced around the world [118]. As of June 2012, the daily price of cocoa beans averaged about £1500 (SDRs/GBP) per tonne on the London LIFFE [119], while the top ten chocolate and confectionery multinationals recorded total net confectionery sales of around £47 billion in 2011 [120].

2.3.1 Composition of Dark Chocolate

Dark chocolate is a colloidal suspension of 65-70% cocoa particles and sugar in continuous fat (cocoa butter) phase [121]. Cocoa particles and cocoa butter are constituted in to the matrix through cocoa liquor, while sugar as well as additional cocoa butter is added subsequently in the process. Particle size of sugar and cocoa particles is controlled to < 30 μm to avoid grittiness and obtain a smooth in-mouth flow. The fat matrix is tempered to obtain crystal homogeneity of polymorphic β Form-V distribution for desired surface gloss, hardness, product stability and melt-in-mouth character [122]. A quantitative relationship of the

compositional recipe of dark chocolate can be defined considering the basic ingredients used in its manufacture i.e. if the proportion of ingredients (in %) are -

S, sugar; *B*, cocoa butter; *M*, cocoa liquor; and *L*, Lecithin

then,

$$S + B + M + L = 100 \qquad 2.5$$

Considering the total content from cocoa source as C;

$$C = M + B \qquad 2.6$$

As a consistency and product quality requirement, the total fat content (F) is between 30 and 40% [123], i.e.

$$F = L + MC_M + B = 30 \rightarrow 40 \qquad 2.7$$

where, C_M is the cocoa butter content (mass concentration) of the cocoa mass (*c.* 0.50 – 0.56). The usual value of *S* for industrial manufacture of dark chocolate is 30 – 50%, and L is 0.2 – 0.5%. Typical formulations of dark chocolate are shown in Table 2-1

Table 2-1 Typical formulations (%) for dark chocolate [124]

Ingredient	Recipe 1	Recipe 2	Recipe 3
Sugar	36	30	39.6
Cocoa Mass (50%)	60	70	46
Cocoa Butter	3.7	0	14
Lecithin	0.3	0	0.4
TOTAL	100	100	100
Cocoa Content	63.7	70	60

2.3.2 Composition of Milk Chocolate

Milk chocolate differs from dark chocolate notably through the presence of milk solids (milk powder) and milkfat. Table 2-2 illustrates a typical formulation of milk chocolate as compared to dark chocolate. The following equation (in %) is valid for milk chocolate formulation –

$$S + B + M + L + W + b = 100 \qquad 2.8$$

where, *S, B, L,* and *M* are as stated above. *W* is the percentage of milk powder and *b* is the percentage of dry milk fat which is optional. Milk chocolate usually contains 40-45% sugar (*S*), and 0.3-0.5% lecithin (*L*).

Table 2-2 Typical formulations (%) for dark and milk chocolate [125]

Ingredient	Dark Chocolate	Milk Chocolate
Cocoa Liquor	40	12
Cocoa Butter	12	19
Milk Powder	-	20
Sugar	47.5	48.5
Lecithin	0.5	0.5

$C = M + B$ remains valid for cocoa content of milk chocolate, and furthermore, taking into account consistency requirements, the total fat content is usually between 30-40%, i.e.

$$F = L + {}_{C_M} M + B + {}_{C_W} W + b = 30 \rightarrow 40 \qquad 2.9$$

where, C_{M} is the mass concentration of milk fat of the milk powder (c. 0.26-0.27). An additional requirement for consistency in milk chocolate is the ratio of cocoa butter to non-cocoa butter fat (R; mass/mass). This governs the degree of fat eutectics responsible in softening of chocolate. As laid down by authority [126], the milk fat content should be at least 3.5 m/m%, with exceptions of 2.5 m/m% made for milk chocolate targeted for sale in tropical climates. The usual values of milk fat content range between 3.5-6.0%; hence, the value of $R + 1$ can theoretically vary as follows –

$$\frac{40}{3.5} \approx 11.4 \geq R + 1 \geq \frac{30}{6} = 5 \quad \text{i.e.} \quad 10.4 \geq R \geq 4 \qquad 2.10$$

The sensory attributes and flow properties of chocolate are strongly dependent on the size, shape and distribution of the particles in the chocolate matrix. Central to sensory character is also the continuous lipid phase, which influences mouth-feel and melting properties. Cocoa butter is an important ingredient for chocolate, accounting for up to 30 to 40% (m/m) of chocolate and binds the other ingredients, forming a crystalline matrix of a stable polymorphic form distribution. It exhibits brittleness below 20°C, and begins softening at 30 – 32°C. It is tasteless, shows sharp and complete melting and Newtonian flow behaviour near body temperature. It plays a key role in giving the chocolate its sharp melting profile and gloss, and dictates its characteristic snap-ability [127]. Cocoa butter may also in part be replaced by milkfat and/or cocoa butter equivalents (CBEs), with permitted quantities governed by legislation.

Chocolate triglycerides are dominated by saturated stearic (34%) and palmitic (27%) fatty acids and monounsaturated oleic acid (34%) [128]. Chocolate is solid at ambient

temperature (20 - 25°C) and melts at oral temperature (37°C) enabling particles to flow past one another and form a smooth liquid in the mouth during consumption [113] [129] [130]. This constrains the types of lipids that can be used. The oral epithelia are sensitive to gradations of smoothness which selects for desirable lipid crystal forms. Differences in the sensory characteristics of these chocolate types can be mainly attributed to the variations in ingredient proportions, blending and processing techniques, and the use of different cocoa-types, and milk powders. Specifications depend on the type of chocolate being produced and its intended use [131].

As a unique food appreciated by many, chocolate provides a sweet taste, comfort and increasingly, a number of health benefits. Despite the high lipid and sugar composition, chocolate consumption at appropriate levels makes a positive contribution to health as a source of antioxidants, mainly polyphenols which predominantly include cocoa flavonoids such as catechin, epicatechin and procyanidins [132] [133].

Chocolate as a luxury food is taken very seriously by its consumers and its perceived quality is deemed to be of particular importance. Composition of chocolate products is controlled by specific regulations in addition to general quality and safety criteria in the European Union and other major countries [134]. The latest EC Community Directive establishing standards for composition and labelling of chocolate and chocolate products is Council Directive 2000/36/EC. The key compositional requirements for chocolate products define the minimum percentage contents for dry cocoa solids, dry non-fat cocoa solids and for cocoa butter. The Directive defines dark (plain) chocolate as – "*a product obtained from cocoa products and sugar, containing not less than 35% total dry cocoa solids, including not less than 18% cocoa butter, not less than 14% of dry non-fat cocoa solids",* and milk chocolate as – "*a product obtained from cocoa products, sugars and milk or milk products, which contains not less than 25% total dry cocoa solids; not less than 14% dry milk solids obtained by partly or wholly dehydrating whole milk, semi- or full-skimmed milk, cream, or from partly or wholly dehydrated cream, butter or milk fat; not less than 2.5% dry non-fat cocoa solids; not less than 3.5% milk fat and; not less than 25% total fat (cocoa butter and milk fat)".*

In both products a maximum of up to 5% vegetable fat/CBEs are permitted by regulation, some of which are – illipe, shea, palm-oil, sal, kokum gurgi and mango kernel [113].

2.3.3 Chocolate Processing

2.3.3.1 Initial Stages

Cocoa harvesting operations till date remain traditional, small-holding scale, and are carried-out manually by skilled workers. Beans are removed from the cocoa pods by hand, and at this stage, could contain up to 65 wt% moisture [116]. They reach maturity after 4 - 6 months and contain two cotyledons (nibs) that yield cocoa mass for chocolate manufacture, or when pressed, cocoa butter and cocoa powder [135]. Fermentation techniques, namely heap fermentation and box fermentation, and further cocoa processing result in complex chemical reactions, which are necessary for good final flavour and textural properties in chocolate [116] [117]. The type of fermentation technique used, contributes towards the characteristic flavour-notes of chocolate. It results in complex aerobic and anaerobic microbial reactions and chemical changes as a result of enzymatic and protein-polyphenol reactions [123]. These have been reviewed in detail by Fowler [135] and Dimick and Hoskin [136]. Drying limits mould growth during transportation and storage, reducing bean moisture content to around 8%. Sun drying is favoured for flavour development and can be carried out above or on hard surfaces, with differences in air-flow [137] and final moisture content [135]. Reduction and control of moisture is necessary for safe transport of beans across the Atlantic to chocolate manufacturing industries. They are transported under controlled storage conditions to chocolate manufacturing sites, or processed in the country of origin to add value with requirements for traceability in quality assurance [116].

2.3.3.2 Cocoa Bean and Liquid Chocolate Processing

As noted previously, the most basic form of chocolate is dark chocolate which essentially only contains cocoa liquor (cocoa particles and cocoa butter), sugar and cocoa butter. It may also contain added flavours such as vanillin. For the manufacturing of milk chocolate, milk powder and milkfat are eventually added. Milk chocolate may typically contain more sugar, and as a consequence, less cocoa solids. Chocolates may also contain emulsifiers which act as surface active agents to stabilise the blend of hydrophilic sugar and the hydrophobic fats.

A typical manufacturing process is illustrated in Figure 2-9, and can be described as follows -

1. Fermented, dried and cleaned cocoa beans are roasted for removal of moisture and development of flavour. There are three main methods for roasting – whole bean roasting, nib

roasting and liquor roasting. Figure 2-8 illustrates the differences in sequence of unit operations between these roasting methods. Roasting is carried out either as a batch process or a continuous process, and involves contacting beans/nibs with hot metal surfaces or zones with controlled hot air circulation [138]. As a result of roasting, moisture content falls to < 3% and Maillard reactions of amino acids from fermentation protease activities yield flavour-active aldehydes from precursors with chocolate flavour and aroma notes. Roasting temperature (90 – 140°C) and time (45 – 60 min for whole bean or nib roasting; 2 – 5 min for liquor roasting) influence nib composition, and so does rate of moisture loss and whether moist or dry roasted [117].

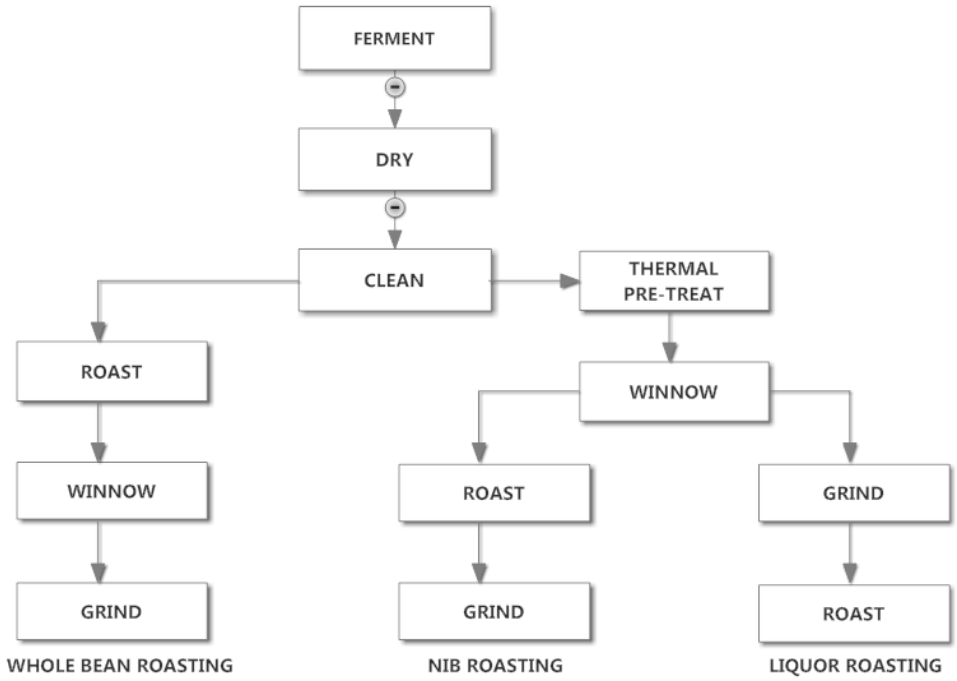


Figure 2-8 Flow diagram illustrating three different methods of roasting cocoa.

2. After roasting, the nibs or the liquor is subjected to grinding. The main purpose of grinding is to make the cocoa particles small enough so that they can be made in to chocolate. Grinding of nib cells also releases cocoa butter fat forming cocoa liquor with particle size up to 30 – 50 μm, while for production of cocoa powder, fine grinding is particularly important. Nib and liquor grinding in modern chocolate manufacturing industries is mainly done in impact mills, disc mills and/or ball mills [138].

3. Three pathways can be used from here onwards:

A) Typically, 70 – 90% cocoa butter can be collected by pressing cocoa liquor. The highest quality cocoa butter for chocolate manufacture is obtained by pressing the liquor in a horizontal press. The press operates as a continuous operation, wherein 40 – 50 MPa pressure results in separation of a press cake containing 8 – 24% fat. Cocoa powder is produced by milling the press cake. Most cocoa powder is produced with a fat content of 20 – 25%. Lower fat ranges are also available, e.g. 15 – 20% or 10 – 12%. Fat-free varieties are also produced and sold for low-fat or fat-free products [139].

B) The cocoa liquor can be mixed with other ingredients such as sugar, cocoa butter, milk fat, vanillin, milk powder (or evaporated liquid milk) and emulsifiers (typically soy lecithin and/or Polyglycerol polyricinoleate; PGPR). Further grinding (refining) and dehydration of the mixture leads to formation of aggregates called *chocolate crumbs*. The crumbs can then be mixed with cocoa butter and/or other compatible fats to produce chocolate. Chocolate crumbs have gained wide acceptance in chocolate manufacturing as the antioxidant properties of cocoa gives protection to the milk-containing crumbs consequently giving them long storage-life. Moreover they just need to be mixed with cocoa butter to produce chocolate. Crumbs contain only up to 0.8 – 1.5% moisture. Hence, the level of water activity it too low for microbial growth. Dehydration during crumb manufacture takes place in multiple effect plate dryers, and at this stage all necessary ingredients are present to promote Maillard reactions. This gives them a characteristic brown colour and caramel-like flavour notes [113].

C) In the third pathway, cocoa mass can be mixed with other ingredients such as cocoa butter, sugar, vanilla, milkfat, milk powder and emulsifiers. The typical method is to mill the solid ingredients together and then mix them with the liquid ones (ingredient pre-mixing). This method leads to different flavours than if all the ingredients are mixed together. This is followed by refining of the pre-mix and conching operations required to produce smooth liquid chocolate with desirable flow properties, flavour and texture. Refining and conching operations and their significance in development of liquid chocolate microstructure are explained in the following sections.

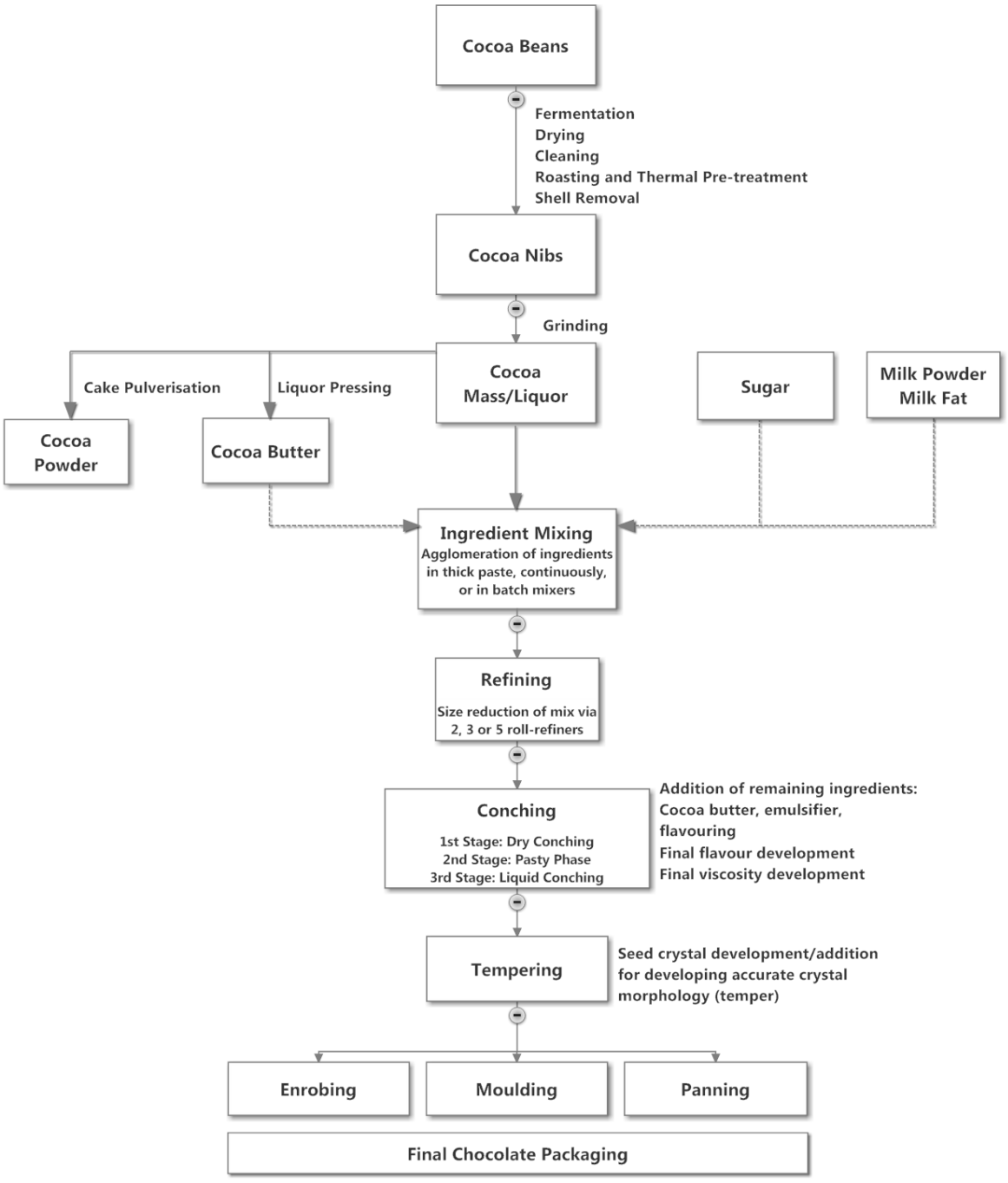


Figure 2-9 Schematic diagram of the bean-to-bar chocolate making process.

2.3.3.3 Chocolate Refining

The aim of the chocolate refining process is reduction of particle size of solid particles (sugar, cocoa particles and milk powder) to a target optimum (typically 15 – 30 μm) and coating existing and newly formed particle surfaces with fat. Accurate particle size reduction and optimisation is critical to develop desired flow properties in molten chocolate, consequently influencing textural attributes, efficiency of processing operations, and quality of chocolate.

Refining the mixture of chocolate ingredients is typically carried out in two-roll (pre-mixing) and five-roll refiners [139].

Roll refiners consists of a vertical array of hollow cylinders, temperature controlled by internal water-flow and pressed together by hydraulic pressure (Figure 2-10). The feed-rate determines the throughput and the fineness of the particles in the liquid chocolate, and can be adjusted by varying the feed roll-gap at a constant roll-speed or vice versa. A thin film of chocolate is attracted to increasingly faster rollers, travelling up the refiner until it is removed by a knife blade. Comminution of particles mainly occurs by mechanisms of abrasion, clipping and fragmentation depending on their mechanical properties. This is facilitated by stresses caused by radial forces exerted by the rollers and frictional forces tangential to the matt surface of the rollers [124].

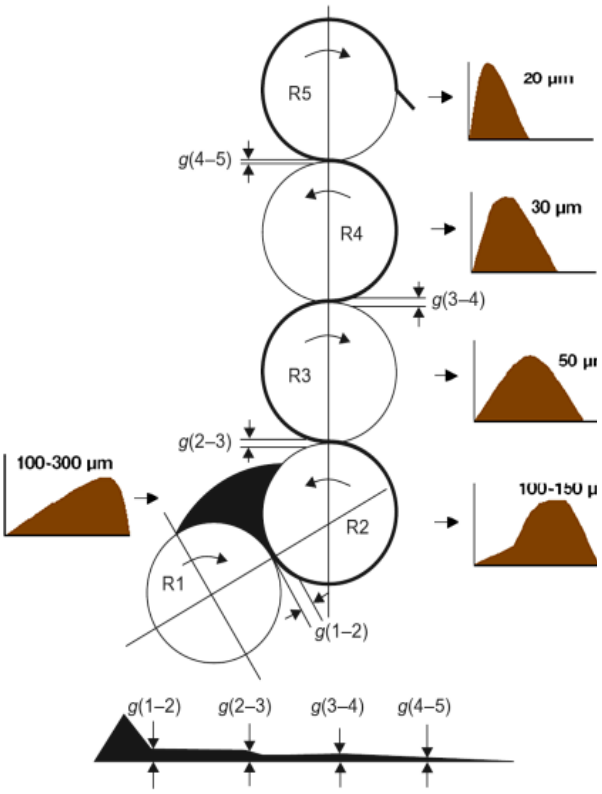


Figure 2-10 Schematic diagram of a 5-roll refiner used for refining chocolate mass; R = roller and g = gap.

Fragmentation of particles and coating of new surfaces with lipid, activates particle surfaces which then absorb volatile flavour compounds from cocoa components. The degree of reduction in a five-roll refiner is usually 5-10, resulting in reduction of particle size from 100-150 µm to a maximum particle size in the range 15-30 µm [113]. The relationship between final maximum particle size and specific flow-rate is linear [140], with theoretical fineness being defined by –

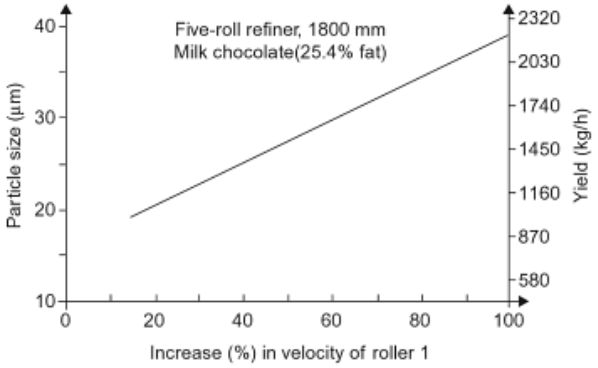


Figure 2-11 Milk chocolate particle size and yield as a function of velocity increase of roller-1 of a 5-roll refiner [124].

$$S_O = 2m' / \rho(v + v_{i+1})^b \qquad 2.11$$

where, So = theoretical maximum particle size equivalent to film thickness/gap, m' = mass flow-rate, ϱ = chocolate density (approximately 1.2 kg/m^3), v = speed of the circumference of rolls i and i+1, and b = roll length. A relative increase in velocity of a roller with respect to the subsequent one results in increase in throughput at the expense of coarser refining (Figure 2-11).

Refiners not only affect particle size reduction and agglomerate breakdown but also distribute particles throughout the continuous fat phase coating existing and newly formed surfaces with fat. It also is the first-step in the process contributing significantly in developing flavour homogeneity in the molten chocolate matrix.

2.3.3.4 Chocolate Conching

Once the chocolate mass has been refined to desired particle size it is processed to a smooth flowing viscous liquid through a process known as conching. Conching aims at acquiring desired flavour development and accurate flow properties, while contributing to the melt-in-mouth character of chocolate [138]. There are many types of conches currently in operation in the chocolate industry. All operate under similar principles: time, temperature, atmospheric aeration, shear and agitation.

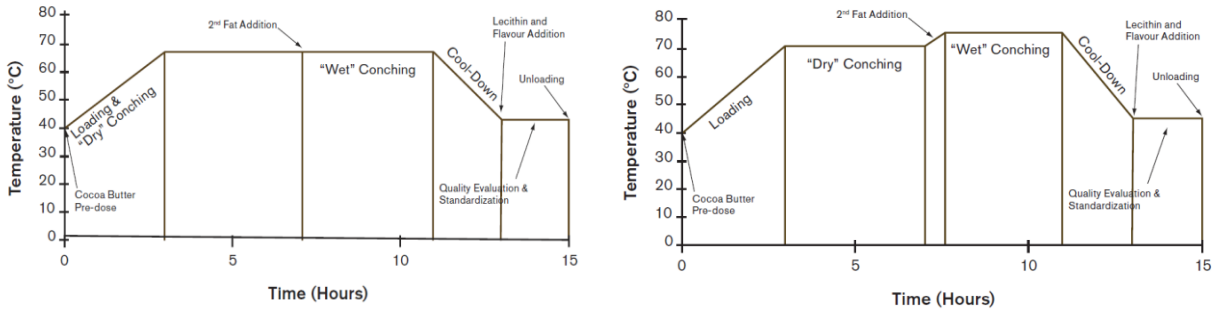


Figure 2-12 Typical time-temperature process curves representing sequential stages involved in dark (left) and milk (right) chocolate conching [141].

Traditionally, conching was carried out in long conches which consisted of a granite trough containing the chocolate mass, and a granite roller which moved back and forth shearing the chocolate. These resembled the conching device first invented by Rudi Lindt in 1878. These have now been completely replaced by multi-bladed horizontal rotary conches which have accurate temperature control, high throughput, and circumvent sedimentation and accumulation problems associated with long conches and rotary vertical conches [138].

To get a well processed chocolate it is desirable to let it pass through three conching stages – 1) dry conching, 2) pasty phase, and 3) wet/liquid conching (Figure 2-12). These stages are optimised for time, temperature and shear-rate so as to obtain the desired flavour and flow properties in liquid chocolate. During conching, chemical and physical changes are induced in the chocolate mass resulting in reduction of moisture and viscosity. It also facilitates loss of volatile acids and development and migration of flavour molecules (Figure 2-13). The dry conching phase may take place between 40-70°C and results in moisture removal which in turn aids in removal of undesirable acidic flavours. This is done by conching the chocolate mass at increased temperatures when it is still in the powdery-state. As the temperature rises with continuing shear, more fat melts and results in a paste-like consistency of the chocolate mass in the conche. In this form there is high probability of shear-action coating the particles with molten fat; an important aim of conching. Modern refiners are extremely efficient and usually no further particle size reduction occurs during conching. Although, breakage of particle aggregates takes place through the shearing action resulting in formation of new surfaces to be coated with fat.

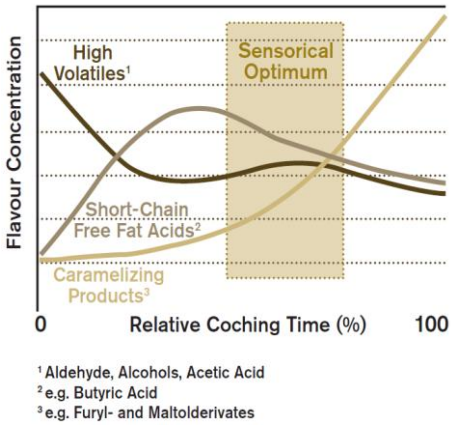


Figure 2-13 Flavour development during chocolate conching [141].

The final function of the conche is to ensure that the chocolate has correct flow properties for the next processing stages, which in turn will depend on the type of coating or moulding machinery being used subsequently. Therefore during the final liquid conching stage, final additions of fat and emulsifier are made. This result in desired flow properties and lead to the formation of a thinner liquid chocolate, while little further mixing takes place. The final stage also involves cool-down of the mass to approximately 40°C and validation of flavours and viscosity at this temperature at which further storage usually occurs. Modern conches have the capacity of processing 5 – 10 tonnes of chocolate in less than 12 hours. So in summary, the conching process reduces moisture content, breaks-up particle agglomerates, removes volatile acids and flavours, reduces bitterness, facilitates flavour development through Maillard reactions, emulsifies the particles with fat and reduces/optimises chocolate viscosity.

2.3.4 Rheological Behaviour of Chocolate

Flow behaviour of liquid chocolate has a major effect on its processing and eating quality. It has critical implications on efficiency of mixing, pumping, enrobing, depositing and moulding operations along with textural characteristics of the final product, and hence, is necessary to control.

As a concentrated suspension of non-spherical particles in a continuous fat phase, chocolate exhibits a temperature-dependent, non-ideal pseudo-plastic behaviour characterised by an apparent *yield stress* and *plastic viscosity* [142]. Without particle interactions other than Brownian motion and hydrodynamic forces, the shear-thinning behaviour of chocolate could scale with the shear strain and be Newtonian at high strain rates [143] [144] [145]. Although, its compositional and microstructural character manifests itself through factors like particle aggregation, polar surface/phase interactions, hydrodynamic, gravitational and Brownian forces, giving chocolate its characteristic rheological behaviour [143] [146].

2.3.4.1 Yield Stress and Plastic Viscosity of Chocolate

Yield stress is a material property which denotes the transition between pseudo-solid and pseudo-liquid behaviours – related to amount of energy and/or minimum shear stress required to initiate flow [147], while *plastic viscosity* is not different from the high shear apparent viscosity, and is related to the energy required to keep the fluid in motion. Both these entities are manifested through the mechanism of dynamic structuring which the suspended particles undergo depending on the shear regime which they are subjected to. Figure 2-14 depicts the typical shear-thinning behaviour of chocolate and schematically shows the underlying mechanisms of particle structuring during this behaviour. Under 'low shear' or rest conditions, particles bump-up against each other, particle-particle interactions and gravitational forces are dominant, causing the chocolate to have an apparent yield stress. Although, once the applied shear exceeds the characteristic yield stress, particle structuring is triggered, and chocolate ceases to behave like a solid. With increasing shear stress, the structuring effect is further developed, causing particle orientation along the shear-field, and as a consequence the viscosity drops. At a fixed shear stress, developing structure would also lead to decrease in viscosity with time, until a shear-stress specific equilibrium structure is reached. A steady state plateau in viscosity is reached if equilibrium has been established between structure break-down and rebuilding. Upon ceasing the shear which caused the breakdown, the material reforms its internal network, and the viscosity recovers. This phenomenon is demonstrated by

chocolate melts, and is called *thixotropy*; an ability of time-dependent structure-disruption and recovery upon application or removal of shear, respectively [148].

Yield stress, or the yield value of chocolate relates to inclined surface coatings, enrobing, shape retention, pattern holding, feet and tails and presence of air bubbles [113], i.e., low shear rate properties. Characteristic of its dependence on particle-particle interactions and specific surface area of particles which form sample-spanning stress-bearing paths and structures [121], particle size, emulsifiers and moisture content affect chocolate yield stress [143].

On the other hand, plastic viscosity or high-shear apparent viscosity relates to mixing operations, coating and depositing properties, pumping characteristics, filling of rough surfaces and thin or thick sensory perception [143], i.e. high-shear properties. Plastic viscosity is dependent on particle size distribution, volume fraction of particles, their shape, extent of particle coating with fat and the viscosity of the continuous phase [147]. Apparent melt viscosity of chocolate in aqueous oral solutions influences flavour attributes and moreover, governs mouth coating, lubrication, deposition, and the way chocolate flows in the mouth, i.e. texture. Thus, rheological parameters often give information related to oral behaviour and related sensory characteristics of chocolate.

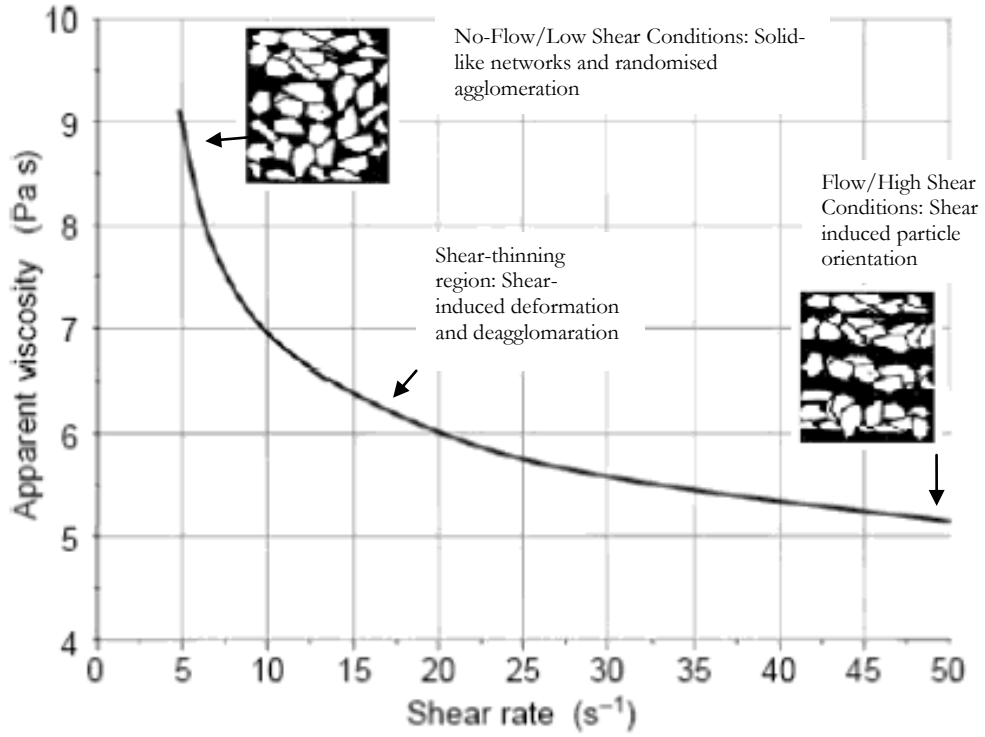


Figure 2-14 Typical rheogram of apparent viscosity versus shear rate for chocolate illustrating shear-thinning behaviour as a result of particle structuring (ordered alignment) (Adapted from: [113]).

2.3.4.2 Expression of Molten Chocolate Flow Behaviour

The flow behaviour of pseudo-plastic foods has been often characterised by the power law model with or without the yield term. (Equation 2.12 and 2.13). It basically establishes a relationship between shear stress and shear rate for a pseudoplastic material wherein the viscosity is shear-dependent.

$$\tau = K \cdot D^n \qquad 2.12 \qquad\qquad \tau - \tau_o = K \cdot D^n \qquad 2.13$$

Here, τ is the shear stress, D is the shear rate, τ_o the yield stress, K is the consistency index, and n is the flow behaviour index. For pseudoplastic foods the flow behaviour index is less than unity; the smaller its magnitude the greater is the pseudoplasticity. Without the yield term i.e. Equation 2.12, is known as the *Ostwald-de Waele* model, while that with the yield term i.e. Equation 2.13, is known as the *Herschel-Bulkley* model [149].

As for the importance of characterising the yield stress term for the shear-thinning nature of chocolate and its relationship to shear stress and viscosity, models which allow for the characterisation of this relationship and the evaluation of the parameters of concern have been used for describing chocolate flow behaviour [150].

The Bingham model was the first attempt at describing the flow behaviour of molten chocolate:

$$\tau = \tau_o + \eta_{pl} \cdot D \quad \text{or,} \quad D = \frac{1}{\eta_{pl}}(\tau - \tau_o) \qquad 2.14$$

where, τ is the shear stress (in N/m^2), D is the shear rate (in s^{-1}), τ_o is the yield stress or yield value (in N/m^2), and η_{pl} is the plastic viscosity (in Pa.s). As the viscosity of chocolate is generally measured in concentric cylinder rotational viscometers, because of the yield stress and of the viscometer geometry, the laminar flow of chocolate between the inner and outer cylinder is realised [151] only when:

$$\tau > \frac{r_2^2}{r_1^2} \cdot \tau_o \qquad 2.15$$

where,

r_1 = radius of the inner cylinder;
r_2 = radius of the outer cylinder.

Steiner [151] has shown that the Newtonian rate of shear at the inner cylinder D_N is given in this case by the relationship:

$$D_N = \frac{1}{\eta_{pl}}\left[\tau - \frac{2r_2^2\tau_0}{r_2^2 - r_1^2}\log_e\frac{r_2}{r_1}\right] \qquad 2.16$$

The following year, Steiner [153] suggested the use of Casson's (1957, 1959) relationships which allow to observe a better linear relationship between $\tau^{0.5}$ and $D^{0.5}$ [152]. This model was originally developed for printing ink.

$$\tau^{0.5} = K_o + K_1 \cdot D^{0.5} \qquad 2.17$$

For rotational viscometers, it is necessary to take into account the ratio $a=r_1/r_2$ and Equation 2.17 then becomes:

$$(1+a)D_N^{0.5} = \frac{1}{K_1}\left[(1+a)\cdot\tau^{0.5} - 2\cdot K_o\right] \qquad 2.18$$

Using this equation, $(1+a)D_N^{0.5}$ is plotted against $(1+a)\tau^{0.5}$; data from different rotational viscometers for the same chocolate should lie on a straight line with a slope $1/K_1$ and the intercept on the abscissa equal to $2K_o$ or b (Figure 2-15).

According to Steiner [153], the plastic viscosity η_{CA} (Casson plastic viscosity) and the yield stress τ_{CA} (Casson yield value) are:

$$\eta_{CA} = \left[\frac{1}{slope}\right]^2 = K_1^2 \qquad 2.19$$

$$\tau_{CA} = \left(\frac{1}{2}\cdot I\right)^2 = \left(\frac{b}{2}\right)^2 = K_0^2 \qquad 2.20$$

where, I = intercept

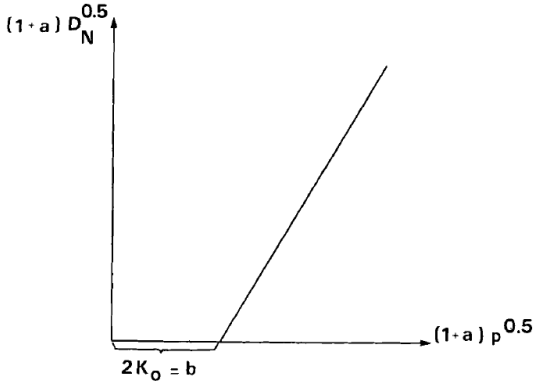


Figure 2-15 A schematic plot according to the Casson equation.

The Casson relationship was adopted as the official method for measurement of chocolate viscosity by the International Office of Cocoa and Chocolate [146] [154], until about a decade ago [155]. This was due to the fact that the Casson model has shown particular unreliability at low shear rates [143]. Nevertheless, it is still widely used within chocolate industry globally. The International Office of Cocoa,

Chocolate and Sugar Confectionery recommended that the apparent viscosity of chocolate should be reported in at least 5 points (shear rates) so as to cover low, medium and high shear rate ranges which are of relevance to chocolate processing [155]. In practice, most chocolates can be characterised by two measurements, one at 5 s^{-1} for low-flow situations and to approximate the yield value, and the second one between 10 s^{-1} and 20 s^{-1} for higher flow rates [150].

As one can imagine, flow behaviour of liquid chocolate is influenced by factors which define its microstructure, such as, composition (fat content, type and content of emulsifier/s, moisture, type and structure of milk solids), particle size and distribution, temperature, degree of temper, and processing operations (e.g. conching time and vibrations). Consequently, these factors are also the ones important in partially governing oral behaviour of chocolate and its texture - a sensory manifestation of change in structure. These factors are discussed in detail in Section 2.4 from a '*structure-function relationship*' point of view.

2.3.5 Polymorphism, Crystallisation and Tempering of Cocoa Butter in the Continuous Phase – *Process-Structure-Property Relationship*

2.3.5.1 Polymorphism of Cocoa Butter

Cocoa butter (CB) is a blend of TriAcylGlycerols (TAGs), which result from the triple esterification of glycerol with three fatty acids. Due to its homogeneous TAG composition, cocoa butter can almost be considered as a pure material [156]. More than 80% of the total TAGs in CB are symmetrical and made up of Palmitic (P) C16 : 0, Stearic (S) C18 : 0 and Oleic (O) C18 : 1 which have very similar chain lengths. A typical composition is POS - 36.6%, SOS – 27.3% and POP – 17.0%. CB, because of its relatively simple composition, is highly polymorphic (similar composition, different molecular arrangements) [157].

Polymorphism is the ability of a molecule to crystallise in a number of different crystal packing configurations of variable thermodynamic stability [158]. Three principal polymorphic forms in food fats, in order of increasing thermodynamic stability, are alpha (α), beta prime (β') and beta (β). Their sub forms include sub-α (or ϒ), β'1, β'2, pseudo-β', sub- β, β1 and β2 [114] [159]. Each of these are associated with a different crystal morphology, chain packing/geometric arrangement and thermodynamic stability, consequently demonstrating different melting points and other structure-related physical properties (Table 2-3 and Figure 2-16) [158]. Polymorph-polymorph transitions usually occur, and take place through solid-

solid transition or melt mediation. α-crystals are the least stable, least densely packed and exist as small, fragile, transparent, platelet-like crystals, about 5 μm in length. Natural fats with a great compositional TAG and fatty acid variety will usually exist as stable β' crystal form. Transformation from α to β' in many cases is slower and that to β is unlikely. β' crystals are small, delicate needle-like crystals 1-2 μm in length. In CB, the β' form is generally transient. The β-form has a relatively higher melting point, forms dense and compact crystal structures, resulting in the most thermodynamically stable lattice. Fats with little compositional variety (e.g. CB) are stable in this form. β needles can measure upwards of 50 μm in length. In chocolate manufacturing, β Form V is the target of tempering to produce a desired continuous phase character to achieve high product quality and stability [114].

Table 2-3 An example of crystal polymorphs of CB, their molecular and chain packing arrangements,. means of formation and melting points (M. pt.) [122]

Form	Polymorph	Molecular Packing	Chain Packing	Common means of development	M. pt. (˚C)
I	sub-α (ϒ)	Orthorhombic	Double	Rapid cool from melt	16-18
II	α	Hexagonal	Double	Cool from melt at 2˚C/min	21-22
III	β'2	Orthorhombic	Double	From Form II stored at 5-10˚C	25.5
IV	β'1	Orthorhombic	Double	From Form III stored at 16-21˚C	27-29
V	β2	Triclinic	Triple	Transformation of Form IV	34
VI	β1	Triclinic	Triple	From Form V (weeks to months at room temperature)	36

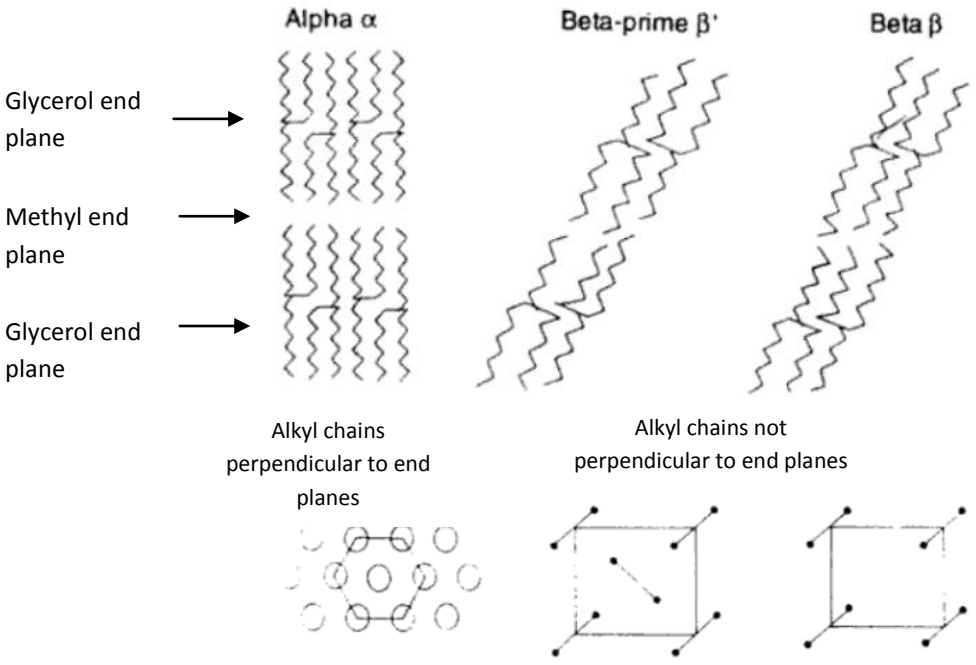


Figure 2-16 Crystal packing of triglycerides. 1) Arrangement of alkyl chains for α, β, and β' polymorphs. 2) Projection looking on to the ends of chains (parallel to the direction of alkyl chain) [113].

2.3.5.2 Crystallisation of Cocoa Butter and Tempering

Solidification of chocolate is due to the crystallisation of CB. Crystallisation proceeds through two different events; nucleation and growth. The nucleation behaviour of fats is heterogeneous [160]. Once seeds of a certain size are formed, or are externally added, they act as nuclei of growth of a micro-heterogeneous fat crystal network. This growth is mediated by molecules migrating from the melt to the crystal surface. The growth phase is generally slower than the nucleation phase [160] and this can be mainly explained by 2 factors – 1) Crystal growth requires the organisation of a structure and therefore longer time is needed for the TAGs to fit in the crystal lattice, and 2) Competitive adsorption may happen between TAGs for a similar place on the crystal surface. This competition consumes time and is why multi-component fats crystallise more slowly than pure ones (fat eutectics). When equilibrium between the solid and liquid fat is achieved, growth ceases and crystallisation ends.

As mentioned earlier, CB can form different crystal types and this is mainly dependent on steric or energetic compatibility of TAG molecules and fatty acid side chains, as well as on the time/temperature gradient they are subjected to. The higher the temperature and the longer the crystal formation time, the denser and more perfect molecular ordering occurs [158] [161]. This is demonstrated in Figure 2-17 and Table 2-4 which also presents the nomenclature and melting ranges for polymorphs as investigated by various authors.

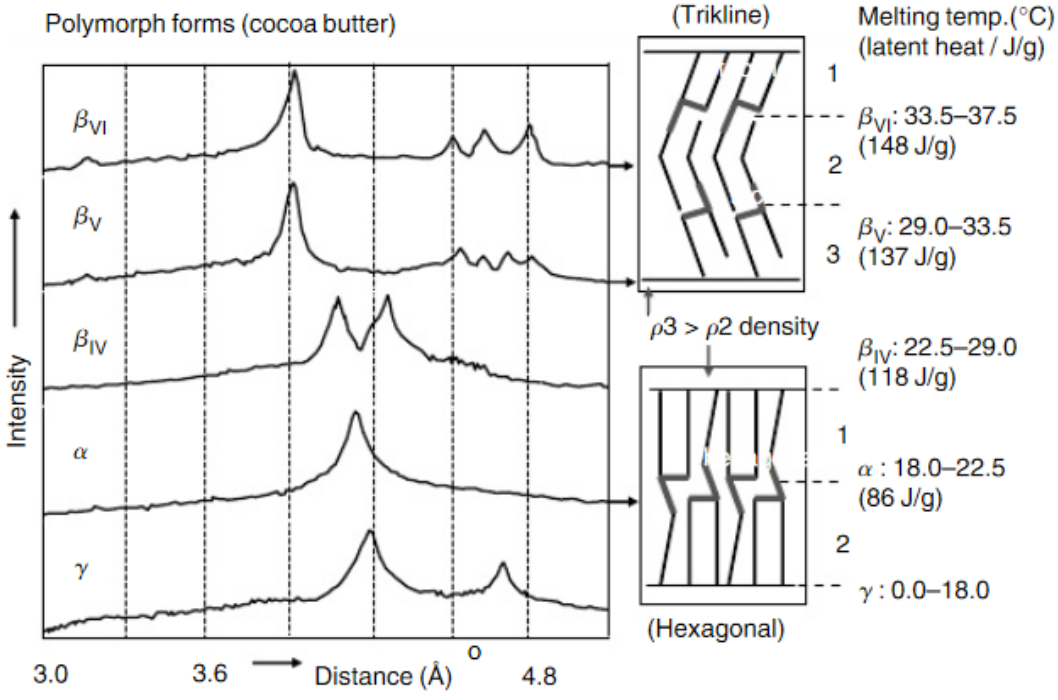


Figure 2-17 Characteristics of CB polymorphs [158].

Table 2-4 Melting temperature/temperature ranges of cocoa butter polymorphs. 1-Vaek [162], 2-Duck [163], 3-Wille and Lutton [164], 4-Lovegren et al. [165], 5-Dimick and Manning [166] (Onset peak max), 6-Windhab and Zeng [167] (Melting range)

T (˚C) 1	T (˚C) 2	T (˚C) 3	T (˚C) 4	T (˚C) 5	T (˚C) 6
ϒ 18.0	ϒ 18.0	Sub-α/I 17.3	VI 13	13.1	ϒ 13.0 - 18.0
				17.6	
α 23.5	α 23.5	α/ II 23.3	V 20	17.6	α 18.0 - 22.5
				19.9	
		β'/III 25.5	IV 23	22.4	III 22.5 - 27.0
				24.5	
β" 28.0	β" 28.0	β'/IV 27.5	III 25	26.4	βIV 27.0 - 29.0
				27.9	
β 34.4	β' 33.0	β/V 33.8	II 30	30.7	βV 29.0 - 33.5
				34.4	
	β 34.4	β/VI 36.3	I 33.5	33.8	βVI 33.5 - 37.5
				34.1	

Due to its relatively simple composition, CB has a characteristic melting behaviour in that, it melts over the relatively narrow range of temperature between room temperature and that of the mouth. Although, once a constituent of the continuous phase of chocolate, it is important to attain a homogenous polymorphic (β Form V) character to achieve this characteristic melting behaviour which associates itself with desired mouth-feel (texture). This is achieved through the process of *tempering*.

Tempering is the next-in-line unit operation to conching. The objective of tempering is to develop a sufficient number of homogeneously dispersed, stable seed crystals in the chocolate mass. These crystals then act as nuclei (seeds) to encourage the total fat phase to crystallise in a stable polymorphic form during the cooling stage following moulding, enrobing or coating [168]. The important effects of tempering the liquid chocolate mass are – 1) optimisation of the yield value and viscosity and stability of chocolate mass for moulding, coating or enrobing, 2) good surface gloss and colour, 3) good snap (in effect a particular fracture toughness which consumers associate with quality), 4) smooth and fast melting at body temperature, and 5) good heat stability [123] [128] [169]. To ensure only the desired polymorph is present in the chocolate mass, the tempering operation involves the following steps –

1. *Complete melting* of chocolate mass by heating to 50˚C; removal of all crystal memory/history.
2. *Cooling and crystallisation*; nucleating different polymorphs and beginning crystal growth.

3. *Melting-out unstable crystals*; as different polymorphs are randomly nucleated during the cooling stage, it is necessary to heat the mass to a temperature where only β_V polymorph is present (the Form β_V is the most stable polymorph crystallisable directly from melt) [170] [171] [172].

The process parameters influencing tempering are 1) the temperature profile and 2) the shear applied to the chocolate mass [173]. Holding time (about 400s) at the cooling temperature of 22°C, high local shear rates (usually 500 - 12,000 s^{-1}), and reheating temperature below 33°C have been found to promote optimum tempering [158].

To reproduce the process at an industrial scale, device such as the one presented in Figure 2-18 are used, and temperature of the stages are typically set to follow the tempering sequence shown in the figure.

1. After removal of all crystal memory by heating to 50°C, the liquid mass is gradually cooled in the first zone (pre-cooling zone) of the temperer to about 32°C to remove sensible heat from the melt.

2. In the second zone (cooling zone) of the temperer, the low temperature ($T \approx 22$ - 27°C) ensures that a consequent number of stable and unstable crystals are formed.

3. In the third zone (reheating zone) of the temperer, the temperature of the seeded mass is raised to a point where the low melting/unstable polymorphs are melted out, leaving a optimally tempered (in seed crystal β Form V) chocolate mass to be used for further moulding, coating or enrobing operations.

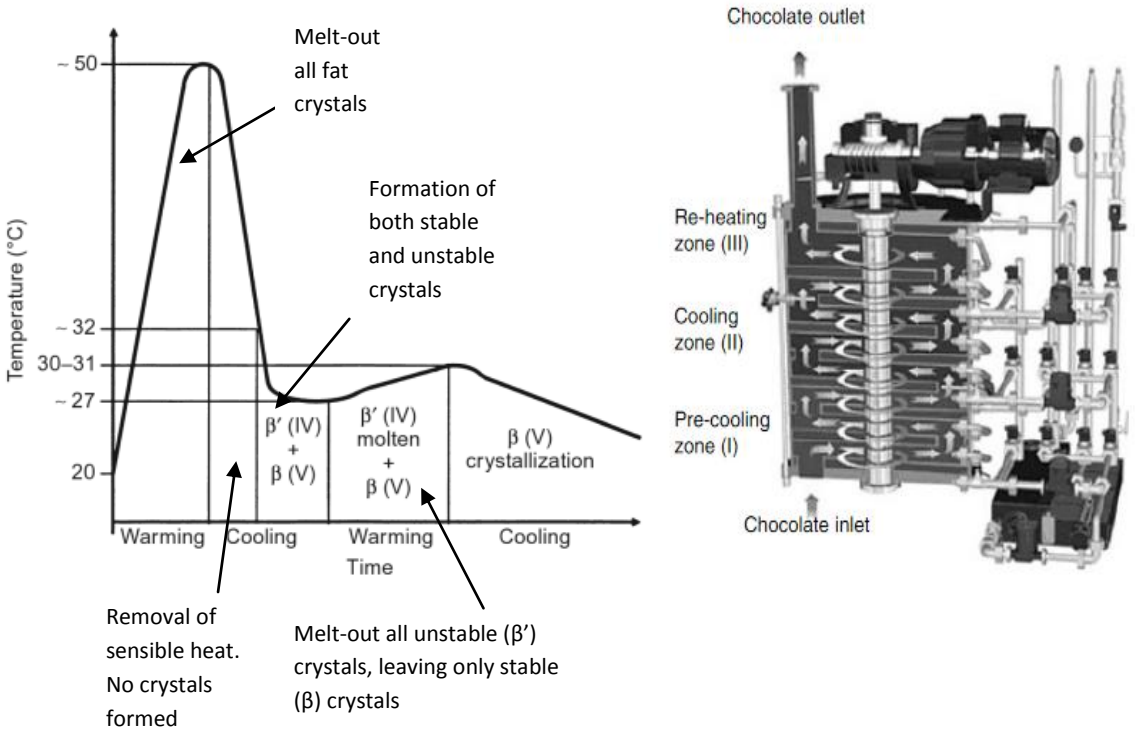


Figure 2-18 A continuous vertical stir-/shear temperer with baffled retention zones (right) and characteristic temperature vs. time sequence for tempering chocolate (left) (Adapted from [158]).

Stirring blades in the temperer to shear the chocolate are essential for producing high nucleation rates under low residence times, with more stable crystals at higher temperatures than normal. Typically, for conventional tempering the amount of form V nuclei present in the melt at the end of tempering is between 2 – 4%, and hence with the reduction in liquid fat, there is a consequent rise in viscosity. There is a wide range of different tempering devices commercially available with different combinations of shear rate, temperature and residence time distributions to adapt for different chocolate recipes [158]. There are also relatively new tempering processes like seed tempering which produce very finely dispersed CB nuclei (rich in SOS, and already in a stable form) contained in the CB melt. Such suspensions contain 10-20% solid fat and only if 0.2-1% is added to untempered chocolate, it still guarantees a good temper and excellent product characteristics [167]. Incomplete, or bad tempering, results in dull appearance, crumbly texture, and inaccurate melting behaviour through development of lower melting polymorphs, unstable crystal growth and as a consequence the solidification, contraction and setting properties may be severely compromised, and even whitish surface spots or a streaky greyish-white defect known as 'fat bloom' may occur [122] [174].

2.4: Structure-Function Relationships in Governing Factors of Importance to Oral Behaviour of Chocolate

The previous sections highlighted how main chocolate processing operations play a role in the formation of desired microstructure. Like in all foods, microstructure of chocolate relates to its texture, which is nothing but the sensory manifestation of the structural elements defining its physical properties, and most importantly influencing the complex envelope of chocolate quality. The most important aspects of texture in chocolate are perceived during its physical transformation in the mouth, and consequently, the structural elements and their interactions which define chocolate microstructure and its physical character, are the ones pivotal in governing its oral transformation behaviour.

2.4.1 Role of the Particle Phase

Depending on chocolate-type and recipe, the particle phase concentration in the final product can usually range from 60 – 75%. Consequently, the property of size distribution of the suspended solids and their ingredient interactions play a defining role in chocolate rheological properties, which in-turn relate to oral flow behaviour and textural perceptions [146].

As discussed in Section 2.3.3.3, while the output of the refining operation is a particle size distribution (PSD), it is important to understand the relationship between PSD and chocolate flow properties. Typical flow curves for chocolate can be divided into three or four shear rate/shear stress regimes [142] [143]. At shear stresses near the yield value, particle-particle interactions – friction or 'network' effects dominate, with the closely packed particles behaving like aggregated 'super-structures' with shape, surface area and surface properties becoming particularly important. Once flow is initiated, hydrodynamic effects become significant and particle-packing volume relevant. In general, it is known that for particulate suspensions like chocolate, viscosity decreases with increase in particle size, particles becoming more spherical, broadening of PSD, and decrease in solids volume fraction [146]. Although the Casson model has its limitations [143], it gives useful insights into chocolate flow behaviour in the first two stress regimes.

As chocolate is refined finer, increase in the number of particles to interact with each other causes the yield stress to increase. It has been demonstrated that the effect on yield stress is much greater than for plastic viscosity (Figure 2-19). Yield stress normally correlates with specific surface area, which is not the case with plastic viscosity, however where very

large particles are present the plastic viscosity may increase again because of an increase in bound-fat or the way the particles pack themselves.

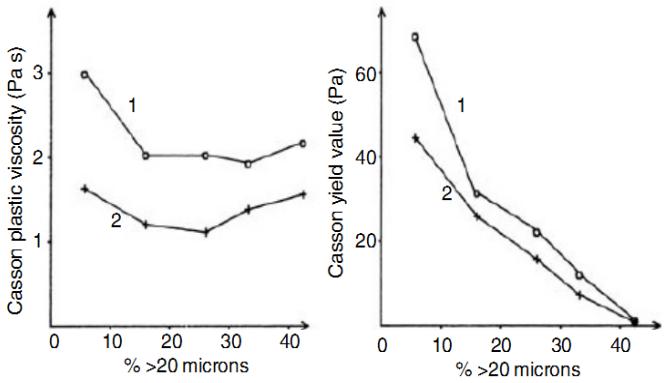


Figure 2-19 Influence of particle fineness on Casson parameters in two chocolates measured at 40ºC with 0.25% lecithin: (1) 30% fat; (2) 32% fat [113].

In investigating competing effects of particle surface area and packing density on Casson parameters, Fischer [175] has shown that for a similar mean diameter, wider/bimodal distributions resulted in much lower plastic viscosity but higher yield values when compared to narrower/unimodal distributions, respectively. The effect was exaggerated at lower fat contents; while differences in yield stress persisted until 45% fat, little differences in plastic viscosity were observed above 35% fat. Related results have been noted by Servais *et. al.* [176] for blends of fine and coarse particles influencing relationship between packing efficiency and shear viscosity with yield value closely related to mean particle diameter and particle surface area but not packing fraction.

These observations can be attributed to smaller particles, which although have been shown to improve sensory properties, result in an increase in plastic viscosity and yield value due to increase in surface area and surface-surface contact points (increase in bed packing efficiency). Particle packing becomes important upon flow initiation and therefore affects the plastic viscosity component. Yield value on the other hand is affected largely by inter-particle contacts and consequently has been shown to have linear dependency on mean particle size, or more accurately, on specific surface area [121] [177].

Chocolates have to be refined to a particle size of less than about 30μm so that they are not perceived gritty [116]. Particle size not only influences sensory perception of coarseness, but also flavour, melt, colour and gloss [113] [178] [179]. Through a one of its kind study, Mongia [178] showed that sweetness, flavour and effort required to melt, manipulate and swallow chocolates was related to PSD and flow behaviour. Their results demonstrated that as the average particle size decreased, the yield value, intensity and time to reach

maximum flavour, and the effort to melt, manipulate and swallow increased, implying the finer chocolate had longer oral residence times. Thickness scores were highly correlated to Casson yield value (r=0.97) and to mean diameter over the volume distribution (r=-0.99). Through multivariate regression, they also concluded that yield value and mean particle size were more significant contributors to textural perceptions than, for example, plastic viscosity or shape of the PSD.

2.4.2 Role of Fats

Fat (both cocoa butter and milkfat) acts as a "binding" continuous phase in chocolates, coating particle surfaces, filling void-spaces, and holding the particulate microstructure together when in the solid form. When in liquid form, it enables the chocolate to flow [180]. Fat influences the flow behaviour of chocolate by governing degree of interparticle interactions and packing efficiency of the matrix, in that, as the fat content increases (increase in continuous phase volume fraction/solid loading reduction), distance between particles increase to cause a drop in viscosity and reduction in melt-related mechanical properties like firmness, spreadability and consistency of chocolates [177]. This has been demonstrated by Afoakwa and co-workers investigating the effect of fat content in relation to PSD on rheological [121], textural [181] and mechanical [169] [177] properties of chocolate.

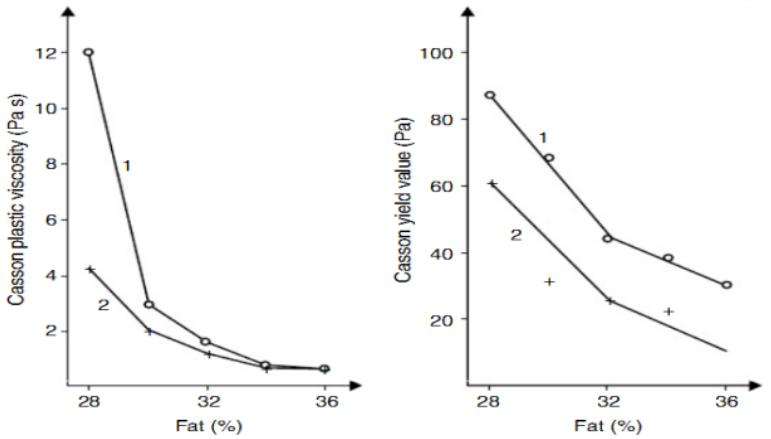


Figure 2-20 Effect of fat content on Casson parameters of two milk chocolates measured at 40°C with 0.25% lecithin: 1) fine chocolate (5.7% particles > 20μm); 2) moderately coarse chocolate (16% particles > 20μm) [113].

It is the free-fat that enables the chocolate to flow, while the trapped-fat source does not aid. It has been demonstrated that the effect on flow properties of additional fat is much exaggerated at lower total-fat content (e.g. about 25%) as compared to higher (above 35%) (Figure 2-20) [182]. Hence, the yield value which is mainly due to inter-particle interactions, is less affected than that by the addition of fat.

Milkfat can be added to chocolate through roll-dried or spray-dried milk powder and/or through the use of compatible fractions [65] [183]. Depending on the form it is added-in, milkfat influences flow properties accordingly. It has the same effect as cocoa butter when in the liquid form at temperatures greater than or equal to oral temperature [123]. Spray-dried whole milk powder (WMP) is a 'low-free-fat system' in which most of the fat remains bound, on the contrary, replacing it with skim milk powder (SMP) and anhydrous milk fat (AMF), so that the milkfat is available freely, is considered as a 'high-free-fat-system'. Utilising either of these results is modification of textural attributes accordingly [113] [184].

Figure 2-21 shows the effect of low-free-fat WMP system and high-free-fat SMP + AMF system on plastic viscosity of milk chocolate. It can be seen that the use of higher levels of free milkfat/total fat (SMP+AMF system) out-weighs the WMP system in terms of rheological advantages. However, this advantage is countervailed by the softening (eutectic) effect arising from reaction of high-level of free-milkfat with cocoa butter. This is depicted in Figure 2-22 for the similar systems used in milk chocolates. The underlying mechanism leading to this effect is detailed in Section 2.4.7.

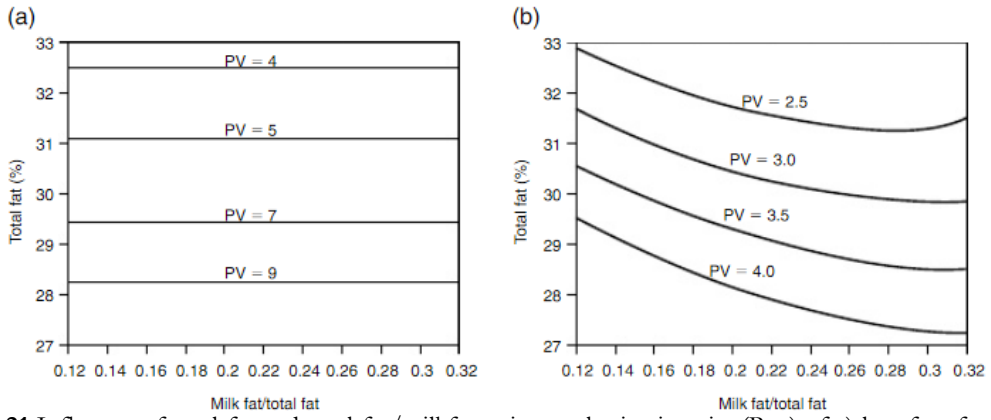


Figure 2-21 Influence of total-fat and total-fat/milkfat ratio on plastic viscosity (Pa.s) of a) low-free-fat (WMP); b) high-free-fat (SMP+AMF)-based milk chocolate.

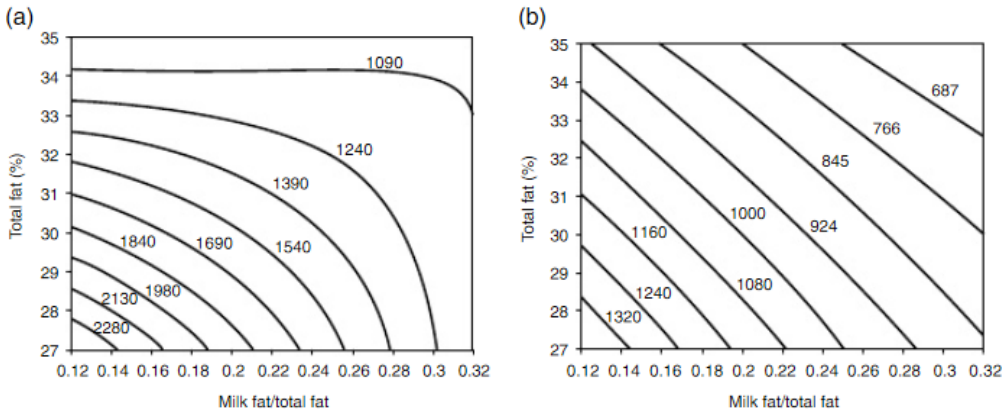


Figure 2-22 Influence of total-fat and total-fat/milkfat ratio on chocolate hardness (g) of a) low-free-fat (WMP); b) high-free-fat (SMP+AMF)-based milk chocolate [113].

2.4.3 Role of Surface-Active Agents

In the continuous fat phase of chocolate, sugar being lipophobic and hydrophilic will not dissolve, so the surfaces have to be coated with fat. Moreover, the presence of a high volume fraction of cocoa and sugar crystals creates resistance to flow due to frictional forces, which have to be overcome to a certain degree to bring about the desired flow character in liquid chocolate. Both these functions are facilitated by surface-active agents (SAAs), which allow the fat content of the chocolate to be reduced while maintaining desired flow behaviour [123] [185].

The most common SAA in chocolate manufacturing is lecithin, which has been used since the 1930s [186]. Commercial soy lecithin is a mixture of natural phospholipids and soy oil [146], having phosphotidylcholine as its most active surface component. It displays both lipophilic and hydrophilic properties. The very little amount of moisture in chocolate is absorbed by the sugar surfaces, in turn increasing friction between them. The hydrophilic group of lecithin attaches itself to the water molecule by monomolecular adsorption, leaving the other end suspended in the fat phase, thus reducing friction, increasing mobility and lowering viscosity [187].

This aids flow behaviour to such an extent that only as low as 1% lecithin can be added [123]. Bouzas and Brown [146] reported that just at 0.5%, about 85% of the sugar was already coated. Lecithin dramatically affects yield value and plastic viscosity in chocolate, and when added between 0.1 - 0.3%, reduces both these parameters and enhances moisture tolerance. As with cocoa butter addition, the higher the viscosity, the more effective is the addition of the lecithin (Figure 2-23). This has also been shown through an excellent study by Afoakwa *et al.*, [121] which also highlights the relationship of lecithin functionality with varying fat content and PSD.

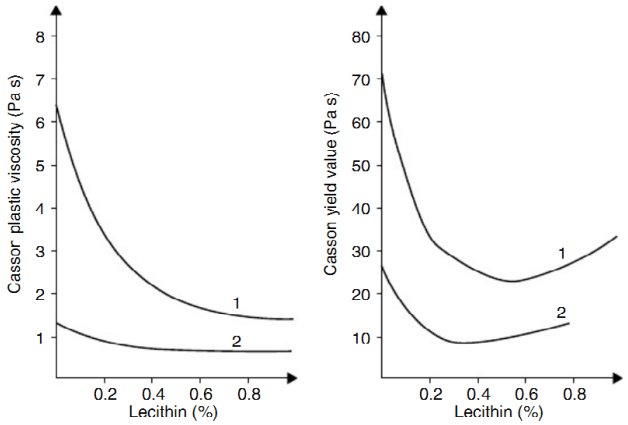


Figure 2-23 Influence of soy lecithin addition on the Casson viscosity parameters in two chocolates: (1) 33.5% fat, 1.1% moisture; (2) 39.5% fat, 0.8% moisture [187].

Unlike cocoa butter however, further additions of lecithin can cause the yield value to increase. This effect is mainly due to the electrostatic attractions between the hydrophilic portions of the 'access' lecithins leading to formation of miscellar structures, or those between the lipophilic portions forming layered structures resulting in reduced effectiveness.

Some of the other SAAs used in the chocolate industry are polyglycerol polyricinoleate (PGPR), Ammoniumphosphatide (YN) and sorbitan tristearate (STS), some of which can be used interchangeably or in combination with lecithin. Although, as most of these SAAs work by coating the surfaces of solid particles, in particular sugar, increase in particle surface area requires a parallel increase in SAAs. In other words, to a large extent, the role of PSD and SAAs has to be considered concurrently in modulating chocolate flow properties [185] [188].

2.4.4 Role of Milk Powder and its Types

Milk powder/s can account for upto 23% of the total mass in typical milk chocolate [189]. The ones explored for use in chocolates include roller-dried and spray-dried WMP, high-fat powders, buttermilk powders, whey powders, and skim milk powder sprayed with AMF or cream [113] [190] [191]. The characteristics of these powders are very different, although they may have similar composition. Table 2-5 summarises the main characteristics of milk powders of specific importance towards properties of final chocolate product.

Table 2-5 Properties of milk powders and their influence on chocolate properties [192]

Properties of milk powder	Properties of chocolate or processing conditions
Particle size and distribution	Flow properties
Particle shape	Refining operations (particle size distribution)
Surface characteristics of particles	Tempering conditions (cocoa butter crystallization)
"Free" fat level	Hardness/snap
Particle density	Bloom stability
Flavor attributes	Flavor attributes

Breakage mechanisms of milk powders during refining depend on their material properties (ductility/elasticity/brittleness), shape, amount of air included in void spaces (vacuole volume; hence density) and the refining conditions themselves [139]. All these factors influence chocolate properties (fluid rheology and mechanical properties of the solidified product). Although, the principle factor linked with milk powder-type which influences textural and rheological properties of chocolate is the degree of free-fat (milkfat) contributed by them [189] [192] [193].

Powders that contain high free-fat, or fat that is easily extractable and can interact directly with the cocoa butter, typically have been desired in milk chocolate manufacturing

[192] [194]. There are several factors that impact the degree of free-fat in milk powders, with the processing conditions (type of heat-treatment) being key to developing a powder with high free-fat. Roller-dried WMPs have characteristically high surface free-fat content, and hence are ideal for use in milk chocolate. Conversely, spray-dried WMP has significantly lower free-fat levels (only 2 to 3%), and does not perform as well in milk chocolate, in that, most of their fat is trapped inside the particles and is not freely available to contribute towards fat eutectics, softening and flow properties [195]. Manufacturers that use spray-dried WMP in their formulation typically use slightly higher concentrations of cocoa butter to keep viscosity down in the desired range. Furthermore, in the cases where SMPs are used, milkfat is externally added towards the latter processing stages, which leads to its 100% availability to help the flow or soften the cocoa butter [196].

Crystallisation of the lipid-phase in combination with the solid dispersed phase (sugar crystals, cocoa solids, and milk solids) governs hardness of chocolate. As will be discussed latter in further detail, addition of milkfat causes a softening effect on cocoa butter and results in softer chocolates [184] [197]. Thus, higher free-fat levels from milk powders are expected to lead to softer chocolates. The packing arrangement of the dispersed phases in chocolate may also determine the mechanical properties of the solidified product (factors such as hardness, snap, etc.). Markov and Tscheuschner [198] and Tscheuschner and Markov [199] documented the effects of various additives on the physical properties of chocolate. Heathcock [200] has shown electron micrographs of different structures of chocolate based on type of milk powder used in the formulation.

2.4.5 Role of Sugars

Initially, sugar was considered as an inert flavour ingredient in chocolate contributing "only" to sweetness. Although that being its principal functionality, when present in the chocolate matrix, sugar is considered as an important structural element contributing towards macroscopic physical properties of the final product. Controlling the particle size of sugar is equally important as that of the other particulate ingredients in chocolate to acquire desired sensory attributes and flow properties. Also as mentioned earlier, sugar crystal surfaces act as the 'binding-site' for emulsifier molecules utilised to control rheological behaviour of chocolate.

Fine crystalline sucrose is utilised at upto 50% in chocolates [124]. Amorphous lactose is introduced into milk chocolate through spray-dried milk powders [189], or can be added

externally as a nutritive carbohydrate sweetener, during the crumb process as a partial substitute to sucrose [193] [201]. Its concentration and the physical state (amorphous vs. crystalline) are the factors particularly affecting post-refining particle sizes, viscosity and requirements of surfactants, with resultant implications on textural and sensory properties [189] [193] [202].

Amorphous lactose in chocolate could crystallise given sufficient moisture content, temperature and time. Aguilar and Ziegler [189] demonstrated that crystallising lactose improved chocolate flow properties, because it resulted in release of entrapped milkfat, alteration of particle size distribution, reduction of particle surface area, and water absorption. In particular, it was demonstrated that milk chocolates with increased amorphous lactose from spray-dried WMP had larger post-refining particle size, reduced viscosity, and a lower requirement for surfactants. While those chocolates with increased crystalline lactose from spray-dried WMP prepared by post-crystallisation had similar post-refining particle size, higher viscosity, and greater surfactant requirement.

A group consisting of these authors [193] have also demonstrated in a subsequent study that the physical form of lactose in WMPs (amorphous vs. α-monohydrate) affected both flavour and texture in milk chocolates. They accredited fundamental changes during conching of chocolate to the crystallisation of amorphous sugar, particularly lactose, above its glass transition temperature. This crystallisation was shown to increase caramel-, milk- and chocolate-like flavours and lower hardness and onset of melt due to liberation of milkfat.

2.4.6 Role of Moisture

Molten chocolate may contain 0.5-1.5% moisture, typically contributed by the cocoa solids. This level of moisture does not affect chocolate flow. However, greater moisture levels are detrimental for chocolate. About 3-4% moisture is enough to aggregate sugar particles to form lumps, absorb onto the sugar surfaces and increases friction, and markedly increase yield value and viscosity. This rise in viscosity is seen for upto 20% moisture, after which, an aqueous phase is formed [203].

Beckett [116] stated that for every 0.3% moisture left after conching, 1% fat has to be added to the molten chocolate.

2.4.7 Impact of Milk-Fat Blending on Continuous Phase Character and Macroscopic Properties

The physical properties of chocolate are largely determined by the composition and physical properties of the underlying fat phase. Composition and quantity of fats which make-up the continuous phase, along with their molecular interactions, dictate macroscopic properties like melting behaviour and solid fat content (SFC) which in turn influence oral transformation and textural perceptions [204].

Cocoa butter and milkfat are the raw materials and major ingredients which make-up the continuous phase of chocolate confections. As described previously, CB is a relatively simple fat and exhibits a characteristic structure-function relationship because of its molecular (TAG) composition and configuration, which relates its crystallisation and polymorphic character with its melting behaviour. This also holds true for milkfat and mixtures of milkfat and CB, in that, the composition and phase behaviour of the individual components play an important role in governing the character and physical properties of the final product [65] [205].

Milkfat is usually present in a large amount in milk chocolate but can also be used at a lower extent in dark chocolate (usually under 5%), typically to reduce possibility of fat bloom [115]. As it is usually present alongside CB in chocolate, the impact of milkfat on CB crystallisation, and the resultant effect of this blend on textural properties of chocolate are of great industrial importance. The TAG composition of milkfat is very heterogeneous and complex as it contains over 100 different TAGs with very broad chain lengths [205]. Milk fat is an association of three largely independent melting fractions which are - high (HMF), moderate (MMF) and low (LMF) melting fractions [113]. These fractions are chemically distinct, with HMF principally containing long-chained saturated fatty acids, MMF containing two long-chain saturated fatty acids and one short chain or *cis*-unsaturated fatty acid, and LMF containing one long chain saturated and two short chain or *cis*-unsaturated fatty acids. Typically the melting curve of untempered native milkfat determined using DSC shows three endothermic peaks corresponding to HMF (>50°C), MMF (35-40°C) and LMF (>15°C). Milkfat can be used in a fractionated form, or as whole, or may also be hydrogenated or interesterified. However the compatibility of milkfat with CB is not caused by the unique milkfat TAGs, but occurs because milkfat does not alter the polymorphic state of CB at typical levels of milkfat addition in chocolate [206] [207].

When milkfat and cocoa butter exist as a blend, their co-crystallisation behaviour becomes a key factor influencing appearance and macroscopic physical properties (melting behaviour, bloom formation, softening) of chocolate. When a blend of these fats is present in chocolate, it is important that the final product sets at a suitable rate and, more importantly, that it has a correct texture and melting behaviour in the mouth [183] [197] [208] [209]. However, the amount of milkfat that can be blended with cocoa butter to make chocolate is limited by the thermodynamic incompatibility between specific milkfat TAGs with that of CB in solid state [206]. They both have different stable crystal forms, and moreover, milkfat alters the temperature at which the specific crystal forms of pure CB occur. Because of geometric constraints and environmental factors that influence kinetics of crystallisation, native milkfat and CB do not form mixed crystals, in that, they crystallise as separate milkfat and CB solids. It also tends to slow the rate of CB crystallisation in mixtures of CB and milkfat and this is expected to occur in chocolates containing the mixture of these two fats. Hence, adding increasing amounts of milkfat alters the physical and functional properties of chocolate including hardness (by altering SFC), crystal packing, ability to temper and melting properties, although controlling its blend concentration and through controlled use of its fractions, specific product properties can be achieved for desired functional behaviour [65] [184] [205].

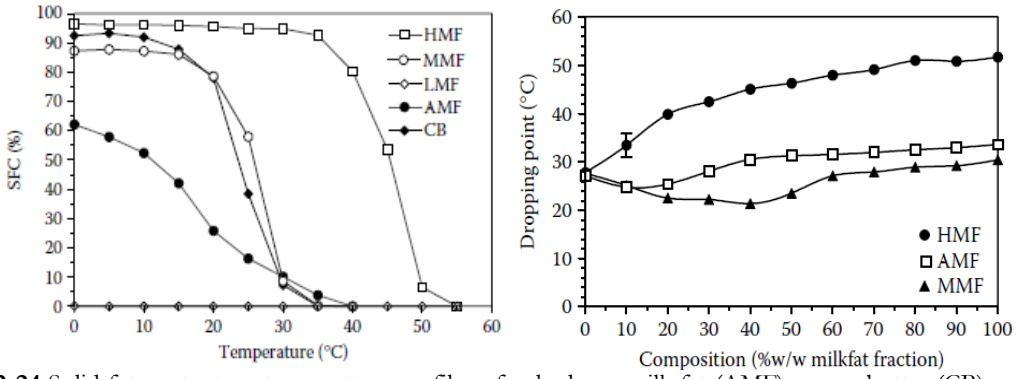


Figure 2-24 Solid fat content vs. temperature profiles of anhydrous milk fat (AMF), cocoa butter (CB) and high (HMF), medium (MMF) and low (LMF) melting fractions of AMF (left), and dropping points of mixtures of HMF, AMF and MMF with CB (right) [65].

Figure 2-24 shows the solid fat content (SFC) vs. temperature profiles of HMF, MMF and LMF, anhydrous native milk fat (AMF), and cocoa butter, and dropping points of CB mixtures with HMF, AMF and MMF. Both HMF and MMF have narrow melting ranges, with MMF showing distinct similarity with CB, while LMF is completely liquid above 0°C. The dropping points of the HMF, MMF and LMF are 51.7°C, 30.4°C, and 27.6°C respectively, while that of native AMF and CB is 34.3°C and 27.6°C respectively. It is also evident how the

mixture of fractions present in AMF gives it a broader melting profile and a much lower solid fat content at ambient temperature as compared to individual fractions - MMF and HMF, and CB.

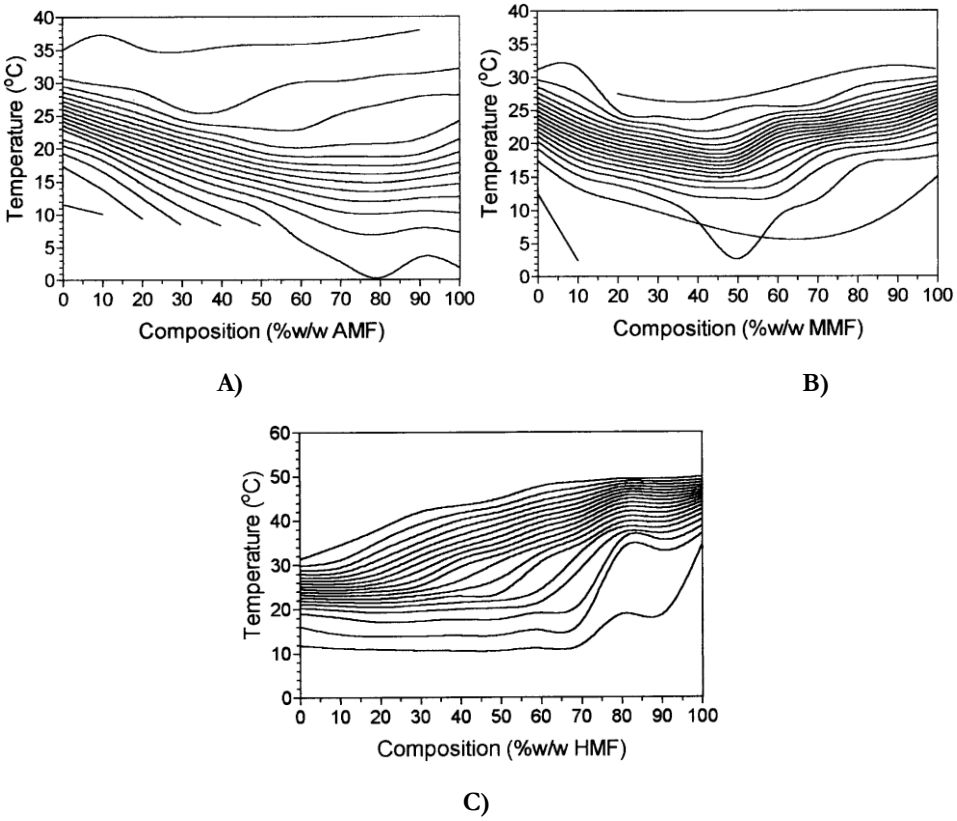


Figure 2-25 Isosolid phase diagrams of mixtures of CB with A) AMF, B) MMF and C) HMF [205].

Timms [206] [210], Marangoni [65], Bystrom and Hartel [211], and Ali and Dimick [212] have shown the effect that different fractions of milkfat mixed with CB have on the phase behaviour of the blends. Isosolid phase diagrams for CB-AMF, CB-MMF, and CB-HMF blends are shown in Figures 2-25a, 2-25b, and 2-25c respectively. LMF TAGs fractionated from, or contributed by native AMF have the effect of diluting the CB and hence induced softening of chocolate because of their low SFCs. As for the similarity between melting profiles of CB and MMF individually, MMF is extremely incompatible with CB TAGs as seen by evident eutectic formation from the dropping points for their mixture over a range of 0-60% (MMF-CB), while from their respective isosolid diagram (Figure 2-25b) it is obvious that any mixture of CB and milkfat's MMF forms a eutectic. Eutectic incompatibility compromises stability and textural properties in chocolate by manifesting itself through softening and changes in melting behaviour. The formation of a eutectic is illustrated by a simplified binary-system phase diagram in Figure 2-26 which basically depicts a decrease in melting point of the blend below that of either of the two individual components, or that the SFC of the blend of two fats becomes lesser at any temperature than a simple summation of

the contributions to the SFC of the individual components. A slight eutectic formation is evident in the AMF-CB system (Figure 9.7). The AMF-CB mixture also results in eutectic formation over a 0-30% range, which is contributed by the incompatibility between MMF and CB.

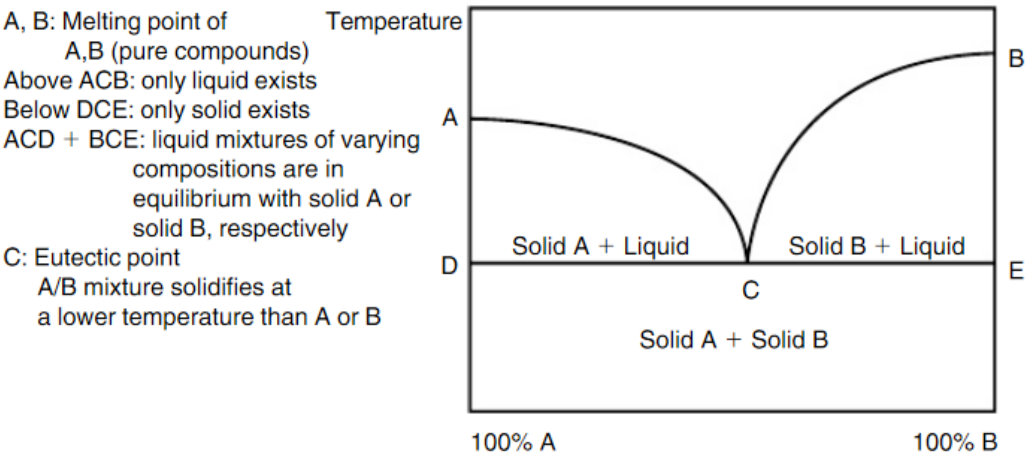


Figure 2-26 Phase diagram of a simple binary system (e.g. cocoa butter and milk fat) [195]

On the other hand, no eutectics are formed between cocoa butter and milkfat's high melting fraction (Figure 9.9). Favourable interactions (strong intermolecular dispersion forces) between the sn-2 oleic acid in CB TAGs and the sn-1 and sn-3 long-chain saturated fatty acids in the HMF TAGs result in their high compatibility with each other and no eutectic formation. For this reason, it is often used with confectionery fats as bloom inhibitor without any effect on product hardness and melting point, or is also added back to AMF in chocolate manufacture for the same purpose.

The level of milkfat to be added to cause changes in the crystal forms of CB is approximately 50% [213]. Extreme incompatibility issues above these concentrations are obvious but of no practical concern in chocolate manufacturing, which restricts milkfat use to around 30% of the total fat. At levels above this the chocolate becomes very soft because of very low solid fat present at these high levels of milkfat, hence may become unacceptable and may also bloom rapidly. Most commercial milk chocolates are made with a ratio of between 12% and 32 % milkfat to total fat. As a general guideline, a minimum of 45% SFC is required to achieve desired physical properties in chocolates and stable products.

CHAPTER 3

Characterisation of Physical Properties and Microstructure of Dark and Milk Chocolate Models

3.1 Context

The scope of this study was to carry out detailed characterisation of physical/material properties and microstructure of the selected dark and milk chocolate products, and outline how the differences in these properties may relate to chocolate composition. Methodologies and instrumentation utilised for characterisation of particle size distribution, mechanical characteristics, rheological properties, melting behaviour and solid fat index (SFI), and microstructure analysis of chocolates along with the results obtained are reported and discussed.

3.2 Materials

Commercially Available High Quality Dark and Milk Chocolate: Chocolates were purchased from Countdown® Supermarket, Palmerston North, New Zealand (Progressive Enterprises Ltd, New Zealand). Chocolate slabs were stored at 20°C; RH:~ 60 – 65% in a dry environment controlled storage facility in original packaging.

Dark and Milk Chocolate Models

1. ***Lindt Excellence® 70% Dark Noir*** - Lindt & Sprungli AG (Switzerland); Slab weight: 100 g (2.5 servings per package; Serving size: 40 g). The chocolate slab mould design consists of 10 cubical sections (36 mm x 40 mm x 6 mm), each weighing approximately 10 g.

   Ingredients: Cocoa mass, sugar, cocoa butter, natural Bourbon vanilla beans (flavour).

2. ***Lindt Excellence® Extra Creamy/Cremoso Milk (Leche)*** - Lindt & Sprungli AG (Switzerland); Slab weight: 100 g (2.5 servings per package; Serving size: 40 g). The

chocolate slab mould design consists of 10 cubical sections (36 mm X 40 mm X 6 mm), each weighing approximately 10 g.

Ingredients: Sugar, cocoa butter, milk powder, cocoa mass, butterfat, lactose, skimmed milk powder, malt extract (barley), emulsifier (soya lecithin), flavouring (vanillin).

Table 3-1 Nutritional Information of Selected Dark and Milk Chocolates*

Australian Nutrition Information (Package size: 100 g)		
	Average Quantity per 100 g	
	Lindt Excellence® 70% Dark Noir	***Lindt Excellence® Extra Creamy/Cremoso Milk (Leche)***
Energy	2180 kJ/520 kcal	2340 kJ/560 kcal
Protein	8 g	6 g
Fat, total	40 g	37 g
- Saturated	24 g	22 g
Carbohydrate, total	33 g	51 g
- Sugars	28 g	50 g
Sodium	60 mg	110 mg

***** *Reproduced from original packaging of chocolates*

Cocoa powder particles of West African origin (Medium-High Alkalized ISCPD11S) – Archer Daniels Midland (ADM) Company Netherlands; (Fat Content – 10 – 12%; Fineness D99.5%: 75 μm sieve; Moisture Content ≤ 3.0%) were obtained from Hawkins Watts Ltd, Auckland, New Zealand, and were used in formulating ingredient mixtures for characterising optical properties using confocal laser scanning microscopy. They were stored at 15 - 20°C in dark and dry environment away from moisture and strong odours, in air-tight containers.

Cocoa Butter - pure prime pressed, deodourised (700-SB) - Archer Daniels Midland (ADM) Company Netherlands (De Zaan®) was obtained from Hawkins Watts Ltd, Auckland, New Zealand, and was used in formulating ingredient mixtures for characterising optical properties using confocal laser scanning microscopy.

3.3 Methods

3.3.1 Particle Size Analysis

Laser diffraction light scattering was utilised for measurement of particle size distribution (PSD) of chocolates. The method aimed at measuring particle size of total non-fat solids present in the chocolates [1] [2]. A MasterSizer® laser diffraction particle size analyser was fitted with a MS small-volume sample presentation unit (Refractive Index 1.590) (Malvern Instrument Ltd., Malvern, England). About 0.2 g of refined dark chocolate was thoroughly melted at 50°C and pre-dispersed in 10 ml refined vegetable oil (RI 1.45) at ambient temperature (20 ± 2°C). This was followed by external ultrasonication in a sonication bath (Sonorex Digitec - DT100, Bandelin Electronic, Germany) for 3 min to ensure particles were independently dispersed and to break any particle aggregates. A sample was then presented to the sample presentation unit (stirrer speed 2000 rpm) until an obscuration of 0.2 was obtained. Size distributions were quantified as relative volume of particles in size bands presented as size distribution curves (Malvern MasterSizer® Software v 5.60). During the procedure particle absorption is set to *Nil*.

The standard operating procedure was set to obtain following PSD parameters -

1. *Specific surface area* - (surface area per unit mass or volume of particle)
2. *Largest particle size* (D_{90}) - (90% of the particles are smaller than this value)
3. *Mean particle volume* (D_{50}) - (50% of the particles are smaller than this value)
4. *Smallest particle size* (D_{10}) - (10% of the particles are smaller than this value)
5. *Sauter mean diameter* D[3,2] – (surface weighted mean diameter); $d_{3,2} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$
6. *Mean particle diameter* D[4,3] – (volume weighted mean diameter); $d_{4,3} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$

3.3.2 Analysis of Mechanical (Instrumental Textural) Properties

Method for analysis of mechanical properties was adapted from [3] [4]**.** A TA-HD Plus Texture Analyser (Stable Micro Systems, Godalming, England) with back extrusion rig and a 40 mm diameter compression disk was attached to an extension arm using a 5 kg load cell (Figure 3-1). Chocolate samples were melted at 50°C thoroughly for 75 min followed by gentle stirring to maintain melt homogeneity. They were then quickly transferred to a back extrusion container (ø 50 mm) and work-done in back extruding 100 mL chocolate was determined measuring force in compression. Six replications were made at a pre-test speed 1.0

mm/s, test speed 5.0 mm/s and post-test speed 10.0 mm/s at 30 mm above sample surface, penetrating 30 mm, then returning to the start position. Mean values were used to obtain a force–time curve (XT.RA Dimension, Exponent 32 software; Stable Micro Systems) for calculation of following instrumental texture parameters –

1. *Firmness* = maximum compression force in extrusion thrust into sample (N);
2. *Consistency* = area within curve during extrusion thrust (N.s);
3. *Cohesiveness* = maximum compression force during withdrawal of probe from sample (N);
4. *Index of viscosity* = area of curve negative region during probe withdrawal (N.s).

Hardness assessments on chocolates using texture analyser with various probes and procedures have been reported previously [5] [6] [7]. The following conditions were applied here. Hardness of solid tempered chocolate was measured using a texture analyser with a penetration probe (needle P2/N) attached to an extension bar. A 5 kg load cell and a metal quadra-pod platform to support the sample were a part of the complete assembly used for measurements (Figure 3-1). Maximum penetration force in 5 different samples (45 mm x 35 mm x 5.5 mm) was determined with two replications per sample at a pre-test speed 1.0 mm/s, test speed 2.0 mm/s, and post-speed of 10.0 mm/s, penetrating 2.5 mm at 20 ± 2°C. Hardness was taken as the peak force gauged in Newton, converting mean values into hardness data using XT.RA Dimension, Exponent 32 software (Stable Micro Systems, Godalming, UK).

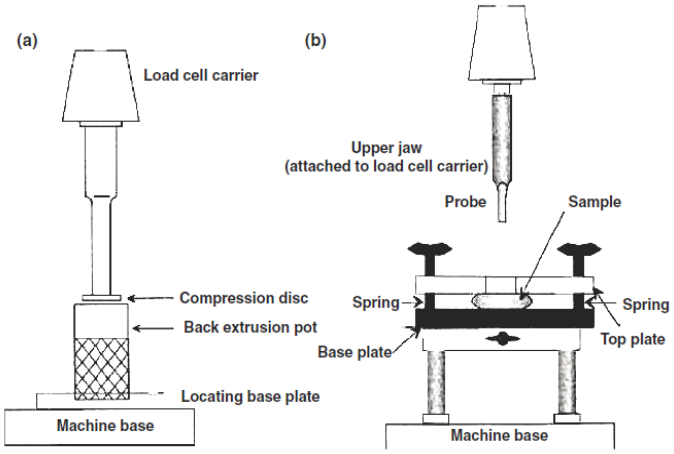


Figure 3-3 Back extrusion rig (a) and penetration test rig (b) used for texture measurements of molten and solid dark chocolate, respectively.

3.3.3 Analysis of Rheological Properties (Flow Behaviour)

Rheological behaviour of chocolates was characterised using steady-shear measurements. All measurements were carried out in triplicates in a shear rate-controlled rheometer (AR-G2, TA Instruments) using a bob and cup DIN geometry, as for IOCCC method [8] with a ratio of inner to outer radius of (r_i/r_o) 0.62 in a coaxial cylinder rotational system. Chocolate samples were incubated at 50°C for 75 min for melting and transferred to the viscometer cup. To avoid possibilities of hysteresis arising from agglomerates, before taking measurements the chocolate melts were sheared between 18 and 50 s^{-1} until shear stress readings were stable. Thereafter, the conditioning step implemented a pre-shear at 5 s^{-1} rate for 15 min at 40°C, before the measurement cycles. Shear stress was measured at 40°C as a function of increasing shear rate from 5 s^{-1} to 50 s^{-1} (ascending ramp) within 300 s, then decreasing from 50 s^{-1} to 5 s^{-1} (descending ramp). A peak hold step was implemented between the ramps at maximum shear rate (50 s^{-1}) for 60 s. Within each ramp 50 measurements were taken.

Temperature of the chocolate samples was controlled during the experiment using a Julabo thermo-regulator (JULABA Corp, Germany). Mean value and standard deviation of triplicate readings were reported. Rheological model fitting was implemented using TA Rheology Advantage Software (TA Instruments). Data was fitted to *Casson* (Equation-1), *Herschel-Bulkley* (Equation-2), and *Bingham* (Equation-3) models -

$$\text{Casson: } \sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} \cdot \sqrt{\gamma} \qquad (3.1)$$

$$\text{Herschel-Bulkley: } \tau = \tau_O + \eta_{pl} \cdot (\gamma)^n \qquad (3.2)$$

$$\text{Bingham: } \tau = \tau_B + \eta_B \cdot \gamma \qquad (3.3)$$

where,

τ, shear stress; τ_O, yield stress; η_{pl}, plastic (shear) viscosity; τ_{CA}, Casson yield value; τ_{CA}, Casson plastic viscosity; τ_B, Bingham yield value; η_B, Bingham plastic viscosity; Υ, shear rate; η, viscosity of suspension; n, flow viscosity index. Rheological parameters (yield stress and apparent viscosity) were deduced from data as recommended by [9] [10] [11]. Value of shear stress at a shear rate of 5 s^{-1} represented yield stress, viscosity at a shear of 30 s^{-1}, apparent viscosity.

3.3.4 Microstructure Analysis

Optical and confocal laser scanning microscopy was used to assess the internal microstructure (particle distribution and particle-fat phase interaction, sugar crystalline networks and particle-particle interactions) of dark and milk chocolate. In addition, procedures aimed at studying particle size distribution of cocoa particles, milk solids and sugar, obtaining information on fat coating of particles, autofluroscence properties, and standardising instrumental procedures (type of laser/s and their intensities, microscope objectives, spectral domains).

3.3.4.1 Optical Microscopy

Assessment of chocolate microstructure using optical microscopy was performed using Zeiss Axiophot Compound Light Microscope (Carl Zeiss, Oberkochen, Germany) fitted with Leica DFC320 Digital Camera (Leica Microsystems GmbH, Wetzlar, Germany). The microscope was operated in differential interference contrast (DIC) or bright field (BF) modes for image acquisition. Image presentation and processing was done using Leica Application Suite Software (LAS Version: 3.3.0) utilising MultiFocus Series (Extended depth of focus) function. 10 g chocolate was melted at 50 °C in a glass beaker to remove all crystal memory. One drop of approximately 100 µl molten chocolate was placed on a pre-warmed glass slide. A cover-slip was gently placed over the sample, and centred to ensure thin and uniform sample thickness. Specimens were placed on a heated (37°C) microscope stage and observed immediately at 20 X and 40 X magnifications. Micrographs were recorded.

3.3.4.2 Confocal Laser Scanning Microscopy (CLSM) of Chocolates and Ingredient Mixtures

Confocal microscopy experiments were performed using a Leica SP5 DM6000B confocal laser scanning microscope (Leica Microsystems, Hiedelberg, Germany) operating in confocal or bright field/polarised mode. Images of representative areas of each sample were taken using a 40X dry or 63X oil immersion magnification objective with a numerical aperture of 0.75 or 1.40 respectively. Confocal illumination was provided by a UV, Krypton/Argon (Kr/Ar) laser, and Helium/Neon (He/Ne) laser. The combinations of laser excitation wavelength and selected emission bandwidths are given in Table 3-2. The confocal pinhole diameter was set to give a resolution of 1.0 µm. Grey level images (8 bit) or RGB colour images (24 bit), 1024 X 1024 pixels in size, were acquired using zoom factors of either 1.0, 1.4, 2.0, or 3.0. Up to 3 simultaneous collection channels spanning fixed emission bandwidths were active for autofluroscence detection, whereas up to two were used for chocolate imaging.

Table 3-2 Excitation source, wavelength, and emission channel configuration for CLSM

Laser/Excitation Source	Excitation Wavelength, nm	Emission Channel Bandwidth, nm
UV Diode	405	410 – 710
Kypton/Argon (Kr/Ar)	488	495 – 710
Kypton/Argon (Kr/Ar)	561	566 – 710
Helium/Neon (He/Ne)	633	640 – 753

Inspection of Ingredient Mixtures for Analysis and Standardisation of Autofluroscence Emission Spectral Bandwidths

Two ingredient mixture systems were inspected for autofluroscence and interference in imaging of ingredient combinations. The strategy was to image the ingredient mixture using a similar laser and excitation wavelength, and check for presence of autofluroscence and interference in a specific emission bandwidth, or combination of collection channels spanning the maximum possible emission bandwidth. The ingredient mixtures assessed were – a) *cocoa butter* + *cocoa particles* and b) *cocoa butter* + *cocoa particles* + *crystalline sugar*. For preparation of all ingredient mixtures, cocoa butter was thoroughly melted at 50°C in a glass beaker placed on a hot plate. A drop (~100 µl) of molten cocoa butter was placed on a pre-warmed convex glass slide, followed by addition of other particulate ingredient/s. The mixture was gently stirred using a toothpick, covered by a glass cover-slip, and analysed in the confocal microscope on a 37°C heating stage.

Assessment of Chocolate Samples

Table 3-3 shows the probes and solvents along with their respective concentrations and target application utilised in the labelling of dark and milk chocolate. Nile Red (EC No: 230-966-0, Sigma Aldrich Ltd) and Fast Green (EC No: 219-091-5, Sigma Aldrich Ltd) were used for localisation of fat and protein in chocolates, respectively. The procedure was adapted from Auty *et. al.* [12], who have applied it for milk chocolate microstructure analysis. A sample of molten chocolate (~ 100 µl) was taken and placed on a pre-warmed microscope slide. Thereafter, 20 µl of the probe or probe mixture was added to the chocolate and mixed gently using a toothpick. A cover-slip was placed on top and light pressure was applied to the cover-slip to ensure a flat horizontal surface. The sample containing slide was then placed on a pre-warmed CLSM heating stage maintained at 40°C for imaging. For dark chocolate, images were acquired at 488 nm excitation, and for milk chocolate images were sequentially acquired at 488 and 633 nm.

Table 3-3 Fluorescence probe(s) used for localisation of fat and protein in chocolates

Chocolate	Fluorescent Probe(s)	Conc., g/L	Solvent(s)	Excitation Wavelength(s), nm	Selected Application
Dark Chocolate	Nile Red	0.02	PEG-300	488	Fat labelling in dark chocolate
Milk Chocolate	Nile Red + Fast Green	0.02 + 0.05	PEG-300 + Distilled water	488, 633	Fat and protein labelling in milk chocolate

3.3.5 Analysis of Melting Characteristics (Thermal Behaviour) and Solid Fat Index (SFI)

The objectives of the Differential Scanning Calorimetry (DSC) experiments were to (i) estimate the solid fat index (SFI) of chocolate samples from the DSC heat flow endotherms, and (ii) measure melting properties of the dark and milk chocolate samples. Thermal analysis of chocolate samples was performed on a TA Q100 DSC (TA Instruments, U.S) with a refrigerated cooling system. Nitrogen was used as the purge gas with a standard flow rate of 50 ml/min. Samples (5 – 7 mg) were sealed in the TA 10 mm^3 aluminium hermetic pans using the standard aluminium pan covers. Each analysis was executed in triplicate (on fresh samples). Samples were placed into the DSC chamber controlled at the respective storage temperature of the chocolates without any delay.

The DSC programme was set-up to heat the chocolate samples from its respective storage temperature (20**°**C) up to 55°C. Initially, three heating rates (0.5; 1; and 2°C/min.) were applied on the dark and milk chocolate samples stored at 20°C. Thereafter, only 2°C/min was utilised for thermal analysis of all chocolate samples so as to avoid recrystallisation caused during heating due to very low rates [13] and to avoid errors associated with thermal-lag as a result of faster heating rates.

Prior to experiments T_{zero} calibration was performed for the cell resistance and capacitance for calculation of calibration constant without pans/samples and with ~ 95 mg sapphire discs. Enthalpy (cell) constant and temperature calibration was performed using indium (T_m: 156.60°C), followed by determination of reference baseline resistance using empty sealed hermetic pans as reference [14]. Data was processed using the TA Universal Analysis 2000 software (Version: 4.5A). Following melting properties were derived from the melting curves – Peak height, onset temperature (T_{onset}), peak temperature (T_{peak}), end temperature (T_{end}) and enthalpy of melting (ΔH_{melt}) [15]. Melting index (T_{index}) was computed as (T_{end} - T_{onset}), as described by [16] (Figure 3-2). For performing peak integration, the inflection point (at the higher temperature region of the melting peak) where the second derivative becomes zero for the first time was determined. Then at the lower temperature region of the melting

peak, the point at which the tangent line contains this inflection point was determined as the end point for integration [17].

Solid Fat Index (SFI) for each chocolate sample was calculated from the area enclosed between the horizontal sigmoidal reference baseline and the melting curve. Change in SFI throughout the temperature ramp was represented by melting integral curves (Integral over all the melting peaks in the endotherm is an indication of the fraction of chocolate fat melted as a function of temperature. Taking the inverse of this integral and converting to percent yields the percent solid).

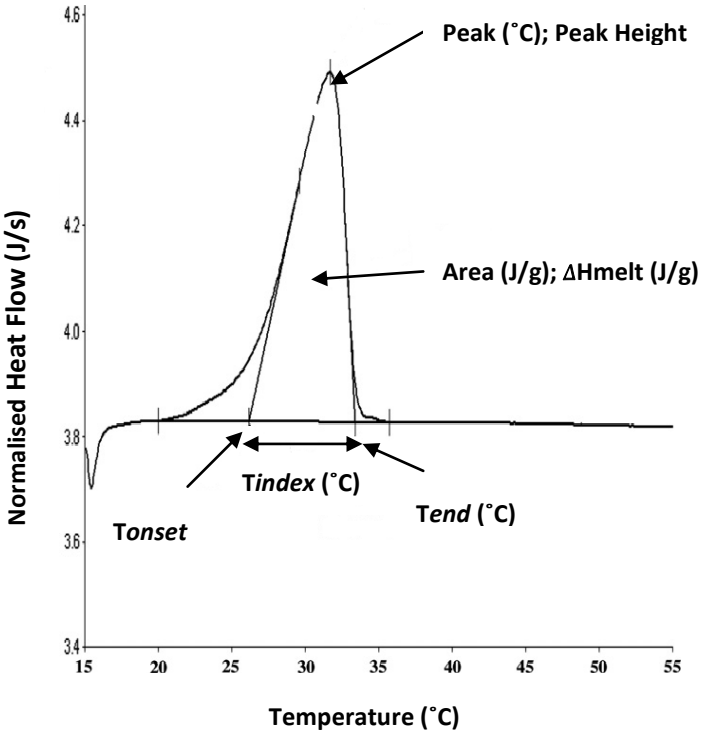


Figure 3-4 Illustration of typical DSC thermogram indicating extracted melting behaviour properties of chocolates

3.4 Results and Discussion

3.4.1 Particle Size Distribution

Laser diffraction was applied to measure the particle size distribution of the model dark and milk chocolate products. PSD parameters obtained for dark and milk chocolates are shown in Table 3-4 and Table 3-5, respectively, and PSD histograms are shown in Figure 3-3 and Figure 3-4.

Table 3-4 Particle Size Parameters of Dark Chocolate (Mean ± S.D)

Smallest Particle Size d(0.1) µm	Mean Particle Size d(0.5) µm	Largest Particle Size d(0.9) µm	Surface Weighted Mean Diameter D[3,2] µm	Volume Weighted Mean Diameter D[4,3] µm	Specific Surface Area m^2/g
4.40 ± 0.22	8.67 ±0.19	19.96 ± 1.64	7.65 ± 0.05	10.13 ± 0.46	0.75 ± 0.04

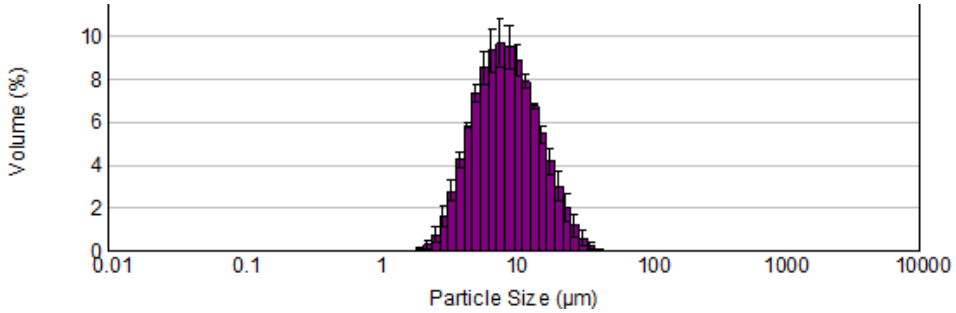


Figure 3-5 Histogram representing particle size distribution of dark chocolate calculated using refined vegetable oil as dispersant by a laser diffraction MasterSizer. Error bars represent standard deviation across three measurements.

Both chocolates demonstrated a unimodal size distribution which was relatively narrower and symmetrical for dark chocolate as compared to milk chocolate. All PSD parameters for both chocolates clearly reflected on the high quality of the products in terms of well optimised processing and control of particle size during manufacture. D_{90} (> 90% finer) values for dark and milk chocolate were approximately 20 µm and 25.5 µm, respectively. These have been reported to correlate with sensory character with micrometer measurements made of the biggest particles [18] [19]. High quality chocolate requires a maximum particle size of 30 µm [20], and at solids > 60% by volume and PSD > 35 µm, the quality becomes unacceptable mainly due to high viscosity and poor texture [21]. Usually in chocolate manufacture, PSD range of 18 – 50 µm covers optimum minimum and maximum sizes with direct effect on texture and sensory character [21], [22], [23].

Table 3-5 Particle Size Parameters of Milk Chocolate (Mean ± S.D)

Smallest Particle Size d(0.1) µm	Mean Particle Size d(0.5) µm	Largest Particle Size d(0.9) µm	Surface Weighted Mean Diameter D[3,2] µm	Volume Weighted Mean Diameter D[4,3] µm	Specific Surface Area m^2/g
4.81 ± 0.10	12.20 ± 0.28	25.51 ± 1.30	9.56 ± 0.12	14.49 ± 0.46	0.59 ± 0.07

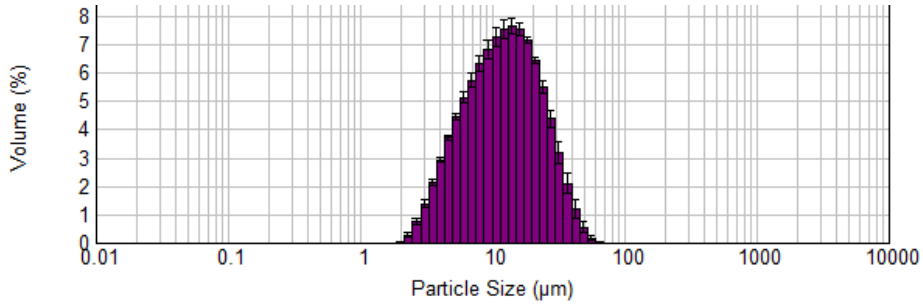


Figure 3-6 Histogram representing particle size distribution of milk chocolate calculated using refined vegetable oil as dispersant by a laser diffraction MasterSizer. Error bars represent standard deviation across three measurements.

Optimisation of PSD in chocolate requires consideration of palate sensitivity. For example, chocolates like those tested, milled to a particle size range of 18 - 25 µm, will have a smoother mouth-feel and texture as compared to a chocolate with a particle size of 30 µm or above, which will be perceived 'coarse or gritty' in the mouth. Traditionally, and in many cases in modern time, European chocolate has been described as having a fineness of 15 – 22 µm, making their target consumers more used to a smoother mouth-feel [24]. Smaller particle size of solids in chocolate results in availability of a larger specific surface area, inherently resulting in a need of larger quantity of cocoa butter to coat all surfaces to obtain desirable viscosity [25].

The milk chocolate size distribution can be seen skewed towards the higher particle size range as compared to dark chocolate (Figure 3-5). The dark chocolate demonstrated lower particle size as compared to the milk chocolate as seen in the comparison of all particle size parameters (Table 3-6). This inherently resulted in a higher particle specific surface area (0.746 ± 0.004 m^2/g) as compared to the milk chocolate (0.598 ± 0.007 m^2/g). As a result of low particle size and high particle surface area, use of relatively high cocoa butter/cocoa butter + milk fat contents in selected chocolates (total fat content - dark chocolate: 40%, milk chocolate: 37%) is justified to achieve good flow properties.

Table 3-6 Comparison of Particle Size Parameters for Dark and Milk Chocolate (Mean ± S.D)

PSD Parameters	***Lindt Excellence® 70 % Dark Noir***	***Lindt Excellence® Extra Creamy /Cremoso Milk (Leche)***
Smallest Particle Size **d(0.1) µm**	4.40 ± 0.22	4.81 ± 0.10
Mean Particle Size **d(0.5) µm**	8.67 ± 0.19	12.20 ± 0.28
Largest Particle Size **d(0.9) µm**	19.96 ± 1.64	25.51 ± 1.30
Surface Weighted Mean Diameter **D[3,2] µm**	7.65 ± 0.05	9.55 ± 0.12
Specific Surface Area **m²/g**	0.75 ± 0.04	0.59 ± 0.07
Volume Weighted Mean Diameter **D[4,3] µm**	10.13 ± 0.46	14.49 ± 0.46

d(0.1), d(0.5), d(0.9) represent 10%, 50%, 90% of all particles finer than this size, respectively. d[3,2] and d[4,3] are Sauter mean diameter and Volume weighted mean diameter, respectively.

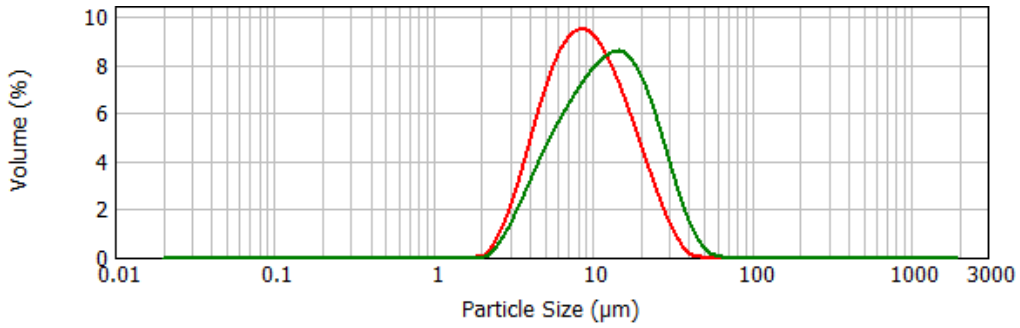


Figure 3-7 Histograms representing particle size distribution of dark chocolate (red), and milk chocolate (green).

3.4.2 Mechanical (Instrumental Textural) Properties

Figure 3-6 shows the textural parameters measured for the dark and milk chocolate using a textural analyser extrusion test on their respective melts. *Firmness, consistency, cohesiveness* and *index of viscosity* were evaluated to ascertain degree of spreadability, consistency and resistance to flow (viscosity).

These mechanical parameters can be considered as an instrumental manifestation of the response of liquid chocolate microstructure under test conditions and can be correlated to specific chocolate texture attributes perceived during mastication. Ziegler and Hogg [22] concluded that such properties are important for chocolate moulding and enrobing and in designing bulk handling systems, and have direct implications on perceived texture in-mouth.

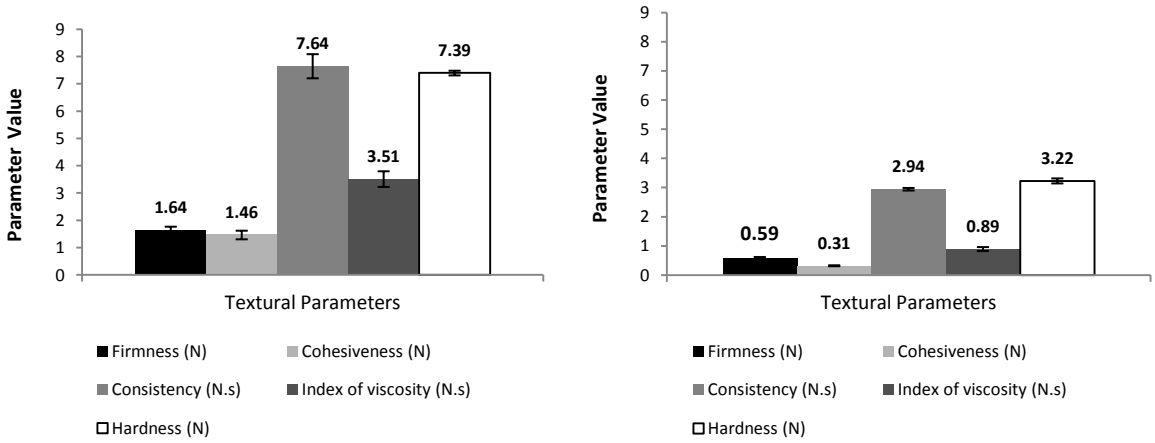


Figure 3-8 Mechanical (Instrumental Textural) parameters for Lindt Excellence dark (left) and milk (right) chocolate. Each parameter has been assessed with a minimum of six replicates using a fresh sample each time (Mean ± S.D).

Chocolate hardness determines the physical rigidity of chocolates and relates directly to sensory properties and may influence oral transformation behaviour. Typical curves obtained during measurements are shown in Figure 3-7 and Figure 3-8.

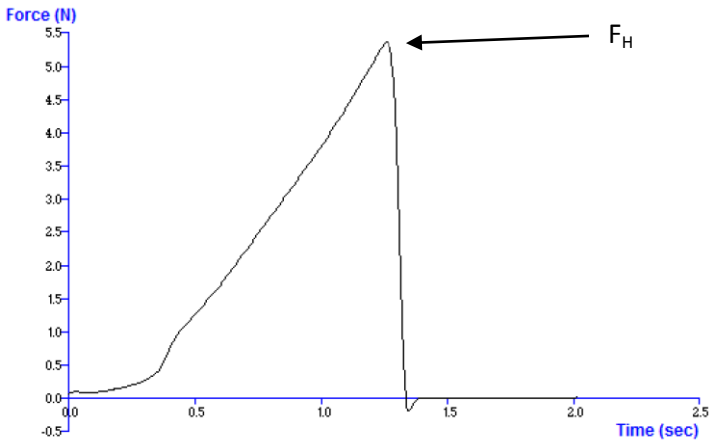


Figure 3-9 Typical penetration curve for solid tempered chocolate. F_H – Maximum penetration force gauged in forward penetration thrust (Hardness, N).

Afoakwa *et. al.* [26] studied the effect of varying particle size distribution and composition (fat and lecithin content) on the textural characteristics of dark chocolate. Their study involved lab-made dark chocolate combination formulations with particle sizes of 18, 25, 35, and 50 µm (D90), fat content - 25, 30, and 35 %, and lecithin content - 0.3 and 0.5 %. Their results indicated that increasing particle size resulted in linear decrease in textural parameters of both molten (firmness, cohesiveness, consistency, index of viscosity) and solid (hardness) tempered chocolate, higher at lower fat and lecithin contents. Textural parameters of selected dark chocolate product (composition: 40% fat, D_{90}: 17.96 µm), correlated well with their findings for dark chocolate with 35-40 % fat, and D_{90}: 18 µm (data not shown).

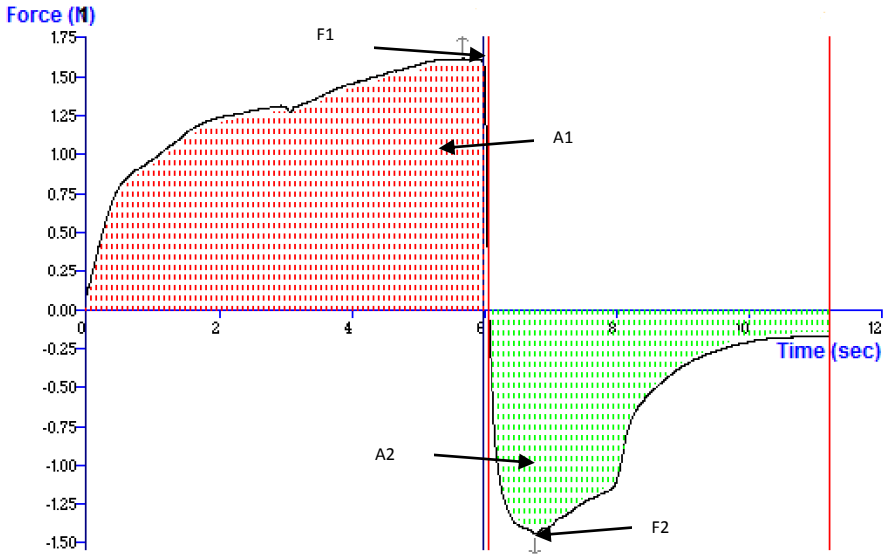


Figure 3-10 Typical back-extrusion curve for molten chocolate. F1 – Maximum compression force in forward extrusion thrust (*Firmness*); F2 – Maximum compression force during back extrusion cycle (*Cohesiveness*); A1 – Area under the curve during extrusion thrust (*Consistency*), and A2 - Area of curve negative region during probe withdrawal (*Index of Viscosity*).

As seen in Figure 3-6, lower values of all textural parameters were recorded for milk chocolate as compared to dark chocolate. These can be justified through compositional differences between the chocolates. One of the chief contributing factors towards these differences is the particle size distribution of both chocolates. Particle size distribution in the chocolate matrix governs degree of particle-particle contact/interaction and structural packing and arrangement [27]. So for a reduced mean diameter, particle number increases to compensate for volume fraction. This results in a parallel increase in specific surface area enhancing particle surface-surface contact to yield higher values of firmness, cohesiveness, and consistency, restricting spreadability and viscosity for a specific solid concentration [26]. Furthermore, if the larger particle size of milk chocolate can be attributed to the presence of the relatively elastic milk solids and a larger quantity of crystalline sugar, the distribution of particle sizes becomes more spread out with a large specific surface area, allowing the smaller particles (mainly cocoa) to fill the spaces between the larger particles, resulting in drastic reduction in the firmness, consistency, cohesiveness and viscosity.

Milk solids (powders) depending on the process used in their manufacture (roller dried vs. spray dried), and their composition (WMP vs. SMP), may contain different levels of free- and/or trapped-fat [25]. This milk fat contributed by the powders along with freely added milk fat is readily available to from a eutectic mixture with the cocoa butter fat resulting in softening of chocolate directly related to lowered SFC values for milk chocolate as compared to dark at ambient temperature [28], reduction in viscosity and firmness [29] [30] [31] [32], and

is famously known for causing variations is tempering, crystallisation and melting characteristics [33] [34].

Furthermore, surfactants like lecithin depending on their concentration, influence textural properties of chocolates through their functionality of reducing viscosity of the chocolate depending on its composition. They act as surface active agents modulating frictional forces, particle-particle and particle-fat interactions contributing towards deagglomaration and dispersibility of the particle phase in suspension [15] [35] [36]. It can be seen that their presence in the milk chocolate may relate to lower values of index of viscosity, firmness, consistency and cohesiveness as compared to the dark variant [4].

Finally, the presence of lactose in the milk chocolate also should be considered, especially in parallel to the presence of surfactants and lactose-type (amorphous vs. crystalline) as a potential influencing factor. The presence of lactose in milk chocolates has been previously shown to influence textural properties especially through its effect on rheological behaviour of molten chocolate [37] [38]. Presence of amorphous lactose from spray-dried milk powders at specific concentrations has been shown to decrease chocolate viscosity, result in an increase in particle size of refined chocolate mass, and lower the concentration of emulsifiers at which the Casson yield value is observed, while the inverse was recorded for crystalline lactose [38]. Although it was not possible to investigate physical form and concentration of lactose present in the milk chocolate, its presence, as opposed to absence in dark chocolate is most certainly an influencing factor for textural properties associated with the molten form (viscosity, consistency, cohesiveness) as well as solid form (hardness).

3.4.3 Rheological Behaviour of the Dark and Milk Chocolate Melts

Important rheological models that have been used to characterise pseudo-plastic flow behaviour chocolate include the Herschel-Bulkley ($\tau = \tau_0 + \eta_{pl} * (\gamma)^n$), Casson ($\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} * \sqrt{\gamma}$) and Bingham ($\tau = \tau_B + \eta_B * \gamma$) forms [11] [39] [40]. These demonstrate compliance over the shear thinning region of the rheogram as measured between shear-rates of 2 s^{-1} and 50 s^{-1} at 40°C and account for the characterisation of yield stress and plastic viscosity for concentrated particulate suspensions. These were applied to characterise the rheological behaviour of the chocolates along with post-2000 ICA recommendations to report yield stress at γ = 5 s^{-1} and apparent viscosity at γ = 30 s^{-1} [9] [18].

Figure 3-9 shows the comparison of dark and milk chocolate rheograms and parameters obtained from model-fitting to flow curves are tabulated in Table 3-7. All models showed a good fit to the chocolate rheograms, with the Herschel-Bulkley model demonstrating the best fit (lowest standard error values: 0.83 – 1.09). As shown in Figure 3-9, milk chocolate proved to be thinner as compared to the dark chocolate over the complete shear rate range. Although, there was not a very large difference between the viscosity parameters of the two chocolate, the milk variant demonstrated lower values of Casson plastic viscosity (η_{CA}: 1.46 ± 0.16 Pa.s), Bingham plastic viscosity (η_B: 2.08 ± 0.07 Pa.s), Herschel-Bulkley plastic viscosity (η_{pl}: 2.906 ± 0.140 Pa.s) and apparent viscosity at $\gamma = 30\ s^{-1}$ (η: 2.67 ± 0.08 Pa.s) as compared to dark chocolate (η_{CA}: 1.56 ± 0.02 Pa.s, η_B: 2.37 ± 0.032 Pa.s, η_{pl}: 4.34 ± 0.17 Pa.s, and η: 3.20 ± 0.03 Pa.s). Values of all measured parameters for the different models, were in good agreement with, and comparable to (in terms of order-of-magnitude), those reported previously for selected commercial chocolates and model chocolates formulated to comparable specifications [2] [8] [11]. Also, it is not surprising to note such low values of plastic viscosity in both chocolate products owing to their relatively high fat contents (dark chocolate: 40 wt% and milk chocolate 37 wt %). This relates to availability of more fat to coat particle surfaces, bringing about a reduction in viscosity.

Lower plastic viscosities in milk chocolate as compared to dark chocolate can be explained through the presence of relatively larger particles (milk chocolate, D_{90}: 25.51 ± 1.30 μm; dark chocolate, D_{90}: 19.96 ± 1.64 μm) over a wider size distribution [26], presence of emulsifiers as opposed to their absence in dark chocolate [25], and possibly through the presence of freely added butter-fat and lactose [41] [38]. When distribution of particle sizes becomes wider with a presence of larger particles resulting in a low specific surface area, lesser surfaces need to be coated with fat resulting in availability of larger free-fat quantity contributing to a decrease in chocolate viscosity by restricting solids packing ability. Moreover, the smaller particles fill spaces between larger ones, reducing viscosity of suspension for any given solid concentration depending on the proportion of large-to-small particles. As for the presence of relatively similar proportions of smaller particles in both chocolates with approximately similar particle sizes (dark chocolate, D_{10}: 4.40 ± 0.22; milk chocolate D_{10}: 4.81 ± 0.10), the size and proportion of large particles becomes a significant influencing factor in modulating rheological behaviour. In addition, as the particles become finer, their number increases with parallel increase in points of contact between particles and in plastic viscosities.

Milkfat has the same effect as cocoa butter on viscosity if added to chocolate at 40°C, but slows-down the setting-rate and softens the final chocolate [25]. The presence of added butter fat contributing to a relatively larger pool of free-fat to coat particle surfaces may also be explanatory of relatively low plastic viscosities observed for milk chocolate. Moreover, it can be stated that crystallisation in WMP results in the liberation of milk fat originally trapped in the amorphous matrix contributing in viscosity reduction [38]. Lastly, the milk chocolate contained soy lecithin as an emulsifier, whereas the dark chocolate did not contain any added emulsifier. This can also be argued as an important factor contributing in relatively lower viscosity of milk chocolate. Emulsifiers like lecithin, function by acting as surface-active agents wherein the hydrophilic-end of the emulsifier molecule attaches to the sugar particle surfaces and the hydrophobic-end is suspended in the continuous fat phase in which the particles are dispersed resulting in a decrease in chocolate viscosity.

Lower yield stress parameters were also recorded for milk chocolate as compared to dark chocolate. Again, very low yield stress values can be attributed to high fat content in both chocolates and the presence of emulsifiers in the milk chocolate. Higher yield stress in dark chocolate can be attributed to the presence of relatively finer particles resulting in a higher specific surface area (cf. Table 3-6), and also to the tendency of these particles contributing in more compact aggregates under low shear or no-flow conditions. Rate of formation and disruption of aggregates in concentrated suspensions are functions of flow induced shear stresses, particle size and their volume fraction, and interaction energy [10]. An equilibrium aggregate size corresponds to each shear rate and relates to the time dependency and thixotropy of such suspensions. Under no-shear conditions, the sample spanning aggregates form or recover and cause an apparent yield stress to arise [42].

Table 3-7 Parameters of mathematical models and ICA recommendation used in characterising rheological behaviour of model dark and milk chocolate (Mean ± S.D)

Chocolate	Casson†		Herschel-Bulkley‡			Bingham*		ICA	
	η_{CA} (Pa.s)	τ_{CA} (Pa)	η_{pl} (Pa.s)	τ_o (Pa)	N	η_B (Pa.s)	τ_B (Pa)	η (Pa.s)	τ (Pa)
Dark Chocolate	1.56 ± 0.02	8.81 ± 0.03	4.34 ± 0.17	17.06 ± 0.255	0.85 ± 0.01	2.37 ± 0.03	24.12 ± 0.17	3.20 ± 0.03	33.74 ± 0.32
Milk Chocolate	1.46 ± 0.16	5.55 ± 0.65	2.90 ± 0.14	14.0 ± 0.07	0.92 ± 0.03	2.08 ± 0.07	17.37 ± 0.34	2.67 ± 0.08	26.65 ± 0.63

Standard Error: ***Dark Chocolate*** - †: 2.031-2.499, ‡: 0.8361-1.098, *: 8.255-9.708; ***Milk Chocolate*** - †: 4.424-5.015, ‡: 0.9235-1.152, *: 4.790-5.183

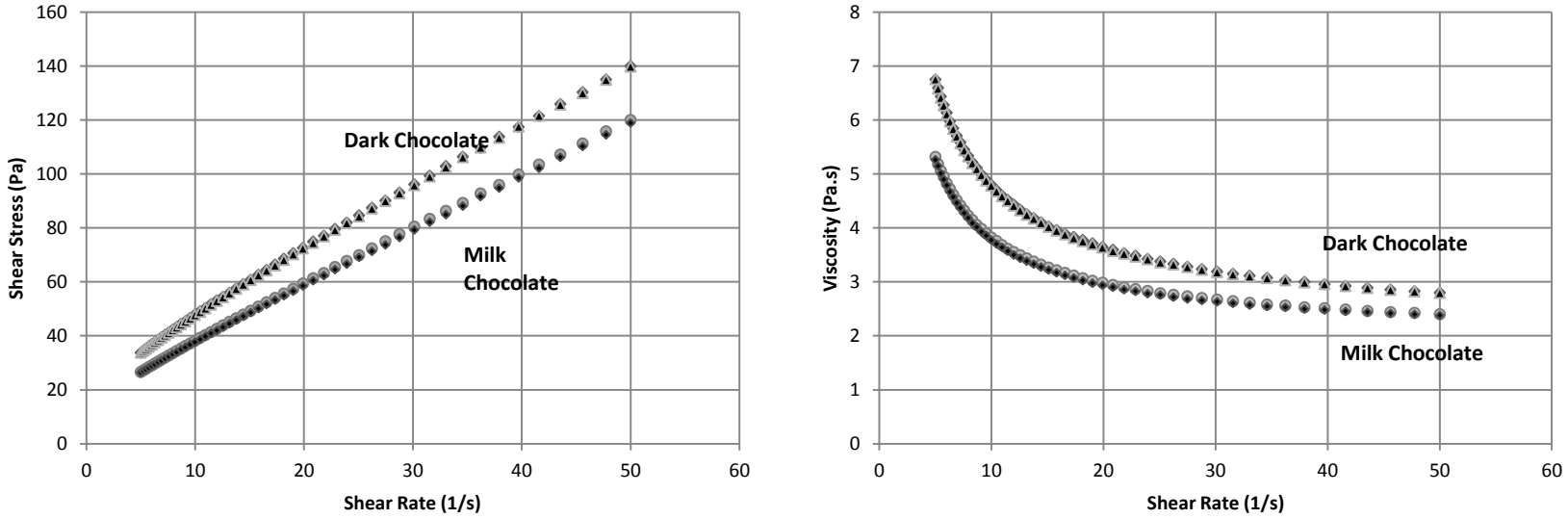


Figure 3-11 Rheograms of shear stress versus shear rate (left) and viscosity versus shear rate (right) of model dark and milk chocolate measured at 40°C. Overlapping curves represent ramp-up and ramp-down measurements of viscosity (right) and shear stress (left).

3.4.4 Chocolate Microstructure

3.4.4.1 Optical Microscopy

Optical micrographs of dark and milk chocolate melts acquired in bright field and differential interference contrast mode are compared in Figure 3-10 and Figure 3-11, respectively. Micrographs revealed a high solids packing density with extensive particle-particle interactions for both chocolates. It was necessary to use molten chocolate samples to obtain thin and even samples necessary for light microscopy. It could be possible that, keeping the cocoa butter molten during imaging influenced the packing and particle interactions as present in the crystalline network of solid tempered chocolate.

Solid particles, mostly ≤ 20 – 30 μm can be seen dispersed in a continuous fat matrix, such that the crystalline network was disperse with a large particle specific surface area. Smaller particles can be observed filling the gaps between the larger, resulting in a high packing density. The micrographs also reveal a uniform coating of fat on the solid particles, where the darker cocoa particles clearly stand-out from the white crystalline sugar particles in the case of dark chocolate, and from the white sugar/milk solid particles in the case of milk chocolate. Mastersizer laser diffraction particle size analysis of the selected dark chocolate yielded a D_{90} value of approximately 20 μm and that for the milk chocolate was about 25.5 μm, with unimodal distribution in the case of both chocolates (c.f Section 3.4.1). Although, it is difficult to infer absolute conclusions regarding differences in PSD between chocolates from the micrographs, in the case of the milk chocolate, the white non-cocoa solids appear to be on an average larger than that in the dark chocolate. As discussed earlier, the contribution of larger particles in the milk variant could possibly be explained through the presence of relatively elastic milk solids particles.

Dense bed-packing density in both chocolates resulting from particle-particle interactions and polar interactions of solid surface-fat, seems to have resulted in flocculation and agglomeration, forming dense pockets of particulates and stress bearing paths. Such compartmentalisation of the suspension matrix, influences flow behaviour and other mechanical properties, and can be thought to have implications on related sensorial attributes of the product. It is also interesting to note that even with high fat content of both chocolates, large flocculation and agglomeration of sugar crystal networks and high inter-particle contact points can be observed. Mechanical properties – firmness and hardness [4], and rheological properties [25] [43] have been previously attributed to depend on fat content in chocolates. Servais *et al.* [10] noted that yield stress was dependent on amount of particle-particle

interactions and specific surface area (number of smaller particles), and originated in mechanical friction and chemical interactions between particles. They also concluded yield value was determined by inter-particle contacts, with a consequent linear dependence on mean particle size and specific surface area.

Higher fat content can be thought to result in less dense sugar crystalline networks and reduced particle–particle interactions, with more open structures and void spaces between crystals and cocoa particles. Although this wasn't clearly visible in the micrographs, it was observed that fat uniformly coated particle surfaces, and filled the spaces between them; a mechanism attributed to reduce resistance in molten chocolate flow with greatest effect at lower particle sizes resulting in a smooth perception of texture [25].

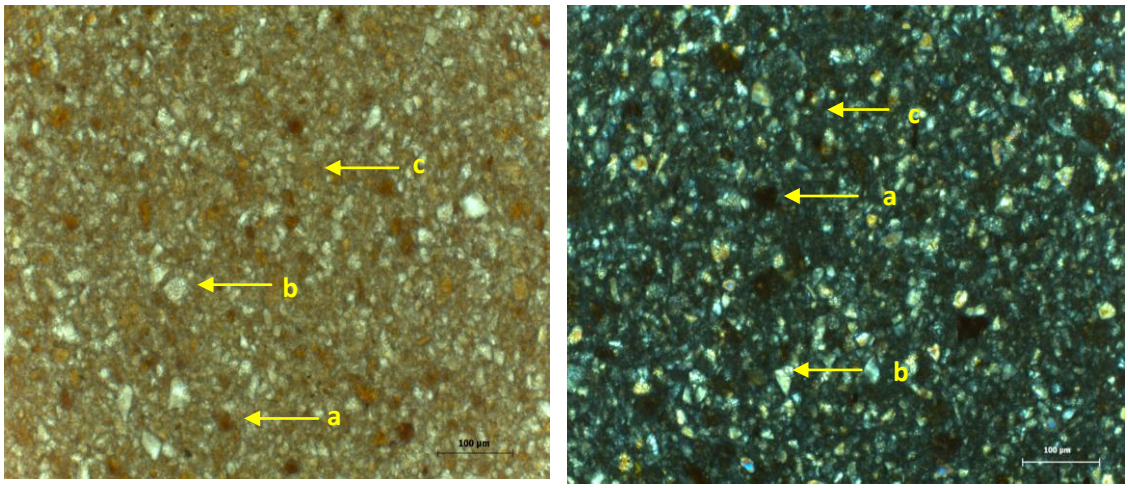


Figure 3-12 Dark chocolate microstructure viewed using bright field microscopy (left) and differential interference contrast (right) mode at 20X magnification. Cocoa particles (a); and sugar crystals (b) are indicated embedded in continuous fat phase (c).

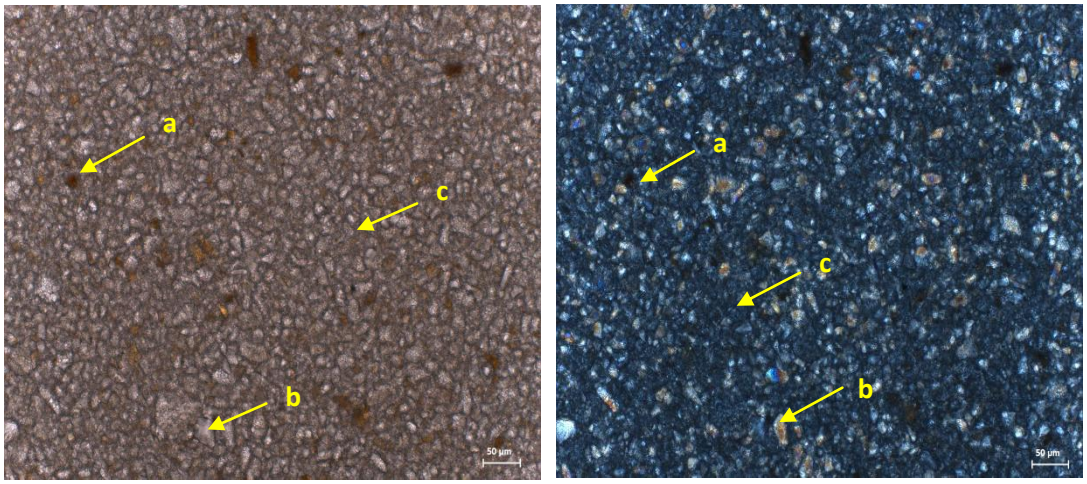


Figure 3-13 Milk chocolate microstructure viewed using bright field (left) and differential interference contrast (right) mode at 20X magnification. Cocoa particles (a) and sugar crystals (b) are indicated embedded in continuous fat phase (c).

3.4.4.2 Confocal Laser Scanning Microscopy (CLSM)

Inspection of Ingredient Mixtures for Analysis of Autofluroscence Emission Spectral Bandwidths

Autofluroscence of cocoa particles encountered during confocal microscopy has been reported previously [44] [45]. Although, information regarding emission bandwidths wherein the autofluroscence is encountered, using possible lasers and excitation wavelengths in CLSM seems absent in open literature. It was necessary to obtain information about these conditions used for confocal imaging, wherein autofluroscence could influence interpretation of images during microstructure analysis of chocolate and latter during CLSM analysis of chocolate boluses. This made it important to estimate CLSM autofluroscence conditions of cocoa particles.

The strategy used here was to image ingredient (cocoa butter, cocoa particles, and sugar) mixtures present in chocolate, and to check the full range of emission channels for autofluroscence, using all possible lasers and UV excitation. Figure 3-12 – 3-15 are the micrographs acquired for cocoa particles suspended in cocoa butter. As can be seen, sharp autofluroscence of cocoa particles is encountered for UV (405 nm), Kr/Ar (488 nm, 561 nm), and He/Ne (633 nm) at respective excitation frequencies. Collection channel windows set on the right of the laser excitation frequency, spanned the full range possible for emission; up to 753 nm. This interestingly proved that regardless of the laser used for excitation of a particular dye, cocoa particle autofluroscence would always be present in analysis using these CLSM conditions. For example, if Nile Red (excitation wavelength; 488 nm) is used for fat staining of dark chocolate and Kr/Ar laser used to excite the dye, autofluroscence of cocoa particles would be detected along with the labelled fat phase regardless of the collection window (wavelength span) used to acquire the micrograph. Moreover it is important to highlight that autofluroscence intensity may largely vary depending on the type and physical nature of the surrounding matrix, its interference may play a critical role in image analysis. Figure 3-16 shows sugar crystals and cocoa particles suspended in cocoa butter. Sugar crystals were clearly visible in the bright field mode, and the cocoa particles were visible as opaque black bodies. Confocal mode utilising Kr/Ar excitation wavelength 561 nm (covering Nile Red, Rhodamine B and Fast Green excitation frequencies) and emission channel 570 – 710 nm, showed autofluroscence of cocoa particles as expected, and sugars as black bodies (no fluorescence) with sharp crystal edges.

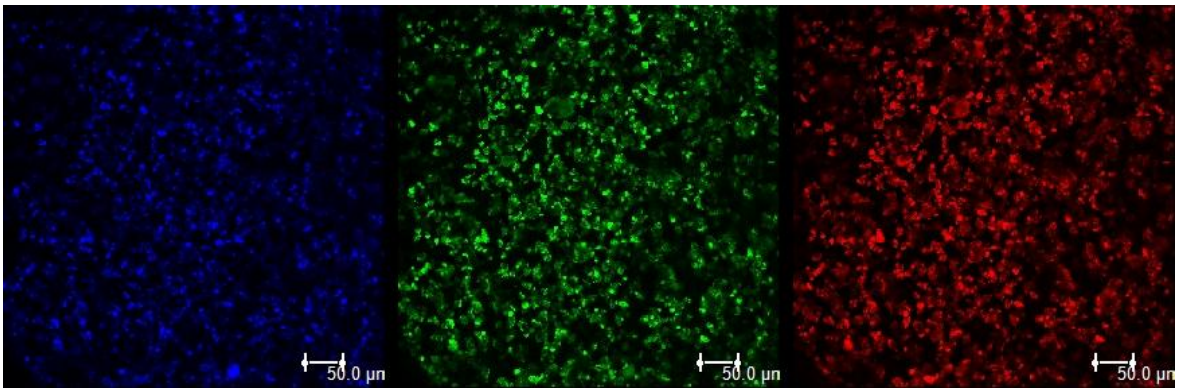


Figure 3-14 Confocal laser scanning micrographs showing autofluroscence of cocoa particles dispersed in cocoa butter. Excitation - UV (405 nm); Channel emission bandwidth – (Blue) 414nm – 502 nm; (Green) 505 nm - 597 nm; and (Red) 599 nm – 710 nm.

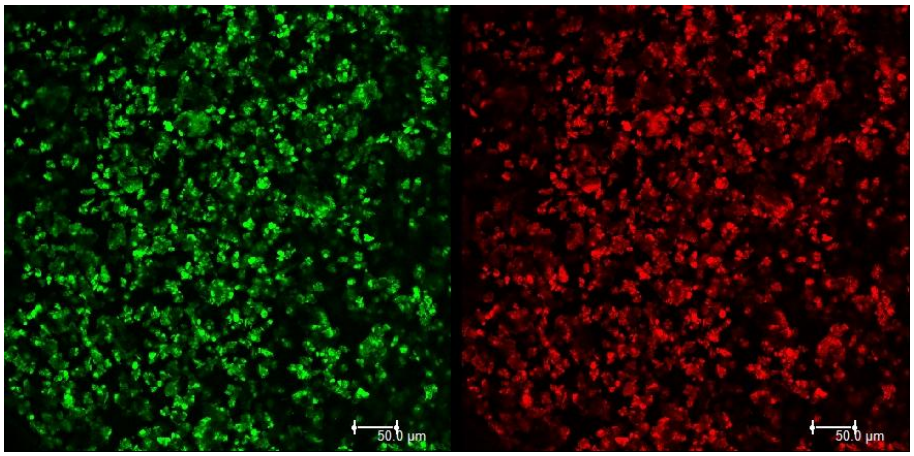


Figure 3-15 Confocal laser scanning micrographs showing autofluroscence of cocoa particles dispersed in cocoa butter. Laser-Kr/Ar (488 nm); Channel emission bandwidth – (Green) 495nm - 597nm; and (Red) 599 nm - 710 nm.

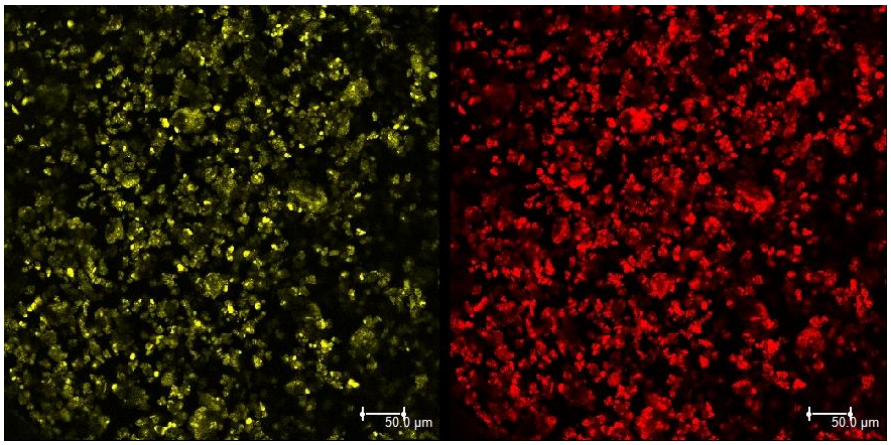


Figure 3-16 Confocal laser scanning micrographs showing autofluroscence of cocoa particles dispersed in cocoa butter. Laser-Kr/Ar (561 nm); Channel emission bandwidth - (Yellow) 566 nm - 597 nm; (Red) 599 nm - 710 nm.

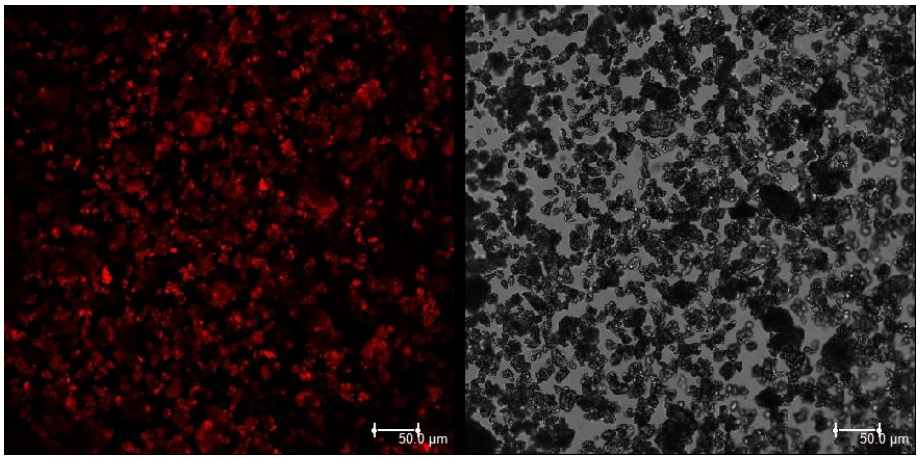
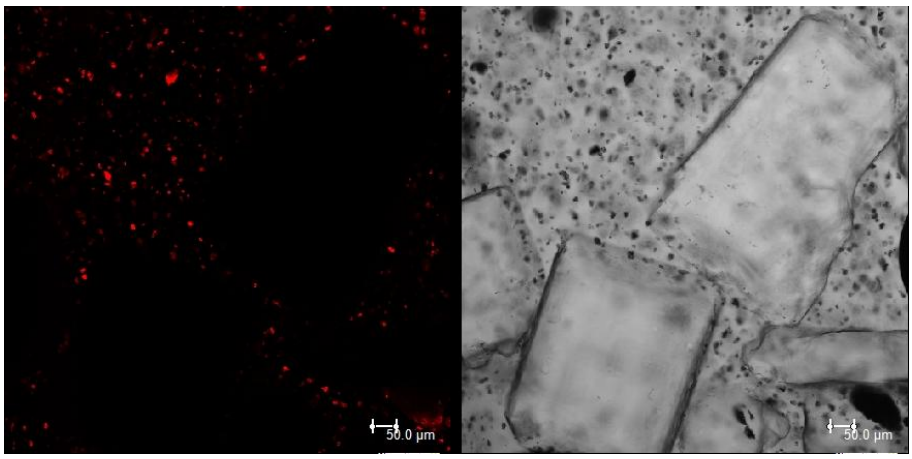


Figure 3-17 Confocal laser scanning micrographs showing autofluroscence of cocoa particles dispersed in cocoa butter. Laser-He/Ne (633nm), Channel emission bandwidth 640 nm -753 nm (left); Bright field mode (right).



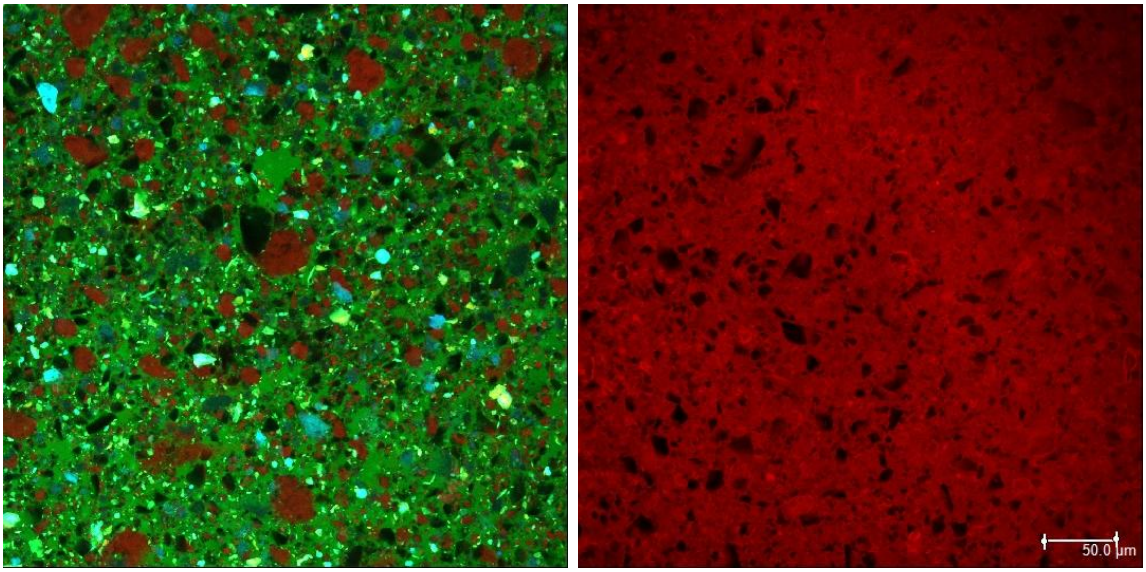
A **B**

Figure 3-18 Confocal laser scanning micrographs of cocoa particles and sugar crystals dispersed in cocoa butter. Laser-Kr/Ar (561 nm); (A) Channel emission bandwidth 570 nm – 710 nm; and (B) Bright Field mode

Assessment of Dark and Milk Chocolate Samples

Figure 3-17 A and B show the microstructure of molten milk and dark chocolate respectively, with fluorescent stain mixture - Nile Red + Fast Green used in the case of milk chocolate and fat stain Nile Red used to label dark chocolate. Figure 3-18 and 3-19 show surface micrographs of solid dark and milk chocolate respectively, both stained using nile red. Nile red preferentially partitions towards more hydrophobic species (fat), while fast green is protein specific and thus its targeted use was for preferential localisation of hydrophilic milk solids. As chocolate is a fat continuous food system with dispersed milk solids in the case of milk chocolate, PEG-300 and water were used as probe solvents for nile red and fast green, respectively.

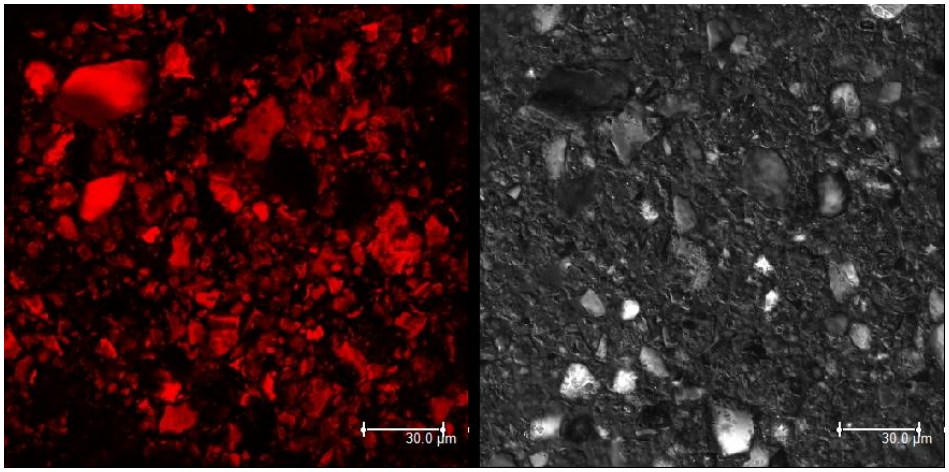
It can now be postulated that autofluroscence of cocoa particles may be partially involved for the background contrast in both chocolates, although the intensity may be diminished as compared to previous micrographs because of the crystalline fat matrix and higher concentration of other ingredients. All CLSM micrographs for dark and milk chocolate revealed presence of dispersed particles ≤ 30 μm in a continuous fat matrix forming a composite concentrated suspension microstructure. In the case of both chocolate, many of the unstained sugar crystals can be seen with sharp edges (Figure 3-17A, B), where background corresponds to the hydrophobic environment of the chocolate and is due to the lipophillic stain diffusing into the liquid portion of chocolate fat. Also as seen in the micrographs, cocoa particles stood-out in bright red (dark chocolate) and blue (milk chocolate) due to their autofluroscence under the imaging conditions. It is interesting to indicate at this stage that as all the dispersed particles (cocoa, milk-solids and sugar) in the chocolates are coated with fat which in turn may be nile red labelled, the black particles observed in the micrographs are due to a particular z-depth in imaging, i.e. no stain diffusing into the sugar particles. Hence, one must be careful in interpreting the particle-type based on these observations, while the strong background contrast caused by the excitation of the lipophillic stain my lead to difficulties in interpretation.



A **B**

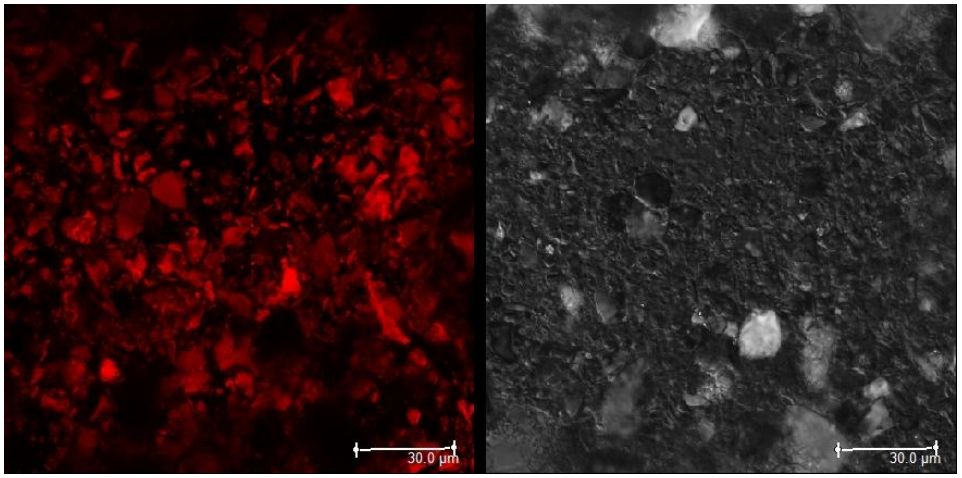
Figure 3-19 Confocal laser scanning micrographs molten milk chocolate (A) labelled with Nile Red + Fast Green, and dark chocolate (B) labelled with Nile Red. (**Milk Chocolate - Colour key**: Green/background contrast-Fat, Red-Milk Solids, Blue-Cocoa Particles, and Black/unstained-Sugar crystals; **CLSM Settings**: Laser-Kr/Ar (488/633nm), emission bandwidth 411nm - 489nm and 515nm – 635nm, Objective – 40X1.25 Dry. **Dark Chocolate – Colour Key**: Background contrast/ Red – cocoa particles, Red continuous – Fat, Black – Sugar crystals; **CLSM Settings**: Laser-Kr/Ar (488nm), emission bandwidth 536 nm - 612nm (Nile Red excitation and autofluroscence interference, Objective – 40X0.75 Dry).

The dual excitation CLSM micrograph of milk chocolate (Figure 3-17A) also revealed a dispersion of milk solids, sugar crystals, cocoa solids and fat by negative contrast, while autofluroscence of cocoa particles was also encountered under the imaging conditions and helped in interpreting their localisation. Interpretation of the components seen in the micrograph was enabled from the separate single excitation images, wherein the milk solids containing the protein were preferentially stained by fast green, cocoa particles were identified from by bright autofluroscence under UV, fast green and nile red channels, and sugars and fat phase were identified as in the case of dark chocolate.



A **B**

Figure 3-20 Confocal laser scanning micrographs solid dark chocolate labelled with Nile Red. A) Laser-Kr/Ar (561 nm), Channel emission bandwidth 571 nm - 701nm (Nile Red excitation and autofluroscence interference); B) Polarised mode. Objective – 63X 1.40 Oil Immersion.



A **B**

Figure 3-21 Confocal laser scanning micrographs solid milk chocolate labelled with Nile Red. A) Laser-Kr/Ar (561 nm), Channel emission bandwidth 571 nm - 701nm (Nile Red excitation and autofluroscence interference); B) Polarised mode. Objective – 63X 1.40 Oil Immersion.

Optical sectioning and surface micrographs of both chocolates also revealed high degree of particle-particle interaction (contact points) and surfaces of crystalline sugar particles coated with fat (Figure 3-18 and 3-19). The images in Figure 3-18 and 3-19 are high-magnification (630x) image of chocolates, re-crystallised after stain diffusion. There is a great variety in the morphology of the particles found in both chocolates, with particles as small as 5 μm visible, with relatively larger sugar particles present in milk chocolate and compared to dark. If a drawback to CLSM can be thought of in context of chocolate microstructure analysis, it is that stains must be used. Stain incorporation is most easily achieved into molten chocolate to acquire good samples for microscopy; nevertheless these may be subsequently re-crystallised as done in this study. Although using crystallised solid chocolate samples can be associated with lack of image details and quality. Liquefaction of the samples on the other hand can circumvent these problems, but may also damage the innate ordering of the structural elements generated during industrial chocolate making, especially in assessment of fat crystal morphology in solid chocolate obtained through an accurate tempering and cooling regime.

3.4.5 Melting Behaviour and Solid Fat Index (SFI)

Melting endotherms and extracted melting behaviour parameters of dark and milk chocolate are compared in Figure 3.20 and Table 3.8. The peak onset corresponds to the temperature at which a specific crystal form begins melting; peak maximum, that at which the melting rate is maximum; and end of melting, complete liquefaction of solid fat crystals. All these are related to the fat crystal-type in the continuous phase. Peak height, position and resolution are related to sample composition and crystalline state character [46]. Both chocolate samples exhibited distinct single endothermic transitions between 20°C and 40°C; a range expected for chocolate melting profiles. Heat flow was recorded to gradually and consistently increase to the onset temperature (T_{onset}), and then progressively more rapidly until the peak maximum (T_{peak}) was attained. Thereafter, it decreased to the end temperature (T_{end}) indicating complete liquefaction of chocolates.

Thermograms of the dark and milk chocolate were distinctly different. They evidently differed in peak shape and size, suggesting characteristic differences in crystallinity and degree of crystallisation as reflected through the melting properties of both chocolates. As expected, milk chocolate demonstrated lower values for all melting parameters apart from $T_{index,}$ as compared to the dark chocolate. Reflecting on an optimal processing and storage temperature

history, T_{end} values for both chocolates were in the range of 31.0 to 33.5°C indicating similar β Form V polymorphic stability.

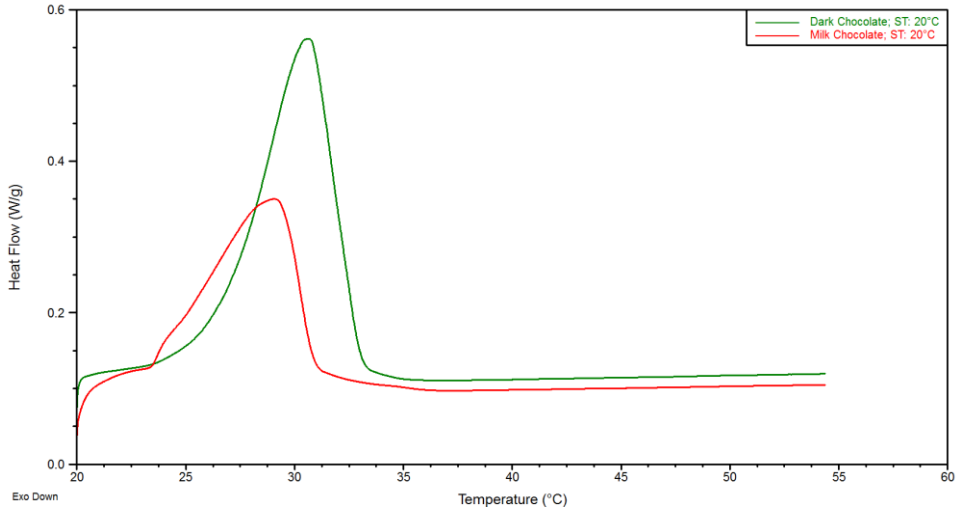


Figure 3-22 DSC thermograms for dark (green) and milk (red) chocolate stored at 20°C prior to measurements. (ST = storage temperature)

Table 3-8 Melting properties of dark and milk chocolate (Mean ± S.D)

Chocolate	Storage Temperature	Tonset (˚C)	Tindex (˚C)	Tend (˚C)	Tpeak (˚C)	ΔHmelt (J/g)	Peak Height (W/g)
Dark Chocolate	**20˚C**	26.4 ± 0.06	6.84 ± 0.04	33.24 ± 0.03	30.62 ± 0.10	54.39 ± 1.00	0.43 ± 0.09
Milk Chocolate	**20˚C**	23.73 ± 0.84	7.55 ± 0.79	31.29 ± 0.06	29.22 ± 0.16	36.47 ± 2.64	0.24 ± 0.01

Although, differences in melting behaviour of chocolates with variation in factors like PSD, emulsifier and fat concentration have been reported previously [15], fat blending is arguably the overriding factor contributing to the differences in melting properties observed here. As discussed previously (cf. Section 2.3.5 and 2.4.7), the melting behaviour of chocolates is largely determined by the underlying fat phase. The thermodynamic incompatibility between milk fat and cocoa butter resulting from compositional differences, geometric constraints and environmental factors influences kinetics of co-crystallisation in blends of milk fat and cocoa butter TAGs resulting in formation of a eutectic – a decrease in melting point below that of the individual components [28] [34]. This influences oral behaviour of the chocolate which is constituted from the blend, and makes it important to control the amount of milkfat and/or milkfat fractions which can be present in the chocolate, along with the tempering conditions so that a stable product of desired quality and textural properties is obtained [47] [48].

It can be noticed that the T_{onset} (inflection point) for the major endothermic transition for milk chocolate (T_{onset}: 23.7 ± 0.8 °C) takes place at a lower temperature as compared to the dark chocolate (T_{onset}: 26.4 ± 0.06 °C). Moreover, average T_{peak} values for the milk and the dark chocolate were 29.2 ± 0.16 °C and 30.62 ± 0.10 °C, respectively, and average T_{end} values were recorded at 31.29 ± 0.060°C and 33.24 ± 0.03°C for milk and dark chocolate, respectively. This implied that even though initiation of melting for the milk chocolate was earlier than the dark chocolate, the continuous phase in both products was tempered to homogeneous β Form V morphology.

Likewise, lower values of ΔH_{melt} were recorded for the endothermic peaks between onset and end for milk chocolate as compared to dark. This suggested that the milk chocolate required less energy to complete melting as compared to dark chocolate. Again, this effect can be argued through the presence of milkfat and also possibly through the presence of compatible milkfat fractions added to the milk chocolate, as opposed to presence of only CB in dark chocolate. This property is directly related to the differences in SFC of the chocolates (in this case, at ambient temperature: 20°C) in that, lower energy is consumed for melting lower levels of solid fat in the milk chocolate which relates to the interaction of milk fat TAGs with that of CB, and also resulting from the higher molecular entropy of the continuous phase. Figure 3-21 shows the change in solid fat index (SFI) for both chocolates with increasing temperature. Areas under the curves (AUCs) and their relative fractional change for specific temperatures in the ramp are shown in Table 3-9 and Figure 3-22 respectively.

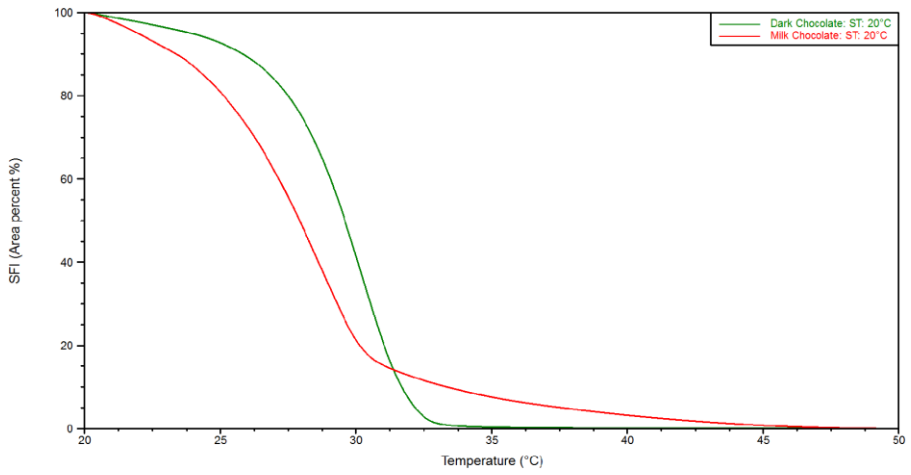


Figure 3-23 Solid fat index (SFI; Area %) of dark (green) and milk (red) chocolates for DSC temperature ramp: 20 - 50°C deduced from the heat flow endotherms

Between 20°C and 30°C, the rate of melting of milk chocolate was faster than the dark chocolate, while between 29˚C and 35˚C the dark chocolate demonstrated a relatively sharper

melting pattern. It was recorded that the first 9°C increase in temperature resulted in ~ 40 % decrease in solid fat content of the dark chocolate ($AUC_{29°C}$: 60.10 ± 1.593 %) as compared to ~ 62 % in milk chocolate ($AUC_{29°C}$: 37.79 ± 3.75 %). ~ 99.5% and 99.0% of the initial solid fat (at 20°C) present in dark and milk chocolates respectively, was melted between 20°C and 45°C. For both chocolates, a steep rate of melting was observed until 32°C, at which 88.4 % and 85.4 % of the initial solid fat was liquefied for dark and milk chocolate, respectively, and the largest relative step change was recorded between 26°C and 32°C corresponding to the endothermic transition discussed earlier.

Table 3-9 Percentage areas under the curves (AUC %) for dark and milk chocolate representing their respective SFI at specific temperatures from 20°C to 45°C (Mean ± S.D)

Temperature (°C)	Dark Chocolate AUC (%)	Milk Chocolate AUC (%)
20	99.9 ± 1.74E-14	99.99 ± 0.005
23	94.04 ± 0.9	91.31 ± 1.4
26	85.97 ± 0.9	74.88 ± 2.4
29	60.10 ± 1.5	37.79 ± 3.7
32	11.51 ± 0.9	14.57 ± 3.4
35	4.22 ± 1.1	8.91 ± 2.2
40	1.77 ± 0.4	3.83 ± 1.1
45	0.41 ± 0.1	0.95 ± 0.3

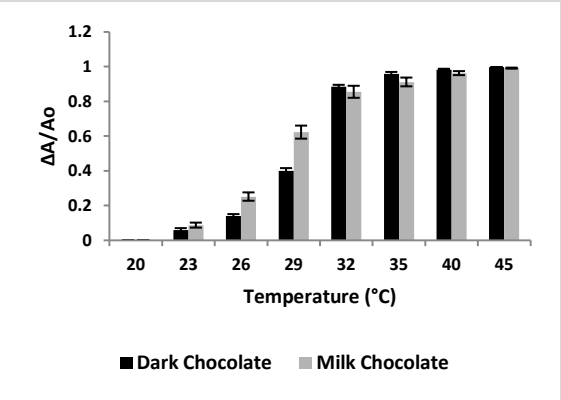


Figure 3-24 Fractional change in AUCs of dark and milk chocolate at specific temperatures from 20°C to 45°C (Mean ± S.D)

Interestingly, from 32°C onwards, it was observed that the milk chocolate consistently showed higher SFI values and a slower rate of melting as compared to the dark chocolate. As seen in Figure 3-21, as opposed to dark chocolate, the SFI curve for milk chocolate shows an inflection at ~ 31°C continuing as a gradually decreasing plateau until 45 - 50°C. This observation can be related to the higher melting point (>50°C) of high melting fraction (HMF) contributed by a specific concentration of native milkfat or externally added to the milk chocolate [48]. Due to higher compatibility of HMF with CB, it does not result in formation of a eutectic, and hence is often added back to AMF to increase and/or adjust hardness and melting point of chocolates [28] [29].

It is now contextual to point out as seen Figure 3-20; the milk chocolate thermogram shows a marginal endothermic transition between 20 - 24°C which is incompletely observed. For scans starting from lower than 20°C (e.g. 0°C) this transition is evident completely (cf. Section 5.3.3.1) and may be related to lower melting fractions (LMF; T_m >15°C) contributed

by the native milkfat. Hence, taking into account both these transitions on the lower (<20°C) and higher (>32°C) melting range, the T_{index} values of milk chocolate are relatively higher than that of dark chocolate. It is important to highlight that even though this indicates that the milk chocolate may take relatively longer to melt completely, the transitions discussed above are marginal with respect to the relative SFC at 20°C, and the major melting zone is within the β Form V region of the fat blend. Hence it may be difficult to infer to what extent these transitions relate to differences in oral behaviour during eating.

CHAPTER 4

Chocolate Bolus Formation and Characterisation of Chocolate Eating Strategies

4.1 Context

The scope of this study was to investigate the variations in eating (mastication and swallowing) behaviour for the selected dark and milk chocolate products, and to screen-out candidates with significantly different chocolate eating strategies for participation in the next stage of research. The study further aimed at highlighting whether individual eating strategies and sensory textural perceptions related to the differences in physical properties between dark and milk chocolate. Furthermore, in this chapter, the process of physical transformation of chocolate during mastication has been discussed and an empirical scale termed as – "Window of Chocolate Mastication" developed through specific oral perceptions of selected subjects is documented.

4.2 Methods

4.2.1 Screening of Chocolate Consumers – Eating Strategies

24 chocolate consumers (age 18-40 years; 11 females and 13 males) were recruited from Massey University, Palmerston North, New Zealand. All subjects were screened through a criterion of good general and dental health. The subjects consumed freshly opened dark and milk chocolate (10 g serving size, cubical section - 35 mm X 40 mm X 6 mm) samples stored at 20°C prior to the study sessions. Each subject participated in 2 sessions lasting up to 40 min each. During these sessions the dark and milk chocolate was analysed in triplicates by each subject, and the servings were according to a randomised cross-over design applied over the total population. A 10 min break was given between samples, and drinking water was provided between servings to clear the mouth of any residual chocolate. For each subject, 4 chewing and 3 swallowing parameters each were determined during the sessions. These were – *chewing parameters*: total number of chews, total chewing time (time from first bite –to- last observable jaw cycle), number of chews until first natural swallow and total chewing rate (calculated); and *swallowing parameters*: total number of swallowing events, time of first swallow and time of last

swallow (time from oral acquisition of sample -to- last swallow leading to major bolus clearance). Chewing parameters were recorded by visual analysis, and the subjects were asked to indicate every swallowing event by a hand gesture to record the swallowing parameters. All samples were served in identical aluminium pie containers, and had similar shape and weight.

Every subject also evaluated the two chocolates in terms of preference (textural preference) and for sensory physical/textural attributes defined in Table 4-1. Preference was evaluated by a paired preference test performed by each subject. They were asked to taste each sample and record which one was most preferred. Sensory physical and textural attributes were evaluated using a series of paired comparison tests. Subjects were asked to taste each sample in the order presented and determine which chocolate had the dominance in the attribute in question. The definition of each attribute and the required eating protocols were fully explained and discussed to ensure all participants understood the assessments prior to commencement of the session. The study was reviewed and approved by the Massey University Human Ethics Committee (MUHEC) (Low Risk Research Involving Human Participants, Application 09/10).

Table 4-1 Glossary of Physical and Textural Attributes of Dark and Milk Chocolate

Physical and Sensory Attributes	Definition
• Hardness	- Force required to break/chew the product at first bite.
• On-set of Melting	- First perception of melting/formation of liquid phase in-mouth.
• Speed of Melting	- Perceived speed of melting of solid chocolate/formation of liquid phase in-mouth.
• Mouth Coating	- Extent of coating of oral cavity surfaces by residual molten chocolate after bolus clearance.
• Cohesiveness	- Agglomeration of particles together to form cohesive-lumps; characteristic of a compact bolus in mouth, opposed to dispersed.
• Stickiness/Adhesiveness	- Adhesion of the product to palate during eating, needing efforts of the tongue to clear/transfer the bolus to the back of the mouth for swallowing.
• Thickness of Melt	- Presence of flow resistance of the product when the tongue is put in contact with the palate during oral processing. As opposed to fluidity.
• Ease of Swallowing	- Product perceived as requiring less tongue and oral muscle-work to swallow. As opposed to difficult.
• Presence of Cohesive Lump/s at Point of Swallow	- Perceived presence of cohesive-lumps of chocolate at first natural point of swallow.
• Physical State of Swallowed Bolus	- Perceived physical state of bolus matter which is swallowed at first natural point of swallow. (Majorly solid, completely liquid, or liquid with presence of solid/semi-solid chocolate particles.)

4.2.2 Data Analysis of Eating Parameters and Selection of Representative Test Subjects

Statistical analysis was performed using software OriginPro 8.6 (OriginLab Corporation, MA, USA). An analysis of variance (ANOVA) test was performed with the data collected for mastication and swallowing parameters to determine if significant differences occurred between the chocolate samples and between subjects. Statistical comparisons were considered to have significant differences for $p \leq 0.05$.

To identify different eating strategy clusters (groups) of dark and milk chocolate, an agglomerative hierarchical cluster analysis was performed. Both chewing and swallowing behaviour parameters were utilised for cluster analysis. The statistical method clusters observations based on their similarity. The Euclidean method was used which applies geometric distance index, while the Ward's method was applied for agglomeration which uses an ANOVA-type sum of squares as a distance measure. Fisher's LSD multiple comparison tests and two factors – sample, subject ANOVA was used for eating parameters differentiating between clusters. Principal component analysis (PCA) was performed to visualise the distribution of subjects with relation to the significantly influencing chewing and swallowing parameters to select three specific test subjects (S1, S2 and S3) varying widely in their eating strategies. Post-hoc Fisher's LSD comparison was used to report differences between selected subjects for each eating parameter for dark and milk chocolate.

4.2.3 Window of Chocolate Mastication

A "*Window of Chocolate Mastication*" was constructed as an empirical scale which maps certain events which occurred during specific points of time, or were short-lived for a specific instance, or prolonged for a certain time-interval with respect to characteristic eating strategies. Subject selected after screening (S1, S2 and S3) dedicated one session each so as to produce data required to construct the window of mastication relative to each of their respective eating strategies and oral perceptions while eating the chocolates naturally. For visual examination of the boluses, subjects were asked to expectorate when they were ready-to-swallow, as well as at fixed times during the masticatory sequence.

4.3 Results and Discussion

4.3.1 Eating Strategies and Sensory Attributes for Dark and Milk Chocolate

Significant differences were found between subjects with regard to the 7 chewing and swallowing parameters considered to investigate overall eating strategies for dark and milk chocolate. Although, while considering if eating strategies changed between the dark and milk chocolate, significant differences were found for 3 chewing parameters (*total number of chews*, $p<0.001$; *total chewing time*, $p<0.001$; and *number of chews until first swallow*, $p<0.001$), and one swallowing parameters (*time of first swallow*, $p<0.001$) as identified in Table 4-2. On an average, higher values of chewing parameters as well as swallowing parameters were recorded for dark chocolate as compared to the milk chocolate.

Table 4-2 Overall mean values (n=24) for mastication and swallowing parameters of dark and milk chocolate. Indicated values (#) are for $p \leq 0.05$.

Parameter overall means	Dark Chocolate	Milk Chocolate	Standard Error	p value
1. Total number of chews	47.46	37.02	2.28	<0.001#
2. Total chewing time (s)	53.58	42.02	3.54	<0.001#
3. Number of chews until first swallow	30.08	21.56	1.63	<0.001#
4. Chewing rate (s^{-1})	0.95	0.96	0.05	0.89
5. Total number of swallowing events	3.78	3.64	0.17	0.06
6. Time of first swallow (s)	27.50	19.71	1.73	<0.001#
7. Time of last swallow (s)	42.50	39.71	2.71	0.39

As seen in Figure 4-1, a noticeable spread is present within the cumulative distribution curves in case of total chewing time and total number of chews. Likewise, the spread was also noticeable for total number of chews until first swallow and time of first swallow for both chocolates. This is likely of the fact that both physical and behavioural factors may be influencing with individuals adapting distinctive eating strategies. It again implied that the chocolates differed greatly in the way they were chewed and for when the trigger to swallow was generated for the first time. Conversely, a distinctly less spread was observed for the distributions of chewing rate and the total number of recorded swallowing events in the whole eating duration. For both chocolates on an average 3 swallowing events were performed for clearance of majority of chocolate from the mouth, with 96% of the population performing 4 or lesser swallows.

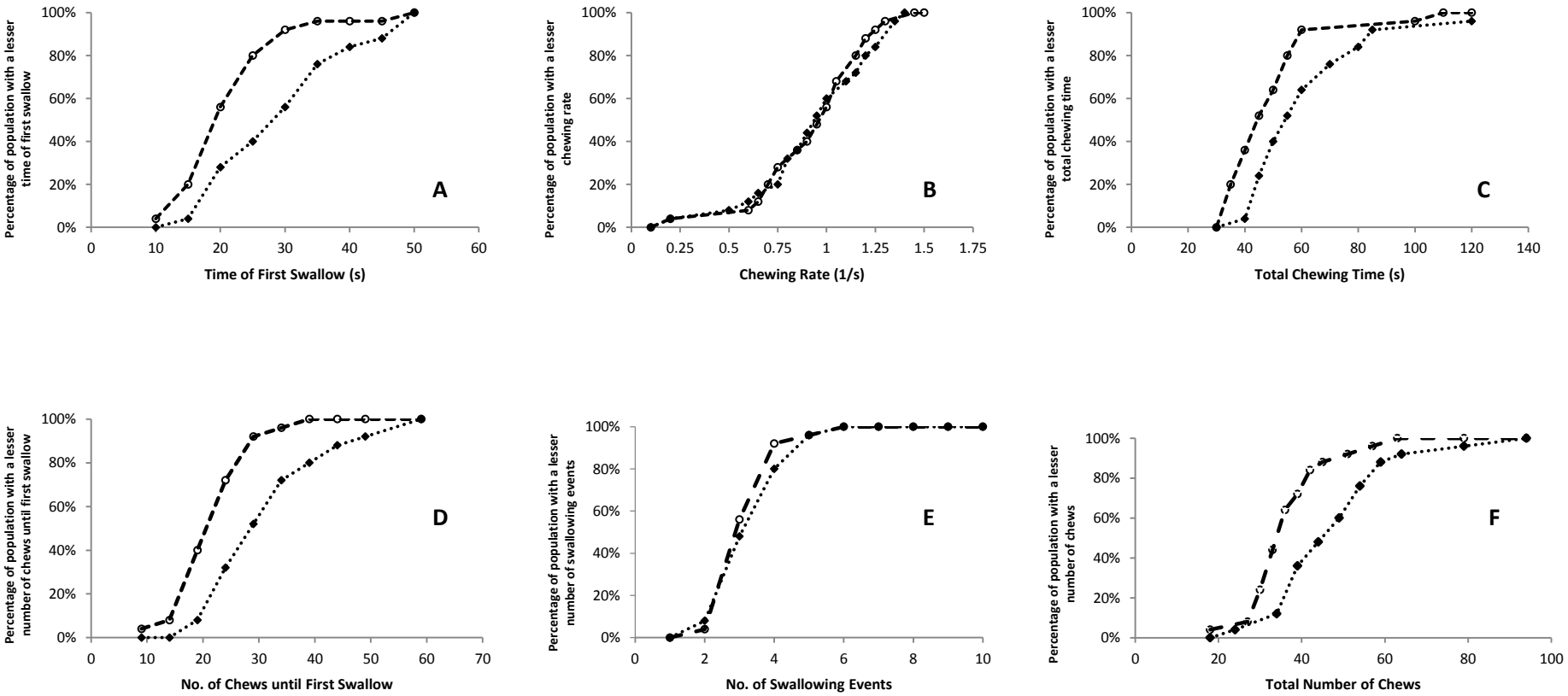


Figure 4-1 Cumulative distribution of mastication (B, C, D, F) and swallowing (A, E) parameters

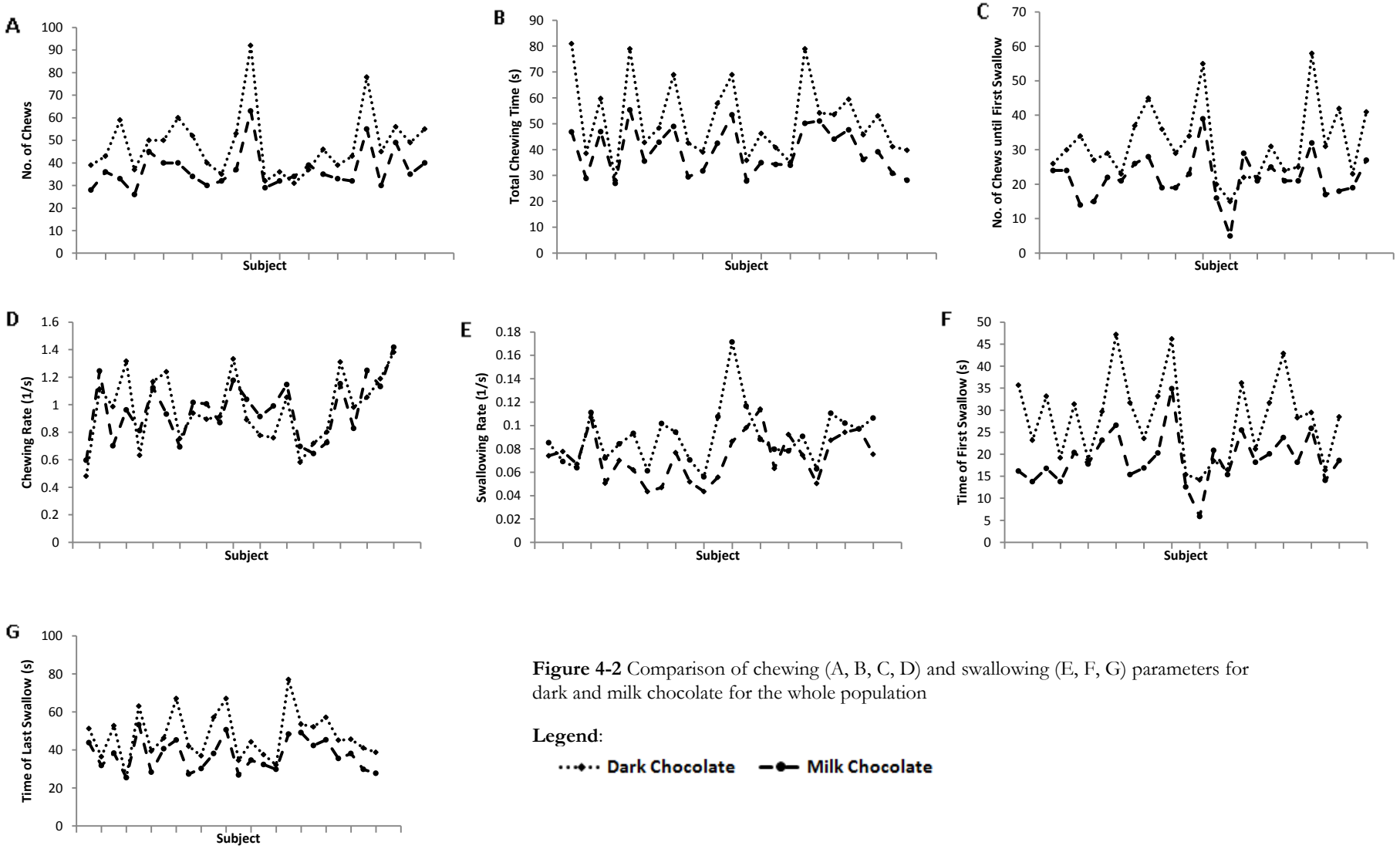


Figure 4-2 Comparison of chewing (A, B, C, D) and swallowing (E, F, G) parameters for dark and milk chocolate for the whole population

Legend:

The average chewing rate for both chocolates was approximately 0.95 s^{-1}, with just 4 % of the population chewing at or below a rate of 0.2 s^{-1}, and 4 % chewing at or over 1.3 s^{-1}. 16% of the population processed the dark chocolate for over 40 sec before swallowing for the first time, as compared to just 4 % for milk chocolate. Also, just 20% of the subjects had a total masticatory time of greater than or equal to 50 sec for milk chocolate as compared to 48 % in the case of dark chocolate.

A comparison of mastication and swallowing parameters of each subject in the population for dark and milk chocolate presented in Figure 4-2. It can be seen that on an average similar mastication and swallowing strategies were observed for eating strategies for both chocolates. This suggested that although the magnitude of a parameter changed within individuals and between chocolates, the general behavioural patterns for chewing and swallowing were maintained by subjects, also indicating adaptation of mastication strategies to chocolate-type along with behavioural dependence.

Results of paired comparison testing for investigating preference and sensory attributes are shown in Figure 4-3. Milk chocolate was preferred more as compared to the dark chocolate ($p \leq 0.05$). All participants reported faster speed of melting and earlier onset of melting for the milk chocolate. As expected, the dark chocolate was reported significantly harder than the milk chocolate. Also, the milk chocolate was reported as easier to swallow, more sticky, although less cohesive, and was perceived thinner in the mouth as compared to the dark chocolate.

It is interesting to note that the observed differences in mastication strategies between chocolates and the sensory results for all attributes in question could well be correlated to the physical properties of the chocolates characterised in Chapter-3 (cf. Sections-3.4.2, 3.4.3 and 3.4.5). Differences in chewing parameters (total number of chews and number of chews until first swallow) between chocolates may be predominantly related to the differences in the physical character of the fat phase in turn relating to differences in hardness and melting behaviour. The dark chocolate with relatively higher solid fat content at room temperature was harder compared to the milk chocolate; consequently it required greater number of chewing cycles to comminute its initial serving as well as the subsequent size-reduced particles during the masticatory sequence. Higher values of melting properties (T_{onset}, T_{end}, T_{peak}, and ΔH_{melt}) resulting from its continuous phase character, may also have contributed through delayed onset of melting, higher energy requirements for melting and lower melting rates for dark chocolate, resulting in higher chewing parameters.

In addition, the swallowing parameters, number of swallowing events performed during eating and time of first and last swallow, can also be explained by the hardness and melting properties of the chocolates, and the rheological properties of the melts. Once again, greater hardness and higher values of melting properties may relate to potentially delayed swallowing events, as the chocolate requires greater number of chews and longer chewing/residence time in mouth to be processed to a swallowable consistency. Again, this results in the dark chocolate through higher energy requirements for melting and lower melting rates as compared to milk chocolate. In addition, differences in rheological and textural properties of the chocolate melts may also contribute to delay of swallowing events and longer oral residence times of the dark chocolate. The higher plastic viscosity and yield stress, as well as firmness, cohesiveness, index of viscosity and consistency of the dark chocolate melts, will increase the time and muscle-work required to prepare the chocolate bolus to a state that can be successfully, and with minimum effort, propelled and swallowed.

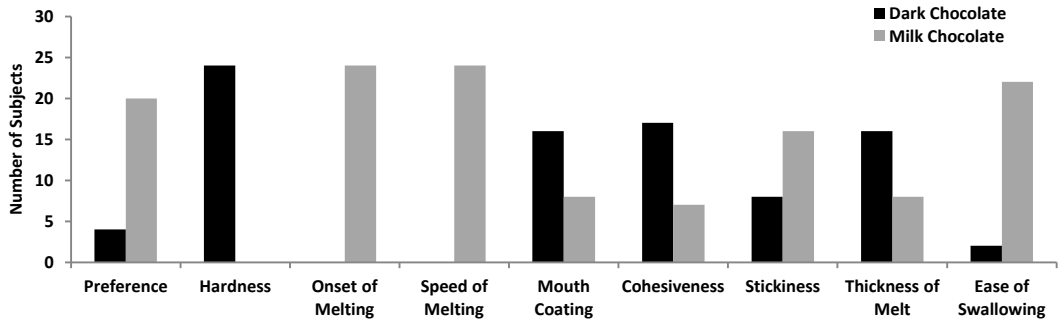


Figure 4-3 Sensory attributes as rated by the whole population (n=24) from the paired comparison test between dark and milk chocolate (each subject assessed the chocolate samples in duplicates in separate sessions).

Furthermore, the mechanical and rheological properties of the dark and milk chocolates and their corresponding melts, were in good agreement with the results of sensory perceptions reported by subjects. The dark chocolate with higher values of instrumental hardness, mechanical/textural properties of melt (firmness, cohesiveness, index of viscosity, consistency), rheological properties of melt (plastic viscosity and yield stress) as compared to milk chocolate, also resulted in higher sensory perceptions of hardness, mouth-coating, cohesiveness, thickness of melt. Likewise, the significant differences in sensory perceptions of onset of melting and speed of melting may well be related to the differences solid fat index and melting behaviour characterised as described previously (cf. Sections – 3.4.5).

Finally, it is noteworthy that while the eating strategies of subjects for dark and milk chocolates appear to be related to the physical characteristics of the chocolate matrices and

their corresponding melts, the possibility that other human-related psycho-physiological factors and product-related organoleptic attributes may also have a dominant involvement in the process. For chocolates, this interestingly highlights a structure-property-oral transformation relationship which forms the underlying basis for texture perception, and how this relationship may relate to variation and adaptation of eating strategies during chocolate oral processing.

4.3.2 Segregation of Candidates According to Chocolate Eating Strategies

Hierarchical dendrograms obtained from agglomerative cluster analysis to segregate subjects based on inter-individual variability in eating strategies are shown in Figure 4-4. Three groups of subjects were identified from cluster analysis of candidates with different traits in eating strategies for dark and milk chocolate. Four and five cluster solutions at lower distances of dissimilarity were also considered, although in these cases, significant similarities were noted in several eating parameters between clusters. Hence, the 3-cluster solution was considered most efficient for segregation of subjects.

All parameters considered for cluster analysis presented significant differences between clusters for both dark and milk chocolate. Tables 4-3 and 4-4 list the mean eating parameter values associated with each cluster and indicate where significant differences exist for both dark and milk chocolate respectively. In the case of dark chocolate, total number of chews, total chewing time, time of last swallow, and for milk chocolate, total number of chews, total chewing time and time of first swallow showed significant differences between clusters and may be considered as the major parameters relating to cluster division.

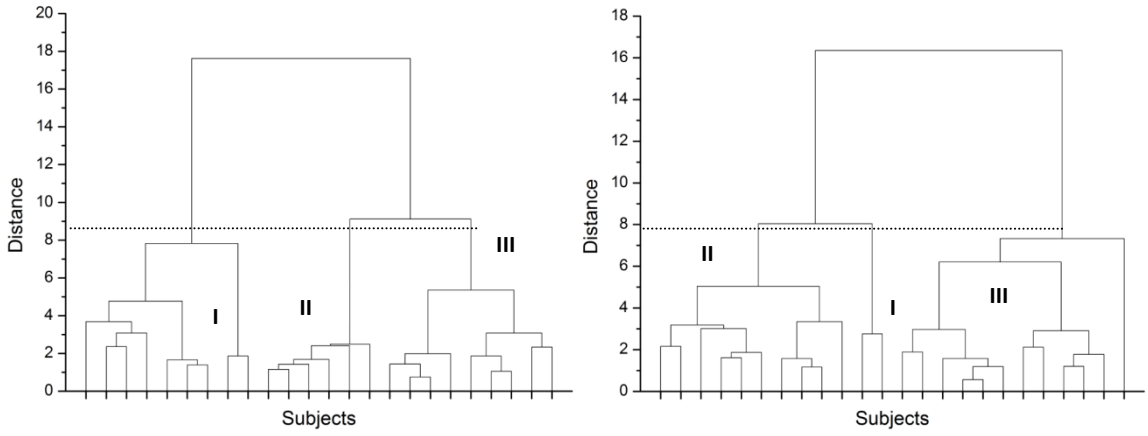


Figure 4-4 Dendrograms from the hierarchical agglomerative cluster analysis for dark (left) and milk (right) chocolate eating behaviour.

Table 4-3 Overall mean values for eating behaviour parameters by clusters for dark chocolate. Indicated values (#) are for $p \leq 0.05$. Values with similar letters are not significantly different at $p \leq 0.05$.

Parameter overall means	Cluster I	Cluster II	Cluster III	p value
1. Total number of chews	57.88[a]	39.91[b]	44.94[c]	**<0.001#**
2. Total chewing time (s)	65.72[a]	36.52[c]	45.31[b]	**<0.001#**
3. Number of chews until first swallow	37.22[a]	26.33[b]	27.00[b]	**0.001#**
4. Chewing rate (s^{-1})	0.90[b]	1.09[a]	1.07[a,b]	0.071
5. Total number of swallowing events	3.88[a]	2.91[b]	4.06[a]	**<0.007#**
6. Time of first swallow (s)	35.57[a]	20.43[b]	21.77[b]	**<0.001#**
7. Time of last swallow (s)	58.68[a]	34.40[c]	43.46[b]	**<0.001#**

Table 4-4 Overall mean values for eating behaviour parameters by clusters for milk chocolate. Indicated values (#) are for $p \leq 0.05$. Values with similar letters are not significantly different at $p \leq 0.05$.

Parameter overall means	Cluster I	Cluster II	Cluster III	p value
1. Total number of chews	59.50[a]	33.66[c]	38.40[b]	**<0.001#**
2. Total chewing time (s)	49.92[a]	31.30[b]	47.32[a]	**<0.001#**
3. Number of chews until first swallow	34.50[a]	19.75[b]	21.65[b]	**<0.001#**
4. Chewing rate (s^{-1})	0.82[b]	1.19[a]	1.02[a]	**<0.001#**
5. Total number of swallowing events	3.00[a]	3.37[a]	3.70[a]	0.165
6. Time of first swallow (s)	28.05[a]	15.69[c]	20.21[b]	**<0.001#**
7. Time of last swallow (s)	47.95[a]	29.76[b]	43.94[a]	**<0.001#**

Cluster I for both chocolates, appears to group subjects with total number of chews, number of chews performed until first swallow, total time spent chewing, and time of last swallow significantly higher than cluster II and III. It was interesting to note that in the case of milk chocolate, cluster I membership was restricted to only 2 subjects, with significantly high time and number parameters of chewing and swallowing as compared to rest of the population. These subjects were also found present in cluster I for dark chocolate which consisted of 8 more subjects who were identified in cluster III for milk chocolate.

Cluster II, in both chocolates, agglomerated consumers who can be considered as relatively quick eaters. The time these consumers retained the chocolates in mouth, total number of chews performed in the masticatory sequence, and the time of last swallow is significantly lower than the others.

Finally, Cluster III is classified as a group which had on an average moderate time- and number parameters for chewing and swallowing, predominantly lying between that of cluster I and II. On an average this group took medium time to eat the chocolate with a moderate chewing rate, with both time of last chew and last swallow significantly higher as compared to cluster II and lower as compared cluster I. Again, it was interesting to note that although all the subjects present in cluster III for milk chocolate were present in cluster III for dark chocolate, all subjects of cluster II for dark chocolate qualified in cluster III for milk chocolate.

Hence, from the previously described adaptive response of mastication strategies by individuals between chocolates, and from the observations of the cross-talk observed between clusters, it was difficult to infer whether cluster formation was independent of chocolate-type, and may be related to predominant physical and sensory differences between the chocolates. Furthermore, it may have been influenced by certain candidates who showed significant variation in some selective eating parameters between chocolates, considered in cluster construction.

4.3.3 Selection of Test Subjects and their Eating Strategies

Although cluster analysis was successful in characterising eating behaviour of subjects for the dark and milk chocolate, with also an aim to screen-out subjects widely varying in their eating strategies, just a random selection of cluster-representing individuals would have been ambiguous because of the factors discussed earlier. Therefore, keeping traits of each cluster in mind, a visualisation of distribution of the complete population with respect to the chief influencing parameters of chewing and swallowing identified through cluster analysis was carried-out.

Principal component analysis (PCA) for eating parameters of dark and milk chocolate is shown in Figure 4-5. PCA for both chocolates resulted in two significant components (eigen value > 1) which explained 60.86% (PC1) and 26.69% (PC2) of the variance in data for dark chocolate and 57.70% (PC1) and 26.02% (PC2) for milk chocolate, respectively. In the case of dark chocolate, the spread of subjects was wider as compared to the milk chocolate, indicating larger variance mainly differentiated across the 1st principal component. Not surprisingly, the chewing and swallowing parameters with the greatest effect on PC1 for both chocolates were those that were significantly different by ANOVA. Clusters of subjects described earlier were also clearly evident in the PCA matrix, and their respective spread/position was related to the eigen vectors defined by the principal component with similar traits as explained earlier.

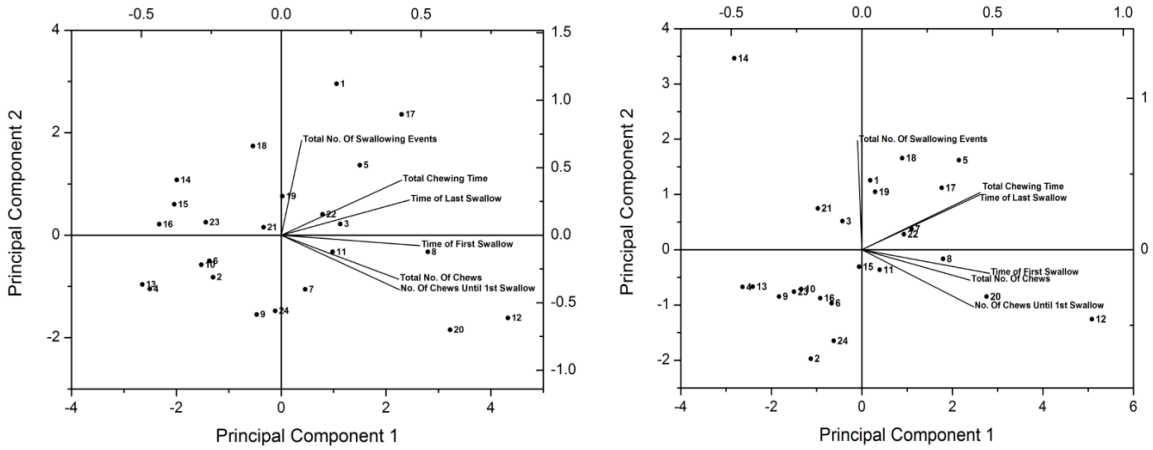


Figure 4-5 Principal component analysis identifying subject positions for loadings of chewing and swallowing parameters [dark chocolate (left) and milk chocolate (right)].

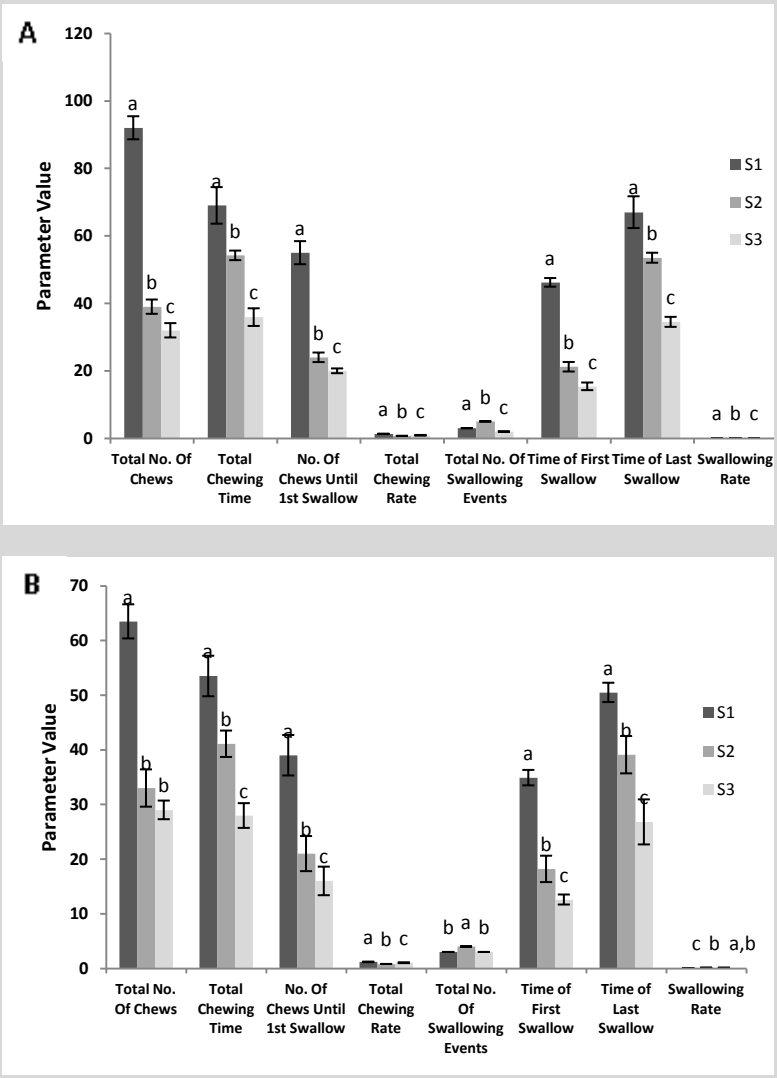


Figure 4-6 Chewing and swallowing parameters for the selected subjects (A: Dark Chocolate and B: Milk Chocolate). Within parameters and chocolate-type, values with similar letters are not significantly different at $p \leq 0.05$

The ambiguity associated with random selection of cluster-representing individuals was once again highlighted here by the spread of the subjects, with exchange of subjects between clusters clearly evident. Although, the sought after aim of the PCA was identification of the position of subjects with respect to the influence of their eating strategy parameters, and to identify three subjects who significantly varied in the eating strategies, but remained consistent in their general eating strategies between chocolates.

Through the PCA, three subjects were identified in accordance to the aim stated above. Here onwards they will be referred to as S1, S2 and S3. Dark and milk chocolate eating parameters of these subjects are shown in Figure 4-6; traits of their eating strategies are as follows -

1. **Subject S1**: (PC1 Score- dark chocolate: 4.323; milk chocolate: 5.081). This subject was identified in Cluster I for both dark and milk chocolate. As compared to rest of the population, this subject had very high values of residence time of chocolates in the mouth, total number of chews, number of chews performed until first major swallowing event, time of first swallow and time of last swallow.

2. **Subject S2**: (PC1 Score- dark chocolate: 0.536; milk chocolate: 0.891). This subject was identified in Cluster III for both dark and milk chocolate. As compared to rest of the population, this subject had intermediate (moderate) values of residence time of chocolates in the mouth, total number of chews, number of chews performed until first major swallowing event, time of first swallow and time of last swallow.

3. **Subject S3**: (PC1 Score- dark chocolate: - 2.647; milk chocolate: - 2.408). This subject was identified in Cluster II for both dark and milk chocolate. As compared to rest of the population, this subject had very low values of residence time of chocolates in the mouth, total number of chews, number of chews performed until first major swallowing event, time of first swallow and time of last swallow.

4.3.4 Description of Chocolate Bolus Formation and the "Window of Chocolate Mastication"

A descriptive approach of interviewing selected subjects (S1, S2 and S3), and observation of expectorates during specific times of their respective masticatory sequences was undertaken to investigate oral transformation of the dark and milk chocolate. Subjects were familiarised with the attributes in question to construct an empirical scale which identified the occurrences and span of certain oral perceptions with respect to their natural eating strategies. The scale is termed as "*Window of Mastication for Chocolate*". As the selected subjects varied in their chocolate eating strategies, and were representative of extremities as well as the mean behaviours in the population, this scale gave a good understanding of the spread of certain perceptions as well as time-scales relevant for these perceptions. Figure 4-7 and 4-8 show the windows of mastication constructed for dark and milk chocolate.

The description of outlined events is as follows -

It is important to point out that serving size is an important parameter which may influence oral residence time, eating strategies, as well as comfort during mastication, hence influencing the window of mastication. If a large portion is served, one usually manually breaks it into smaller pieces, or takes a 'first-bite' as per his or her natural bite-size for comfortable oral accommodation and mastication. These factors have been previously shown to influence subsequent mastication strategies of food and chocolate bars [1]. To control the bias which may result from the serving size, a 10 g serving size (36 mm X 40 mm X 6 mm) was selected as per the criteria of 'comfort in mastication and oral accommodation'.

It was observed that chocolates fractured at first-bite but tend to crumble as opposed to forming a couple of daughter particles. The dark chocolate was reported significantly harder at first bite and for subsequent comminution of size-reduced fragments as compared to the milk chocolate, complying with the instrumental measurement of hardness discussed earlier. As chocolate is a heat sensitive food, initial storage temperature history will affect product characteristics [2], in turn affecting the way chocolate fractures or transforms in the mouth. For this purpose, this study was performed on chocolate samples stored at a similar temperature (20°C).

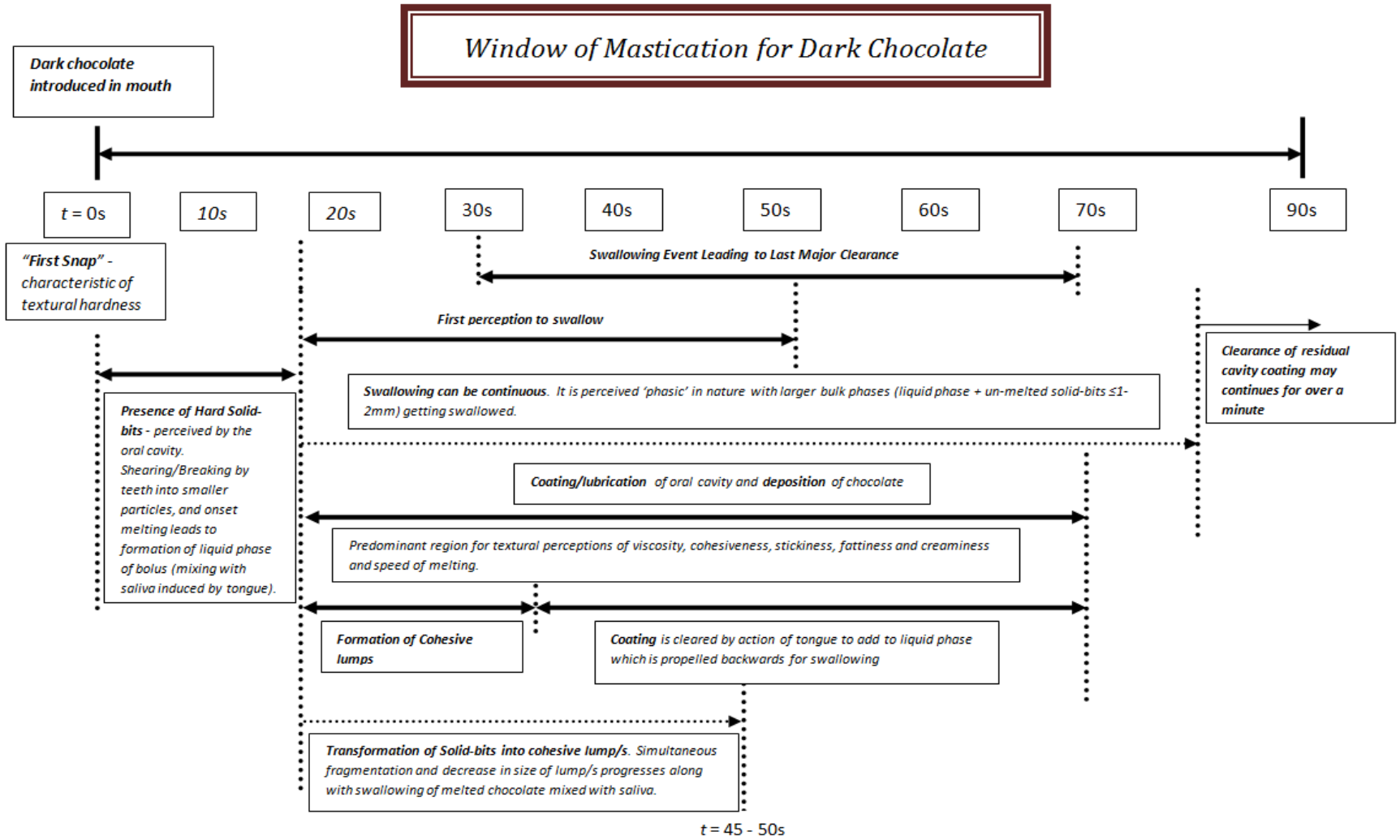


Figure 4-7 The window of mastication of dark chocolate conceptualised based on observations of eating behaviour, perceptions generated in the oral cavity, and observation of expectorates made during mastication studies involving S1, S2 and S3. It proposes a time-frame starting from the first bite until most of the dark chocolate has been cleared from the mouth, and identifies the occurrences and span of specific events during mastication of dark chocolate.

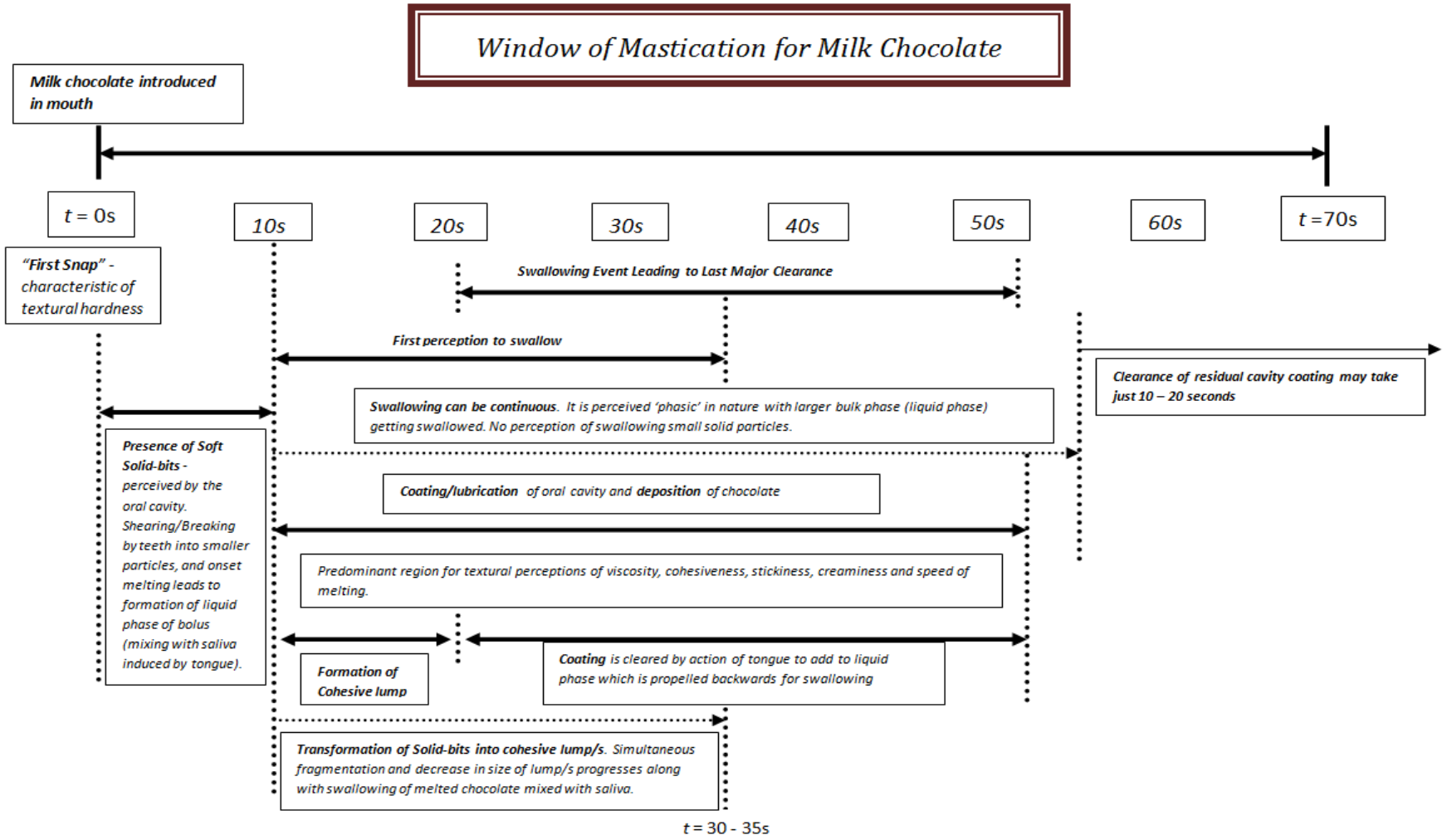


Figure 4-8 The window of mastication of milk chocolate conceptualised based on observations of eating behaviour, perceptions generated in the oral cavity, and observation of expectorates made during mastication studies involving S1, S2 and S3. It proposes a time-frame starting from the first bite until most of the dark chocolate has been cleared from the mouth, and identifies the occurrences and span of specific events during mastication of dark chocolate.

It is interesting to note the wide spread in the oral residence time for both chocolates and the span over which the swallowing events occurred for the selected subjects. The first perception to swallow for dark chocolate ranged from approximately 15 sec to 50 sec, and that for milk chocolate ranged from approximately 12 sec to 35 sec during mastication. Even for the last swallowing event which led to major clearance of bolus, a wide spread of approximately 30 to 70 sec for dark chocolate, and 25 to 50 sec for milk chocolate was possible. This interestingly indicated how differently a similar chocolate-type and/or two widely different chocolate-types can be masticated to a point where the chocolate bolus is perceived ready for swallowing by a respective individual. Considering comparison between chocolates, this also suggests that differences in properties like hardness, melting behaviour and rheological properties of melts, along with eating behaviour may influence the span of physical transformation and the occurrence of related sensory events during mastication of these chocolates.

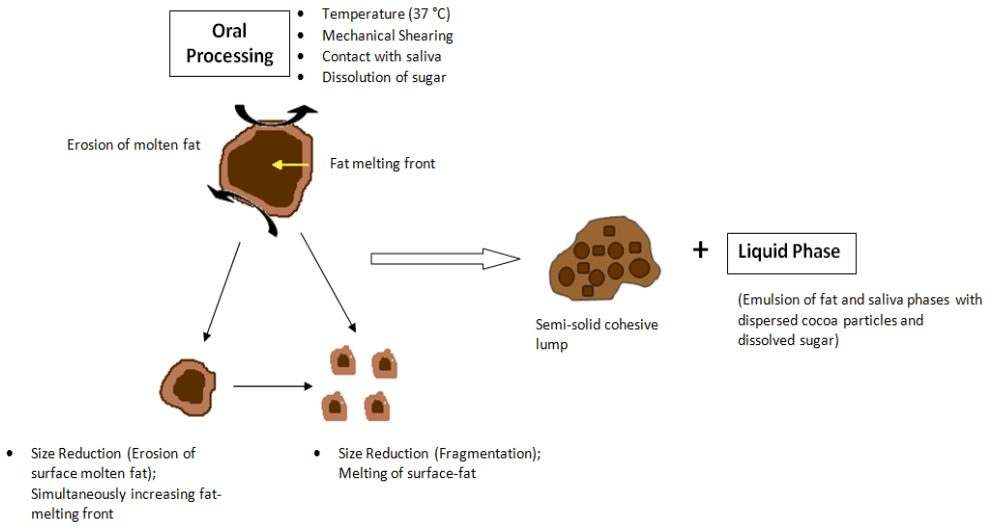


Figure 4-9 Schematic diagram for dynamics of bolus formation during mastication of dark chocolate.

For the first few chewing strokes the effect of melting was reported insignificant by the subjects and the perception of texture was reported mainly reflecting on the hardness and subsequent comminution of size reduced solid particles. Boluses obtained at every 10 sec interval during the masticatory sequence, and at the first point of swallow for dark and milk chocolate from a selected candidate are shown in Figure 4-10. It was observed that, as comminution continues with progress in mastication, melting begins to have a significant effect with the chocolates starting to change phase. With an increase in surface area for heat transfer due to the formation of comminuted particles, melting becomes far more significant. Melting of chocolate and contact/mixing with secreted saliva results in gradual build-up of the bolus liquid phase. Figure 4-9 schematically shows the dynamics of bolus formation during mastication of chocolates, conceived from observation of expectorates.

Figure 4-10 Boluses collected at every 10 sec interval and at first point of swallow (indicated by subject) during mastication of 10 g dark (left) and milk (right) chocolate for a subject from Cluster III. Observe the presence of crumbled bolus (at 10 sec) consisting of size-reduced particles cohesively bound to each other; gradual formation of cohesive lump/s and fat melting (10-40 sec), coexistence of lumps and liquid phase at the point of swallow; and complete liquid phase with homogeneous consistency (50 sec).

Shearing induced by teeth and the tongue, physiological temperature of the oral cavity, and contact with saliva leads to melting of surface fat on the size reduced daughter particles. Furthermore, fragmentation results in formation of new surfaces as particle size decreases. These factors also result in the formation of cohesive bolus lumps containing semi-solid chocolate (Figure 4-10). The lumps may contain solid and partially melted, semi-solid daughter particles bound to each other cohesively due to the action of molten surface fat and the liquid phase (mixture of molten fat and saliva). It was observed that the initiation of formation of cohesive lumps for milk chocolate was earlier as compared to dark chocolate. For milk chocolate, these lumps were also observed to become relatively more compact earlier as compared to the dark chocolate, which also as opposed to milk chocolate consisted fragmented particles cohering with each other for a longer duration.

The subsequent changes in the cohesive lumps mainly result in further size reduction and melting of fat to gradually add to the mass of the in-mouth liquid phase. As further melting of fat continues, the liquid phase of the bolus was observed to gradually build-up with reduction in size of lumps.

It is interesting to consider the time-span (15-70 sec for dark chocolate, and 10-50 sec for milk chocolate) during which the major swallowing events occur for these subjects with different eating strategies, and the overlap of events during this span. During this period, for both the chocolates, 2 – 5 swallowing events were performed by the subjects. Comminution, melting and saliva incorporation resulted in formation of cohesive-lumps which simultaneously decreased in size as mastication continued, and 'melt away' to cause build-up of molten chocolate. Although, only the liquid phase was majorly observed (and perceived) to be swallowed for all subjects, cohesive lumps were observed to coexist along with the liquid phase during the first swallowing event. Furthermore, coating and lubrication of the oral surfaces and differences in textural perception discussed earlier were reported to be mainly dominant during this span.

From the perspective of a "swallowing threshold", the above mentioned observations indicated towards some interesting factors. Firstly, the complete chocolate bolus may not attain a uniform ready-to-swallow state at the first perception to swallow. During the time-frame of the first swallowing event, it may contain a phase (mainly liquid) which qualifies as safe-to-swallow, while the cohesive lumps and larger unmelted solid particles, may not qualify the safe-to-swallow threshold and undergo further processing resulting in size reduction and melting. Secondly, it also suggests that in the case of chocolate, melting behaviour and hardness resulting from the continuous fat phase character, rheological character of the melt, along with physiological and behavioural factors like saliva incorporation and mastication strategies may influence rate of oral transformation and the degree of structure, as well as physical and related-sensory properties of its ready-to-swallow bolus. Hence, with the presence of a liquid (molten) and unmelted/semi-solid phase when the bolus is perceived safe to swallow, it becomes increasingly important to consider how the above mentioned factors affect the physical properties of both these phases to understand potential physical criteria involved in swallow initiation, history of physical transformation leading to the point of swallow, and the properties of a ready-to swallow chocolate bolus. Previously, authors have postulated the role of food structure and behavioural dependence in attaining certain "degree of structure" and "degree of lubrication" in boluses to qualify as safe to swallow [3]. Several others have tested this relationship taking in to consideration food texture [4] [5] [6], and mechanical and rheological properties [7] [8] [9], water content [10], and particle size [11] [12] [13] [14] as key parameters and physical markers characterising a ready-to-swallow bolus.

CHAPTER 5

Physical Properties and Microstructure of Ready-to-Swallow Dark and Milk Chocolate Bolus

5.1 Context

This chapter details the study undertaken to investigate moisture incorporation, physical properties and microstructure of ready-to-swallow dark and milk chocolate boluses formed by selected test subjects (S1, S2 and S3) who differed in their chocolate eating strategies. Firstly, the study aimed at understanding the effect of chocolate-type and individual eating strategies on the ready-to-swallow dark and milk chocolate boluses (Section A). Secondly, the effect of storage temperature on physical properties of dark and milk chocolate, eating and saliva incorporation strategies of selected subjects and the physical properties of ready-to-swallow chocolate boluses was investigated (Section B).

5.2 Methods

5.2.1 Storage Temperature Induced Physical Changes in Dark and Milk Chocolate

5.2.1.1 Storage of Chocolates

Physical changes were induced in the continuous phase character of the dark (DC) and milk (MC) chocolate by storing them at 0°C (DC0 and MC0), 20°C (DC20 and MC20) and 30°C (DC30 and MC30) for duration of 2 weeks. Chocolate samples were placed on plastic trays coated with aluminium foil, followed by covering the trays with cling film. These trays were then placed in controlled-temperature storage facilities (0°C - chiller, 20°C and 30°C - dry rooms) for 14 days. Chocolate samples were utilised for physical (mechanical and thermal) characterisation, mastication and bolus characterisation studies immediately after completion of storage.

5.2.1.2 Analysis of Changes in Melting Properties and Solid Fat Index (SFI)

The impact of controlled storage treatments on melting properties and SFI of the dark and milk chocolate was evaluated using differential scanning calorimetry (DSC). Thermal analysis of the chocolates stored at 0°C (DC0, MC0), 20°C (DC20 and MC20) and 30°C (DC30 and MC30) was carried out as described previously (cf. Section 3.3.5).

5.2.1.3 Analysis of Chocolate Hardness after Storage

To investigate the change in instrumental hardness of chocolates after completion of controlled storage, similar procedure as described previously in Section 3.3.2 was implemented.

5.2.2 Eating Strategies of Selected Subjects

Mastication and swallowing parameters (*chewing parameters*: total number of chews, total chewing time, number of chews until first swallow, number of chews until last swallow, and chewing rate; and *swallowing parameters*: total number of swallowing events, time of first swallow and time of last swallow) were recorded for the selected subjects for all temperature-treated dark and milk chocolate samples following the similar procedure implemented previously (cf. Section 4.2).

5.2.3 Characterisation of Ready-to-Swallow Chocolate Boluses

Each subject participated in a total of 7 sessions to complete this study. 2 sessions were dedicated by each subject to moisture content analysis of boluses, mechanical characterisation of boluses, rheological characterisation of boluses, and 1 session for bolus microscopy, respectively. A session lasted up to 90 min, and for each subject the individual study sessions were carried out on separate days. All chocolate samples were analysed in triplicates by subjects for each study, expect for bolus microscopy wherein samples were assessed in duplicates. 10 g chocolate sample (36 mm X 40 mm X 6 mm) was taken from respective storage facility and weighed accurately using an electronic weighing balance. Subjects masticated the chocolate sample naturally, and were instructed to expectorate the bolus at their first natural point of swallow; each collection being a separate trial with a fresh serving. A 10-15 min break was given between samples, and drinking water was provided between servings to clear the mouth of any residual chocolate. Bolus collection was done in aluminium pie containers (r = 25 mm). Expectorates were collected in two spitting events per trial - first being normal expectoration, and second being clearing the mouth cavity with tongue followed

by expectoration immediately after the first collection. Ready-to-swallow boluses were utilised for characterisation of saliva incorporation (moisture content), physical (mechanical and rheological) properties, and microstructure. The study was reviewed and approved by the Massey University Human Ethics Committee (MUHEC) (Southern A: Application 12/21).

5.2.3.1 Analysis of Moisture (Saliva)-Uptake by Bolus during Mastication

Following procedure was implemented for evaluation of moisture uptake in ready-to-swallow chocolate boluses after mastication –

A 15 min rest-period was given between samples to obtain boluses for quantification of moisture incorporation during which water was used for mouth rinsing by subjects. Weight of container and expectorate was determined using an electronic weighing balance. Wet weight of expectorate (W_E) was determined. Containers with known weight of wet bolus were subjected to vacuum drying at 70°C, 75 mm Hg for 6 hours. Three replicates for boluses for each chocolate sample were analysed for moisture content. After completion of the drying-run, dry weight of expectorates (W_D) was calculated. The bolus water content was determined as a mass fraction and expressed as percentage, i.e.

$$Moisture\ Content = \frac{W_E - W_D}{W_E} \times 100 \qquad (5.1)$$

The total drying time was standardised in a preliminary study which demonstrated that weight-loss was only an additional 1 - 2% when drying was carried out for 8 and 10 hours for 2, 6, 8 and 12 g bolus sample sizes, collected after 30, 50 and 70 sec mastication time under similar drying conditions. For these trials, a subject masticated 10 g dark chocolate for 30, 50, and 70 sec, under a restricted masticatory sequence wherein swallowing was not permitted. Bolus was collected after each mastication time duration and uniformly mixed. 2, 5, 8, and 12 g of expectorate from each mastication time was weighed accurately and stored in weighed containers. Boluses were vacuum dried at 70°C, 75 mm Hg for 10 hours. Weight loss was checked after every 120 min period. Number of hours required for total moisture loss to be ≤ 1 – 2% was recorded.

5.2.3.2 Mechanical Characteristics of Chocolate Boluses

Mechanical properties of cohesive lumps present in ready-to-swallow chocolate boluses collected at the first natural point of swallow were analysed by a reciprocating uniaxial double-

compression procedure using a TA-HD Plus Texture Analyser (Stable Micro Systems, Godalming, Surrey, England). A compression fixture (ø 40 mm) attached to an extension bar (length: 70 mm) using 5 kg load cell was used in the procedure. The procedure was partly adapted from Peyron *et. al.* [1], who recently applied a similar strategy (using TPA analysis, and extracting four TPA-defined parameters) to deduce mechanical properties of wheat-flake cereal boluses.

Subjects masticated the chocolate samples naturally and expectorated the bolus in a flat metal compression container (ø 48 mm). Compression containers were maintained at 37°C in a temperature-controlled laboratory oven. Bolus samples were analysed immediately after expectoration without any delay. The bolus underwent two successive compressions at a pre-test speed of 1 mm/sec, test speed of 1 mm/sec (constant displacement rate), post-test speed of 5 mm/sec and compression ratio of 65% of deformation of the cohesive lumps in the bolus. The probe was set to return to start position at the end of each test, and data acquisition was set at 200 pps. Analysis was repeated for three expectorations, for each chocolate, per subject.

Following parameters extracted from the curve obtained from the double compression test (Figure 5-1) were chosen as a measure of mechanical characteristics of the cohesive lumps (XT.RA Dimension, Exponent 32 software; Stable Micro Systems). –

1. *Firmness* ($F_{max}1$) = maximum force gauged during first compression (N)
2. *Cohesiveness* (AUC3/AUC1) = ratio of area under the curve positive region for compression cycle 1 and 2
3. *Adhesiveness* (AUC2) = area of curve-1 negative region during disk withdrawal (N.s)
4. *Work of Spreading/Compression* (AUC1 in force-distance curve) = area of force vs. distance curve of positive region during first compression (N.mm)

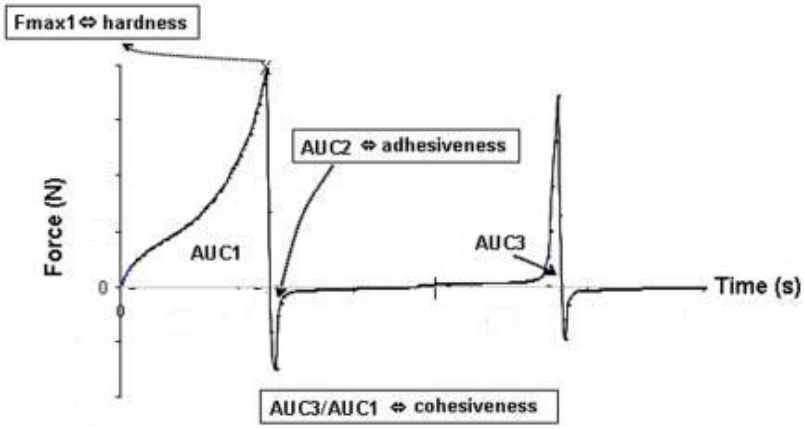


Figure 5-1 Typical force-time curve and extracted mechanical characteristics obtained from uniaxial double compression test performed on chocolate bolus.

5.2.3.3 Rheological Characterisation of Chocolate Boluses

Rheological behaviour of the liquid-phase of chocolate boluses was characterised in a shear rate-controlled rheometer (AR-G2, TA Instruments) using a steel cone-plate geometry CP 4°/40 mm (cone angle of 4° and plate diameter of 40 mm) and gap value of 97 μm between apex of cone and base-plate [12].

Participants expectorated the chocolate bolus in an aluminium pie container through a sieve (ø 1.5 mm) to prevent solid-bits and cohesive lumps to get through. A sample of the liquid chocolate bolus was carefully placed on the rheometer base-plate using a spatula. Excess spillage of the bolus was trimmed-off once the cone was lowered in to place. This ensured that a constant bolus quantity was measured each time. Shear stress was recorded as a function of increasing shear rate from 1 s^{-1} to 100 s^{-1} within 5 min, collecting 50 points per decade.

Measurements were carried out at a temperature of 37°C, controlled during the experiment using a Julabo thermo-regulator (JULABA Corp, Germany). Sealed lids were used to prevent moisture loss during measurements. Mean value and standard deviation of triplicate readings were recorded. Curve analysis and model fitting was implemented using TA Rheology Advantage Software (TA Instruments). The flow behaviour of the boluses was characterised by fitting rheograms to the *Power Law model,* as it appeared to give the best-fit -

$$\textit{Power Law model:} \quad \tau = K \cdot (\gamma)^n \qquad (5.2)$$

where, γ, shear rate; *τ*, shear stress; K, consistency index; *n*, power law/flow viscosity index.

Values of apparent viscosity at shear rates 1, 5, 10, 25, 50, and 100 s^{-1}, and the viscosities obtained from power law fitting were reported.

5.2.3.4 Microstructure Assessment of Ready-to-Swallow Chocolate Boluses

5.2.3.4a Optical Microscopy

Microstructure assessment of dark and milk chocolate boluses using optical microscopy was performed using Zeiss Axiophot Compound Light Microscope (Carl Zeiss, Oberkochen, Germany) fitted with Leica DFC320 Digital Camera (Leica Microsystems GmbH, Wetzlar, Germany). The microscope was operated in bright field mode. Image presentation and processing was done using Leica Application Suite Software (LAS Version: 3.3.0) utilising MultiFocus Series (Extended Depth of Focus) function. Expectorates were collected in 50 ml

glass beakers pre-warmed to 37 °C. One drop of approximately 100 µl sample was taken from the liquid phase of the bolus and deposited on a glass slide placed on a temperature stage controlled at 37°C. A cover-slip was gently placed over the bolus sample. Specimens were observed immediately at 10X and 20X magnifications. Micrographs were recorded.

5.2.3.4b Confocal Laser Scanning Microscopy (CLSM)

CLSM utilising fluorescence dye binding was applied to visualise dark and milk chocolate bolus microstructure to identify distribution of phases and ingredients. Equipment and microscope specifications for the experiment were same as described previously in Section 3.3.4. Milk chocolate boluses obtained after expectoration were dual-stained with nile red (analytical grade, EC No: 230-966-0, Sigma Aldrich Ltd.) for staining the fat phase, and fast green (analytical grade, EC No: 219-091-5, Sigma Aldrich Ltd) for the localisation of the proteins contributed by the chocolate and the saliva phase. Dark chocolate boluses were dual-stained with nile red for staining the fat phase, and rhodamine B (Standard Fluka analytical grade, EC: 201-383-9; Sigma Aldrich Ltd) for the localisation of the saliva phase. PEG-300 was used as a solvent for nile red, and distilled water was the solvent for fast green and rhodamine B. Stain/solvent concentrations were as stated previously. A drop (~ 100 µl) from the liquid phase of the ready-to-swallow chocolate bolus was placed on a convex glass slide, followed by addition of 10 µl of the probe or probe mixture. A cover-slip was gently placed on top, and the slide is observed under the microscope on a 37°C warming stage platform. Micrographs were acquired.

5.2.4 Data Analysis

Statistical analysis was performed using OriginPro 8.6 software (OriginLab Corporation, MA, USA). An analysis of variance (ANOVA) test was implemented on the data collected for eating parameters, bolus moisture contents, mechanical and rheological parameters of the chocolate boluses to determine the occurrence of significant differences. Firstly, the test was implemented to identify subject-effect and the effect of chocolate-type i.e. establishing a comparison between dark and milk chocolate, and secondly, it aimed at investigating whether significant differences occurred between the storage temperature treatments, within-chocolate type. Statistical comparisons were considered to have significant differences for $p \leq 0.05$. Fisher's LSD post-hoc comparison test was used to report significant differences in overall means for the subject-effect and chocolate-type/storage temperature treatment effect.

5.3 Results and Discussion

Section A: Effect of Chocolate-Type and Mastication Strategies on Physical Properties of Ready-to-Swallow Boluses

5.3.1 Mastication and Swallowing Strategies during Consumption of Chocolates

Concerning overall eating strategies, significant differences in all chewing and swallowing parameters were noted between selected subjects (S1 vs. S2 vs. S3), and for all but total-chewing rate and number of swallowing events between chocolates (DC20 vs. MC20) ($p \leq 0.05$). On an average, higher values of mastication parameters (total chewing time, total number of chews, number of chews until first swallow) and swallowing parameters (time of first swallow, time of last swallow, total number of swallows) were recorded for dark chocolate as compared to the milk chocolate (Table 5-1; cf. Section 4.3.2 and 4.3.3).

Table 5-1 Comparison of eating parameters of selected candidates S1, S2 and S3 for dark and milk chocolate stored at 20˚C for 2 weeks (Mean ± S.D)

Eating Parameter	Dark Chocolate			Milk Chocolate		
	S1	S2	S3	S1	S2	S3
Total number of chews	92 ± 3.4	39 ± 2.1	32 ± 2.1	63.5 ± 3.1	33 ± 3.1	29 ± 1.7
Total chewing time (s)	69 ± 2.4	54.2 ± 1.4	35.9 ± 2.6	53.5 ± 3.70	41.1 ± 2.4	27.97 ± 2.3
Number of chews until first swallow	55 ± 3.4	26 ± 1.4	20 ± 2.6	39 ± 3.71	21 ± 3.2	16 ± 2.61
Total chewing rate (s^{-1})	1.33 ± 0.02	0.80 ± 0.07	0.90 ± 0.1	1.29 ± 0.05	0.73 ± 0.12	1.01 ± 0.07
Chewing rate until first swallow (s^{-1})	1.18 ± 0.01	1.13 ± 0.02	1.3 ± 0.09	1.12 ± 0.06	1.15 ± 0.07	1.27 ± 0.12
Swallowing events	3	5	3	3	4	2
Time of first swallow (s)	46.2 ± 1.3	21.2 ± 1.4	15.4 ± 1.1	34.9 ± 1.4	18.2 ± 2.4	12.6 ± 0.9
Time of last swallow (s)	67 ± 3.7	53.5 ± 1.4	34.5 ± 1.5	50.6 ± 1.8	39.1 ± 3.4	26.8 ± 4.1

Amongst subjects, *total oral residence time* and *total number of chews* in the masticatory sequence were in the order S3<S2<S1 for either chocolates. Although, each subject maintained a similar masticatory frequency for both chocolates, significant differences were noted between subjects wherein, S1 had the highest total-chewing rates at 1.33 and 1.29 s^{-1}, S2 had the lowest at 0.80 and 0.73 s^{-1}, and S3 had intermediate total-chewing rates at 0.90 and 1.01 s^{-1} for dark and milk chocolate, respectively. For the complete eating duration, 2 – 4 swallowing events were performed for major clearance of the milk chocolate, while 3 – 5 were performed for the dark chocolate.

Three parameters characteristic of eating strategies until the first point of swallow were – *time of first swallow*, *number of chews until first swallow* and *chewing rate until first swallow*. Amongst these parameters, each subject demonstrated significantly higher values for time of first swallow and number of chews until first swallow for dark chocolate as compared to milk chocolate ($p \leq 0.05$), which suggested that the dark chocolate required relatively greater chewing strokes and oral processing time to be transformed into a ready-to-swallow state. On an average, the time at which the first perception to swallow was generated for the dark chocolate was 15.4 ± 1.1s for S3, 21.2 ± 1.4s and 46.2 ± 1.3s for S2 and S1, respectively. While for milk chocolate it was recorded at 12.6 ± 0.9s for S3, 18.2 ± 2.4s and 34.9 ± 1.4s for S2 and S1, respectively. From sample acquisition until the first perception to swallow, number of chewing cycles recorded for dark chocolate were 20 ± 2.6, 26 ± 1.4 and 55 ± 3.4 for S3, S2 and S1 respectively, while for milk chocolate 16 ± 2.61, 21 ± 3.2 and 39 ± 3.71 chewing strokes were recorded for S3, S2 and S1 respectively.

Moreover, similar to the total chewing rate, the chewing rate exercised by each subject until first swallow did not differ significantly between chocolates, although it was significantly different between subjects. S3 demonstrated the highest chewing rates until first swallow for both chocolates (DC20: 1.3 s^{-1}, MC20: 1.27 s^{-1}), whereas S1 and S2 showed approximately similar chewing rates until first swallow (S1: DC20: 1.18 s^{-1}, MC20: 1.12 s^{-1} and S2: DC20: 1.13 s^{-1}, MC20: 1.15 s^{-1}). This also interestingly showed that subjects gradually adapted their chewing rates to the changing bolus properties, as total chewing rates for each subject was significantly different to their respective chewing rate until first swallow for both chocolates ($p \leq 0.05$).

Importantly, these observations suggest that each subject demonstrated a characteristic mastication strategy to form a bolus which they perceived suitable for safe swallowing. Concerning the effect of chocolate-type on individual mastication strategies, subjects maintained their general mastication strategy regardless of chocolate-type, although they adapted their respective strategy to differences in chocolate texture as reflected by the change in magnitude of mastication parameters. Previously researchers have not only documented such adaptation of eating parameters by subjects to differences in food physical properties for formation of bolus suitable for deglutition, but also preservation of strategies between food textures specifically by maintaining similar masticatory frequencies [2] [3] [4] [5] [6].

The milk chocolate having a lower apparent SFI (at and before 37°C) resulting from the presence of milkfat, is a relatively softer product (c.f Section 3.4.2), while it also

demonstrated melting properties (T_{onset}, T_{end}, T_{peak}, ΔH_{melt}, peak height) relating to an earlier onset and faster rate of melting, as well as lower energy requirements for complete liquefaction (cf. Section 3.4.5 and 2.4.7 for discussions of underlying mechanisms) as compared to dark chocolate. These differences in hardness and melting behaviour between the products can be considered explanatory of the observation that regardless no significant differences in chewing rates of subjects between-chocolates, the milk chocolate is perceived ready-to-swallow before the dark chocolate, and requires lesser chewing activity to be processed to a swallowable state.

Moreover, as compared to milk chocolate, the dark chocolate was more viscous and demonstrated a higher yield stress. It also demonstrated higher cohesiveness, firmness and index of viscosity for its melt character. These differences in melt rheological and mechanical attributes may not only explain the extended oral processing activity required in the case of dark chocolate, but also suggest an increased requirements of oral muscle-work and greater energy expenditure in dilution and manipulation of chocolate melt in the mouth to transform to a swallowable consistency.

5.3.2 Moisture (Saliva) Incorporation and Microstructure of Chocolate Boluses

5.3.2.1 Saliva Incorporation

Figure 5-2 compares the moisture incorporated in ready-to-swallow boluses of dark and milk chocolate for subjects S1, S2 and S3. Significant differences were observed in bolus moisture contents between chocolates (Table 5-2; $p<0.0001$). Moisture content of dark chocolate boluses was higher as compared to milk chocolate for each subject. On an overall average, moisture content of ready-to-swallow boluses of dark chocolate was higher at 40.25 wt% as compared to 32.20 wt% for milk chocolate boluses.

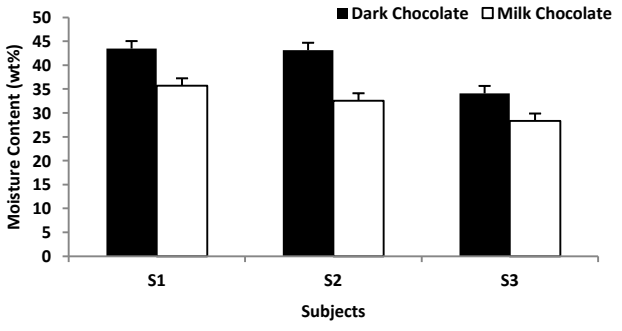


Figure 5-2 Moisture contents of ready-to-swallow dark and milk chocolate boluses for subjects – S1, S2 and S3 (Mean ± S.E.M).

Although, a subject-effect was noted for this parameter (p=0.0004), hydration of chocolate boluses produced by S1 and S2 did not differ significantly within chocolate-type, while boluses produced by S3 had significantly less moisture incorporated in them ($p \leq 0.05$). Moisture contents of dark chocolate boluses obtained from S1 and S2 were very similar at 43.5wt% and 43.14wt% respectively, while that of boluses obtained from S3 was significantly lower at 34.11wt%. Similarly, milk chocolate boluses obtained from S1 and S2 had moisture contents of 35.7wt% and 33.57wt% respectively, while those obtained from S3 had a lower moisture content of 28.33wt%.

Table 5-2 Overall means and analysis of chocolate- and subject-effect for moisture contents, mechanical and rheological properties of ready-to-swallow dark and milk chocolate boluses for subjects S1, S2 and S3.

Variable	Chocolate Effect (Overall Means)			Subject Effect (Overall Means)			
	Dark Chocolate	Milk Chocolate	*P*	S1	S2	S3	*P*
Bolus Moisture Content (wt%)	40.25	32.20	**<0.0001**	39.60^{a}	37.85^{a}	31.22^{b}	**0.0004**
Bolus Mechanical Parameters							
- Firmness (N)	24.03	14.94	**0.04**	7.62^{b}	23.89^{a}	26.95^{a}	**0.003**
- Work of Spreading (N.mm)	46.03	38.54	**0.02**	16.50^{c}	39.43^{b}	70.92^{a}	**<0.001**
- Adhesiveness (N.s)	2.04	4.27	**0.01**	3.25^{a}	2.68^{a}	3.96^{a}	0.263
- Cohesiveness	0.286	0.236	0.22	0.34^{a}	$0.26^{a,b}$	0.18^{b}	**0.020**
Bolus Rheological Parameters (Pa.s)							
APV at $\Upsilon = 1\ s^{-1}$	2.01	2.10	0.77	1.87^{b}	1.50^{b}	2.78^{a}	**0.036**
APV at $\Upsilon = 5\ s^{-1}$	1.05	1.10	0.79	0.96^{b}	0.68^{b}	1.44^{a}	**0.018**
APV at $\Upsilon = 10\ s^{-1}$	0.66	0.71	0.62	0.66^{b}	0.57^{b}	0.94^{a}	**0.009**
APV at $\Upsilon = 25\ s^{-1}$	0.36	0.42	0.22	0.37^{b}	0.26^{b}	0.54^{a}	**0.002**
APV at $\Upsilon = 50\ s^{-1}$	0.23	0.27	0.14	0.24^{b}	0.16^{b}	0.35^{a}	**0.003**
APV at $\Upsilon = 100\ s^{-1}$	0.15	0.18	0.20	0.16^{b}	0.11^{b}	0.23^{a}	**0.004**
Power Law Consistency K	3.13	2.89	0.60	2.63^{b}	2.09^{b}	4.32^{a}	**0.006**

Values in bold are for $p \leq 0.05$; values with same letters are not significantly different by Fischer's LSD at $p \leq 0.05$. APV: apparent viscosity and Υ: shear rate. Power law index *n,* for chocolate boluses (mean ± S.D) – dark chocolate: 0.35 ± 0.02, milk chocolate: 0.45 ± 0.09, S1: 0.39 ± 0.04, S2: 0.39 ± 0.07, S3: 0.41 ± 0.12).

It was observed that for a similar chocolate-type, there was no significant difference within subjects in moisture content of ready-to-swallow boluses over replicates, confirming a good repeatability of bolus preparation for different eating strategies ($p>0.05$). Such within-subject repeatability in saliva incorporation during mastication has been reported previously [7] [8]. In justification of this intra-subject repeatability in moisture incorporation observed in this case, it may be postulated that each subject maintained a similar saliva flow-rate during mastication of both chocolates (saliva flow-rate not investigated in this study) [8].

Consistently higher saliva incorporation during formation of dark chocolate boluses regardless of mastication strategies clearly highlighted a chocolate property-related effect. The mechanical property of hardness has been shown to positively relate with mastication parameters like muscle-work of chewing, number of chewing cycles, and mainly oral

processing (masticatory) duration, and consequently to saliva incorporation in ready-to-swallow food boluses [3] [9] [10]. Significantly higher hardness of dark chocolate which results in greater number of chews, chewing muscle-work and oral processing time for particle size reduction and formation of a bolus suitable for deglutition can be considered a major factor explaining relatively greater saliva incorporation. Also, slower melting rate and higher energy requirements for liquefaction for the dark chocolate may relate to longer prevalence of harder/firmer transient bolus structures (comminuted particles and cohesive-lumps) during mastication. These consequently require greater masticatory activity and oral processing time to melt completely and transform into a swallowable fluid consistency, and may relate to higher saliva content in the dark chocolate boluses. Moreover, the dark chocolate demonstrated significantly higher rheological (yield stress and viscosity parameters) and mechanical (cohesiveness, index of viscosity, firmness and consistency) of the melt, which indicate higher resistance to flow and spreadability. These rheological attributes suggest the requirement of greater oral effort and processing time for manipulation, dilution and structuring in turn linked with higher saliva incorporation in formation of a molten dark chocolate bolus which qualifies the rheological threshold for swallowing [11] [12].

Concerning the subject-effect, the study revealed a dichotomous response in moisture incorporation which grouped S1 and S2 separate from S3. Firstly, even though with similar chewing rates and significant differences in oral residence time until first swallow and number of chewing strokes until first swallow, S1 and S2 incorporated similar amount of saliva in ready-to-swallow boluses within chocolate-type. This predominantly suggested a possible effect related to differences in saliva flow rates between these subjects. Secondly, observations also suggested that for both chocolates, boluses with different moisture contents could be perceived ready-to-swallow by individuals exercising distinct mastication strategies. Although S3 demonstrated significantly higher chewing rates, boluses of either of the chocolates perceived ready-to-swallow by this subject had lower moisture incorporation, which could largely relate to significantly lower oral processing time invested in bolus preparation.

Apart from its primary role in providing certain degree of lubrication and facilitate particle cohesion in the bolus [13] [14] [15], specifically during chocolate oral processing, saliva predominantly plays an important role in modulating bolus rheological behaviour, which in turn relates to product- and subject-dependent sensory texture perceptions and attainment of a safe-to-swallow consistency [16] [17]. This is mainly facilitated by the dilution and structuring effect that saliva provides during bolus formation i.e. by dissolution of sugars,

bringing about reduction in particle phase volume fraction of the bolus, emulsification and flocculation based interactions with fat and other immiscible particulate solids, as well as contributing through its own compositional factors, for example, salivary mucins.

5.3.2.2 Bolus Microstructure

Some of the characteristics of the dilution and structuring of the bolus liquid phase resulting from saliva incorporation and its interaction with chocolate matrix ingredients were clearly observable in the bolus micrographs (Figure 5-3 and 5-4). Bright field micrographs of ready-to-swallow dark and milk chocolate boluses obtained from each subject are shown in Figure 5-3. Figure 5-4 shows confocal micrographs obtained for boluses of milk and dark chocolate for one subject (S2).

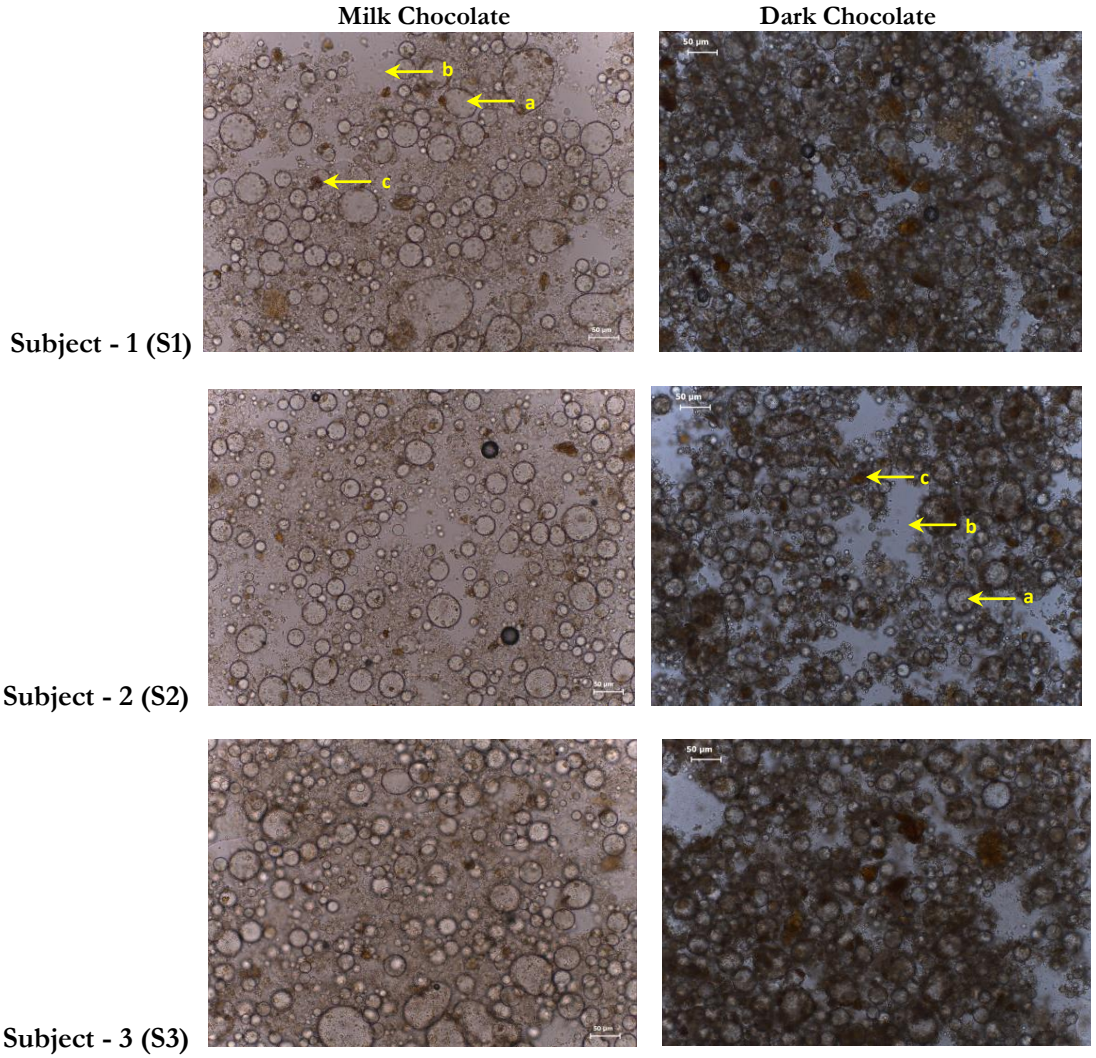


Figure 5-3 Optical micrographs (bright field, 20X magnification) of the liquid phase of milk (left) and dark (right) chocolate boluses collected from subjects S1, S2 and S3. Fat globules ranging from 5 - 50 µm (a), continuous saliva pockets (b), and cocoa particles (c) are indicated. Observe the extensive flocculation of emulsified globules and agglomeration of dissociated chocolate matrix components (fat and cocoa particles) particularly in the vicinity of the flocs in boluses of all subjects. A relatively denser microstructure characteristic of extensive flocculation is also clearly evident in the case of dark chocolate boluses.

Coarse emulsification of fat globules in the liquid phase was very clearly witnessed for boluses of both chocolates. Similar microstructural features for chocolate bolus have been disclosed recently by researchers [18]. Identification of phases was facilitated by the autofluorescence of cocoa particles investigated previously, and the use of fat stain (nile-red) and protein stain (fast green/rhodamine B) for preferential staining of chocolate fat and saliva, through the use of confocal microscopy Figure 5-4. In the case of boluses of both chocolates, globules ranging from approximately 5 to 50 μm, dissolving sugar crystals, and cocoa particles were clearly observable dispersed in continuous pockets of saliva phase and agglomerated molten fat. For a similar chocolate-type, particularly distinct attributes in bolus microstructure were not clearly noticeable between-subjects regardless of differences in mastication strategies and saliva incorporation. Flocculation was clearly observable in all boluses, although dark chocolate boluses had a relatively denser microstructure, with extensive flocculation of globules as compared to milk chocolate.

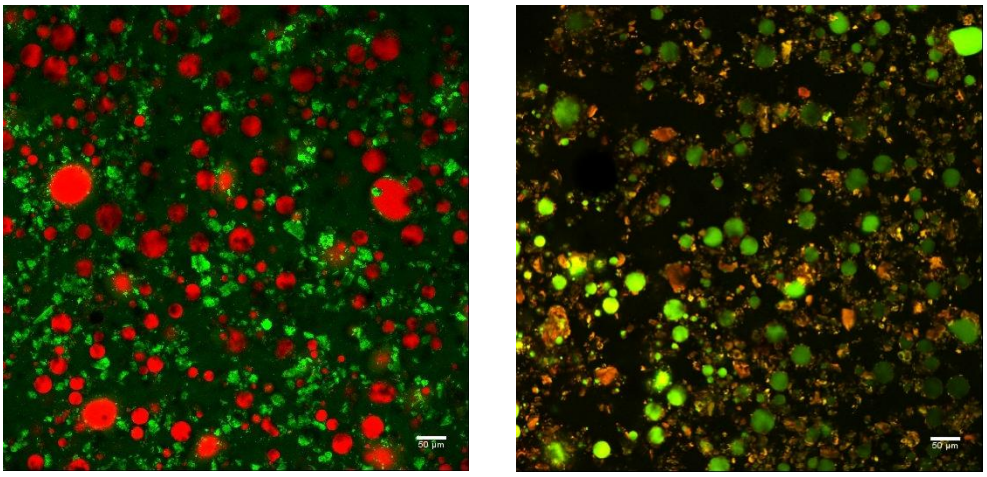


Figure 5-4 Triple excitation confocal micrographs of the liquid phase of milk (left) and dark (right) chocolate ready-to-swallow boluses collected from subjects S2. Milk Chocolate Bolus: *Specifications*: nile red (488nm) and fast green (633nm), 20X 0.70dry. *Colour key*: red – fat globules, fluorescent green (particulate) – cocoa solids/milk solid dispersions, and green (continuous) – saliva phase. Dark Chocolate Bolus: *Specifications*: nile red (488nm) and Rhodamine B (561nm), 20X 0.70dry. *Colour key*: green – fat globules, rust (particulate) – cocoa solids, and dark green (continuous) – saliva phase. Scale Bar - 50 μm.

During oral processing of chocolate, shearing induced by the tongue and teeth in the bolus liquid phase, results in mixing and homogenisation of the saliva and chocolate fat. This facilitates dissociation and emulsification of the fat matrix leading to the characteristic bolus microstructure. Chocolate matrix factors such as composition and viscosity of the fat phase, presence of solid particles and surface active agents, and chocolate melting behaviour, as well as human-related factors of extent of oral processing and masticatory motor functions, saliva flow-rate and viscosity, and presence of salivary mucins can be considered important in contributing towards the characteristic structure of the chocolate bolus. These structural

characteristics as well as extent of liquid phase dilution (saliva-phase volume fraction) in turn can be considered to have important implications on the rheological and oral surface-interaction characteristics of the ready-to-swallow bolus.

5.3.3 Physical Properties of Ready-to-Swallow Chocolate Boluses

Mechanical characteristics of the bolus cohesive lumps and rheological characteristics of the liquid phase of the bolus were quantified as a measure of the overall physical-status of the ready-to-swallow chocolate boluses as influenced by differences in physical properties of the chocolates, mastication strategies of selected subjects and saliva incorporation.

5.3.3.1 Mechanical Characteristics

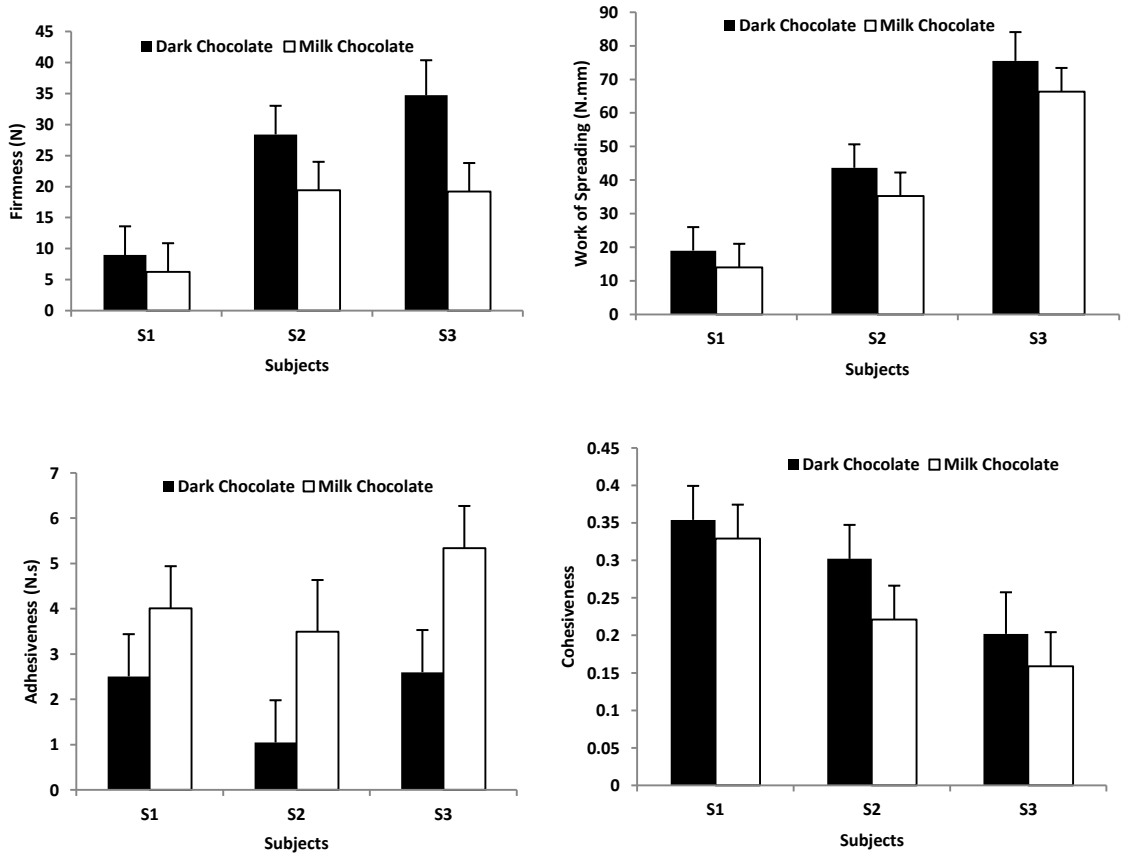


Figure 5-5 Mechanical characteristics (firmness, work of spreading, adhesiveness and cohesiveness) of ready-to-swallow dark and milk chocolate boluses for subjects – S1, S2 and S3 (Mean ± S.E.M).

Mechanical characteristics of the cohesive lumps (firmness, work of spreading, cohesiveness and adhesiveness) were measured as their structural response to controlled deformation. Figure 5-5 and Table 5-2 shows a comparative account of the mechanical properties of cohesive lumps in the dark and milk chocolate boluses obtained from subjects – S1, S2 and S3. An effect of chocolate-type was observed for firmness, work of spreading and

adhesiveness, while cohesiveness did not differ significantly between dark and milk chocolate boluses. On an average, cohesive lumps at the point of swallow for dark chocolate boluses were significantly firmer ($p=0.04$) and required more work of spreading ($p=0.02$) as compared to milk chocolate. On the other hand, those in the milk chocolate boluses were significantly more adhesive ($p=0.01$).

Considering the subject-effect, on an overall average, no statistically significant difference was noted for adhesiveness between subjects ($p=0.263$). This parameter ranged from 1.04 N.s to 2.59 N.s for dark chocolate and 3.49 N.s to 5.33 N.s for milk chocolate. For both chocolates, boluses obtained from S1 were significantly less firm as compared to those obtained from S2 and S3 ($p\leq0.05$), while no significant difference in this parameter was noted between S2 and S3. Firmness of lumps ranged from 8.98 N to 34.71 N for dark chocolate boluses, and 6.26 N to 19.38 N for milk chocolate boluses. In comparison, work of spreading showed a more pronounced increasing trend in the order of S1, S2 and S3 respectively, with significant differences between subjects ($p\leq0.05$) for both chocolates. Parameter values ranged from 18.99 to 75.47 N.mm for dark chocolate boluses and 14.01 to 66.38 N.mm for milk chocolate boluses. Finally, within chocolate-type, cohesiveness of boluses produced by S1 and S3 differed significantly ($p\leq0.05$), although those produced by S2 were not significantly different from the other subjects. As opposed to work of spreading and firmness, bolus cohesiveness demonstrated a decreasing trend in the order of S1, S2 and S3 respectively, with values ranging from 0.20 to 0.35 for dark chocolate boluses, and 0.15 to 0.33 for milk chocolate.

As discussed previously, cohesive lumps are highly inhomogeneous structures consisting of partially-melted/solid chocolate particles agglomerated together by the action of saliva and molten chocolate fat. They are formed as a result of continuous oral manipulation of the chocolate bolus during which particle size reduction, fat-melting and saliva incorporation are concurrent actions. The presence of these lumps at the point of swallow interestingly indicated that only a part of the bolus is ready-to-swallow when the first perception to swallow is elicited. Due to their relatively large size, they do not qualify the particle size threshold of swallowing, and also require further oral processing to transform to a fluid consistency and/or particle size associated with safe swallowing. Nevertheless, as they are a constituent of the ready-to-swallow chocolate bolus, their mechanical characteristics not only indicate the extent of chocolate oral transformation until the point of swallow, but are

also important as bolus physical properties conveying sensory information of bolus structural attributes related to swallow initiation.

Firmness can be defined as the resistance elicited by the structure of the lumps to the applied compression-force, while the work of spreading is the work done in compressing the bolus lumps to a constant value of strain, in this case, 65%. Both these properties may not only relate to the oral muscle-work requirements in further particle size reduction and dissociation of the bolus, but also to the sensory perception of hardness/firmness of bolus at the point of swallow. Even though as compared to the milk chocolate, the dark chocolate underwent greater chewing activity over a longer oral processing time and had higher moisture incorporated in its ready-to-swallow state, cohesive-lumps of the dark chocolate bolus were firmer and required higher work for compression. This indicated that chocolate hardness and melting properties clearly played a dominant role in governing these physical parameters.

The dark chocolate which is significantly harder and slower melting of the two, results in firmer cohesive lumps which also exhibit greater resistance to compression i.e. require higher work in deformation to reach a particular value of strain when compressed. This is possibly due to inherently higher hardness of its comminuted particles and lower molten fat content in the bolus as compared to milk chocolate. Molten fat fills the crevices of the semi-solid lumps and coats the surfaces of the chocolate particles in the bolus, cohesively holding them together as a compact entity while decreasing overall firmness and friction between particles during deformation.

Concerning the subject-effect, the results suggested that firmness and work of spreading of cohesive lumps may be positively related to inter-individual differences in oral residence time and chewing activity, but not necessarily saliva incorporation. For both chocolates, an increase in oral residence time and number of chews invested in bolus preparation seem to have resulted in reduced firmness and work of spreading for the bolus lumps. This maybe largely related to a decrease of solids in the bolus, as melting of comminuted chocolate particles is continuous during oral processing.

Adhesiveness depends on food properties and saliva characteristics, and can be described as resulting from external forces due to attraction between bolus and the oral-cavity surfaces [1]. Significantly higher adhesiveness of milk chocolate boluses as compared to dark chocolate, positively reflected on the related-sensory attribute of stickiness discussed previously (cf. Section 4.3.1). Higher adhesiveness of milk chocolate boluses may be mainly

related to factors like presence of milk ingredients (milk powder and lactose) and significantly larger sugar concentration as compared to the dark chocolate. The adhesion and water binding properties of the milk solids and hygroscopic nature of sugar [19] [20], result in enhanced surface binding and agglomeration potential, in turn resulting in greater adhesiveness of the molten milk chocolate matrix upon contact with saliva. This effect may particularly manifest itself through sensory stickiness [21], greater in milk chocolate as compared to dark chocolate. Interestingly, no subject-effect for bolus adhesiveness indicated that this parameter was independent of the characteristic level of molten fat to that of saliva in the bolus which may have resulted from different mastication and saliva incorporation strategies of the subjects.

Cohesiveness is an empirical property often measured using texture profile analysis through uniaxial compression [22] and its importance has been highlighted in studies investigating bolus flow and deformation behaviour [23]. One must however be cautious that instrumentally measured cohesiveness may not directly relate to its sensory manifestation particularly due to multiple structural, compositional and physicochemical interaction-related factors of the food bolus [24]. It can be described as resulting from forces inducing particles to agglomerate together to constitute the bolus as an entity [25]. It probably reflects on attributes like bolus compactness and perceived stickiness [26].

Previously authors investigating food bolus formation during mastication and factors governing the swallowing threshold have considered bolus cohesion as a parameter of importance [3] [27] [28] [29]. It has been proposed that at the point where cohesion between bolus particles is maximum is when swallowing is initiated, while cohesiveness should be stronger than the adhesion to the oral mucosa to trigger a safe swallow [25]. Expectorated boluses near the swallow point are reported to cohere together, whereas those expectorated well after the natural swallow point fall apart as saliva floods the food bolus causing lack of cohesion. This was clearly observable in the preliminary studies performed to standardise drying time of boluses for accurate determination of moisture content. Here, the masticatory sequence duration was controlled and swallowing not permitted. Consequently, the boluses which were expectorated well beyond the nature point of swallow were disassociated and flooded with excessive saliva as they failed to cohere together as lumps.

Several factors like differences in composition and physical properties between the dark and milk chocolate, related to differences in the dynamics of oral breakdown and phase transformation, extent of bolus wetting by saliva, particle size reduction, and quantity of molten-fat in the bolus at point of swallow may play an important role in influencing bolus

cohesiveness. Considering this complexity, it is evident that adaptation of eating strategies and saliva incorporation demonstrated by each subject may have proved to be important factors contributing to similar bolus cohesiveness achieved between chocolates. In particular, it remains unclear how specific adaptations in eating parameters and saliva incorporation to chocolate texture may circumvent the complex effect of the above mentioned factors and result in boluses of similar cohesiveness. Nevertheless it is noteworthy that this result was common to subjects exercising widely different eating strategies, and that within chocolate-type, cohesiveness of the bolus lumps may not be a physical marker which reaches a consensus at the point of swallow.

5.3.3.2 Rheological Behaviour of Liquid Phase of Chocolate Boluses

Concerning the rheological behaviour of the liquid phase of the ready-to-swallow dark and milk chocolate boluses, typical rheograms of shear stress vs. shear rate and viscosity vs. shear rate are shown in Figure 5-6. Mean viscosities obtained from power-law fitting to the rheograms of dark and milk chocolate boluses of S1, S2 and S3 are compared in Figure 5-7.

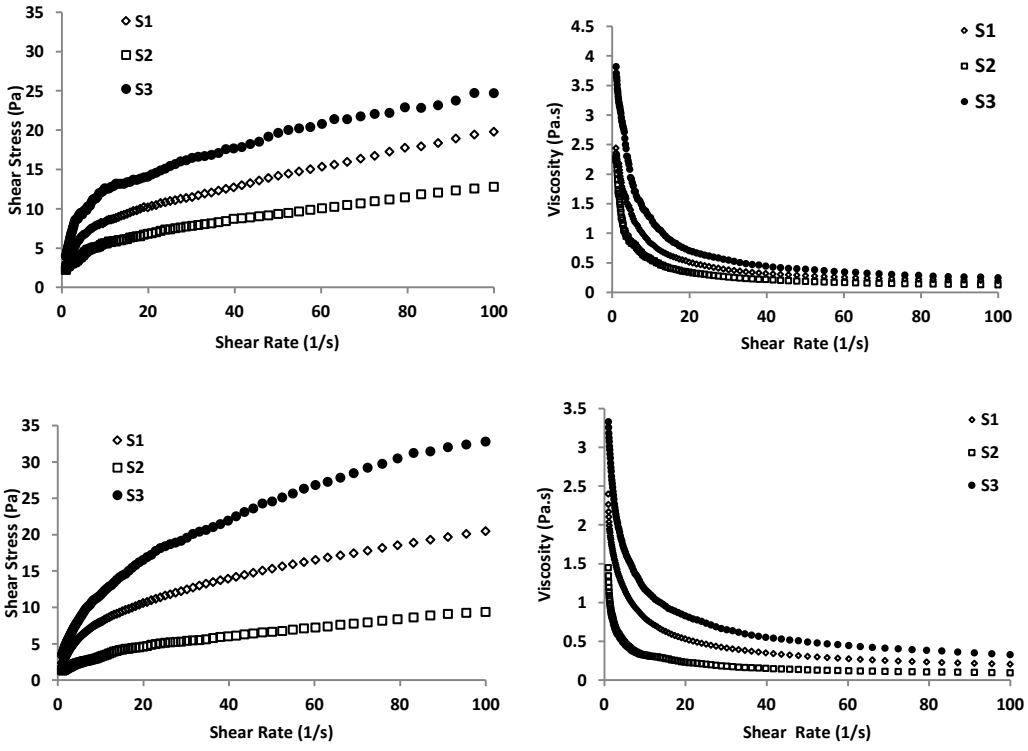


Figure 5-6 Rheograms of shear stress versus shear rate and viscosity versus shear rate for dark (above) and milk (below) chocolate boluses obtained from S1, S2 and S3.

The chocolate boluses demonstrated a shear-thinning behaviour, which was characterised in a shear rate range of 1 s^{-1} to 100 s^{-1}. For both chocolates, boluses of all subjects showed a steep drop in viscosity i.e. the major shear-thinning regions were noted

between 1 to 30 s^{-1}, after which a gradual plateau characteristic of viscosities lower than 1 Pa.s was observed for subsequent shear rates. Interestingly, no significant differences were noted between chocolates in either the mean apparent viscosities from shear rates 1 s^{-1} to 100 s^{-1} or the power law consistency of the boluses obtained from all subjects (p values: 0.14 – 0.79; Table 5-2). Mean apparent viscosity of dark chocolate boluses measured from 1s^{-1} to 100 s^{-1} ranged from 2.0 Pa.s to 0.15 Pa.s respectively, and that for milk chocolate boluses ranged from 2.10 Pa.s to 0.18 Pa.s respectively. Considering the relevance of the yield stress characterised at a low shear rate range, mean shear stresses of dark chocolate boluses between 1 s^{-1} to 5 s^{-1} ranged from 2.23 ± 0.27 Pa to 5.0 ± 0.38 Pa respectively, and that for milk chocolate ranged from 2.15 ± 0.23 Pa to 5.15 ± 0.6 Pa.

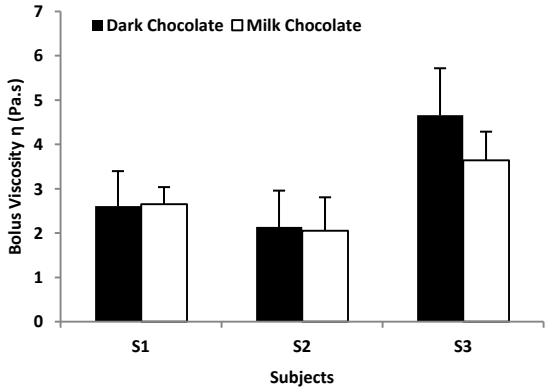


Figure 5-7 Mean viscosities of ready-to-swallow dark and milk chocolate boluses for subjects – S1, S2 and S3 obtained from Power Law fitting (Mean ± S.E.M).

Subject-effect was noted for all rheological parameters (p values: 0.002 – 0.036; Table 5-2). On average, boluses of S1 and S2 did not differ significantly in their rheological parameters, while those produced by S3 were significantly more viscous as compared to S1 and S2 with higher values for all rheological parameters for both chocolate boluses ($p \leq 0.05$). On average, mean bolus consistencies deduced from power-law fitting in the order – S1, S2 and S3 were 2.61 Pa.s, 2.14 Pa.s and 4.66 Pa.s for dark chocolate boluses, and 2.65 Pa.s, 2.05 Pa.s and 3.64 Pa.s for milk chocolate boluses, respectively.

In particular, bolus rheological behaviour of chocolate can be very difficult to detail in that, several influencing factors such as composition, initial melt rheological properties, melting behaviour, saliva incorporation, eating strategies, etc., may play a competing role. Considering this, the within-subject repeatability of attaining similar bolus consistencies for a particular chocolate-type was particularly noteworthy. It may interestingly suggest that individuals maintain a 'memory' of mastication and saliva incorporation strategy in relation to

chocolate textural properties which they implement during oral processing to achieve a common bolus consistency which may be associated with a swallowing threshold. A mouthful of food does not always form one swallowable bolus. Small quantities of food may be propelled anterior of the tongue to the oropharyngeal region as it has met the criteria for swallowing [30] [31]. Particularly, during chocolate oral processing this phenomenon holds true as the bolus liquid phase is ready-to-swallow, while the cohesive lumps are amidst the transformation process. Hence the rheological behaviour (consistency and flowability) of the bolus liquid phase is a physical factor of particular importance in relation to the swallowing threshold.

As discussed previously, differences in composition and related-physical properties between the two chocolates result in the differences in sensory-textural perceptions related to the characteristic oral transformation of chocolate structure. It was expected that these differences between the chocolates, in particular between rheological and mechanical (instrumental textural) properties of the melts, melting behaviour and hardness would reflect through differences in rheological properties of the bolus liquid phase at the point of swallowing mainly resulting from distinct oral transformation dynamics and ingredient interactions with saliva. Interestingly, results indicated that regardless of different composition and physical properties (and related-sensory textural attributes), each subject processed the chocolates to boluses of similar liquid phase viscosities i.e. no effect of chocolate-type was noted for bolus liquid phase rheological parameters.

Observed adaptation of eating and saliva incorporation strategies demonstrated by subjects to compensate for differences in chocolate texture can be considered towards explaining these results. The dark chocolate was harder and slower melting amongst the two chocolates. Also, in its molten form, the dark chocolate demonstrated higher viscosity, yield stress and mechanical attributes as compared to the milk chocolate. These factors not only relate to the characteristic dynamics differentiating the oral transformation of the two chocolates, but also particularly to requirements of increased oral effort, oral processing time and saliva incorporation which each subject invested in attaining a swallowable consistency for the dark chocolate similar to that of milk chocolate. Moreover, it is noteworthy that the presence of a higher proportion of soluble solids in the milk chocolate, in particular sugar and milk solids may be considered an important factor relating to shorter oral processing times and lesser saliva incorporation for adequate dilution to achieve a ready to swallow consistency.

Results further suggested that for individuals exercising different chocolate eating and saliva incorporation strategies, the chocolate bolus could be perceived ready-to-swallow at different rheological consistencies, which are possibly characteristic of inter-individual swallowing thresholds. It was clear that lower saliva incorporation over a very short oral processing time invested in bolus preparation may lead to the formation of a relatively viscous bolus with a high yield stress. This was observed in the case of S3 regardless of high chewing rates which the subject demonstrated in the case of both chocolates. This result could be attributed to lack of mixing and dilution of the chocolate fat with saliva which results in a reduction of bolus viscosity. Furthermore, although S1 and S2 had comparatively different mastication strategies but incorporated similar amounts of saliva for the formation of ready to swallow boluses, produced boluses with relatively similar viscosities. Saliva incorporation has been previously revealed to influence extent of change in mechanical and rheological properties of boluses of different foods [7] [24] [32].

Section B: Effect of Storage Temperature-Induced Physical Changes in Dark and Milk Chocolate on Eating Parameters and Physical Properties of Ready-to-Swallow Boluses

5.3.4 Storage Temperature-Induced Physical Changes in Dark and Milk Chocolate

The physical properties of dark and milk chocolates after controlled storage for 14 days at 0°C (DC0 and MC0), 20°C (DC20 and MC20) and 30°C (DC30 and MC30) were investigated using DSC and TA texture analyser. This study provided an understanding of changes in melting properties, solid fat index (SFI) and hardness implicated by low (0°C) and high (30°C) storage temperature relative to 20°C.

5.3.4.1 Changes in Melting Behaviour and Solid Fat Index (SFI)

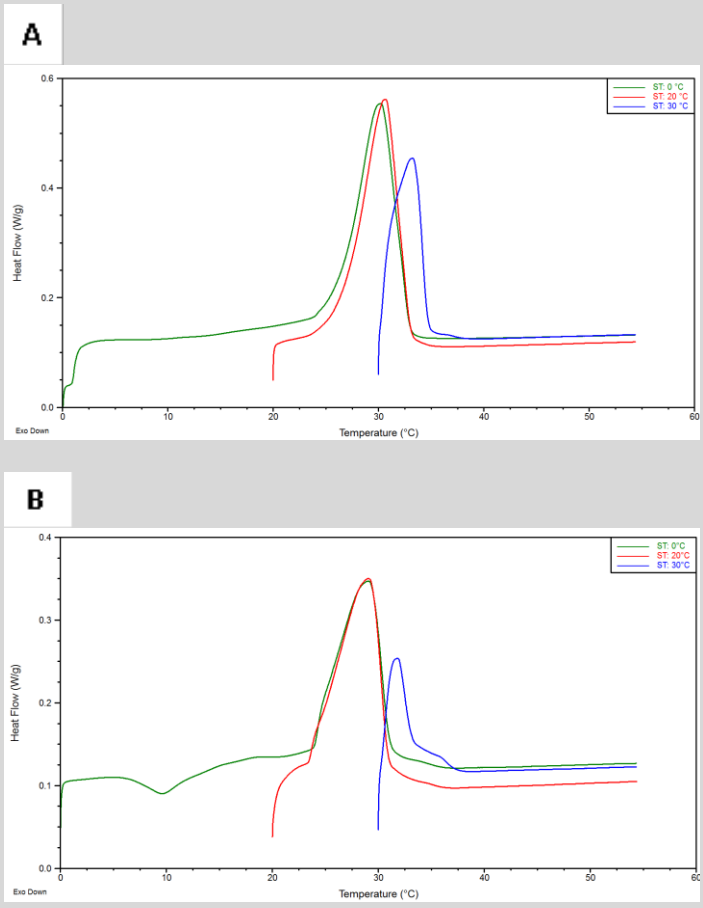


Figure 5-8 Overlay of DSC thermograms for dark (A) milk (B) chocolate after 2 weeks storage temperature treatments at - 0°C (green), 20°C (red) and 30°C (blue), respectively. (ST = storage

A comparison of DSC thermograms and extracted melting behaviour parameters of dark and milk chocolate stored at 0°C, 20°C and 30°C are shown in Figure 5-8 and Table 5-3, respectively. Similar to the case of chocolates stored at 20°C (DC20 and MC20) discussed earlier (cf. Section 3.4.5), MC0 and MC30 demonstrated lower values for all melting parameters apart from $T_{index,}$ as compared to the dark chocolate stored at respectively similar temperatures (DC0 and DC30). It can be noticed that the T_{onset} (inflection point) for the major endothermic transitions for MC0 (T_{onset}: 23.756 ± 0.125 °C) and MC30 (T_{onset}: 30.106 ± 0.080°C) takes place at a lower temperature as compared to the DC0 (T_{onset}: 25.383 ± 0.005°C) and DC30 (T_{onset}: 30.266 ± 0.005°C) respectively, implying earlier initiation of melting for milk chocolate compared to dark chocolate regardless of

storage treatments. Furthermore, average T_{peak} values for all temperature treatments for milk chocolate remained approximately 2°C lower than those of the respective treatments for dark chocolate.

Considering the effect of storage temperature within chocolate-type, for both dark and milk chocolate stored at 0°C and 20°C, no significant change was observed in the major endothermic transitions between 20 - 40°C, with the peaks almost superimposing over each other. Melting parameters associated with the major endothermic transition; T_{onset}, T_{end}, T_{peak}, T_{index} and peak height were not significantly different between these temperature treatments for both dark and milk chocolate ($p>0.05$). T_{onset}, T_{end} and T_{peak} values for all temperature treatments for milk chocolate (MC0, MC20 and MC30), and those for DC0 and DC20 were all in the range expected for β Form V polymorphic distribution of solid fat. T_{end} for DC30 was recorded at 34.89 ± 0.02°C suggesting possible re-crystallisation of some liquid fat in Form VI after elevated temperature storage.

Table 5-3 Melting properties of dark and milk chocolate after respective storage temperature treatments (Mean ± S.D)

Chocolate	Storage Temperature	T_{onset}* (°C)	T_{index}* (°C)	T_{end} (°C)*	T_{peak} (°C)*	ΔH_{melt} (J/g)*	Peak Height (W/g)*
Dark Chocolate	0°C	25.38 ± 0.005^b	7.33 ± 0.08^b	33.21 ± 0.08^b	30.32 ± 0.09^b	66.96 ± 2.98^a	0.431 ± 0.004^a
	20°C	26.40 ± 0.06^b	6.38 ± 0.04^b	33.24 ± 0.03^b	30.62 ± 0.06^b	54.39 ± 1.07^b	0.439 ± 0.009^a
	30°C	30.26 ± 0.005^a	4.78 ± 0.02^a	34.89 ± 0.02^a	33.24 ± 0.11^a	31.57 ± 0.08^c	0.329 ± 0.004^b
Milk Chocolate	0°C	23.75 ± 0.12^b	7.62 ± 0.08^b	31.37 ± 0.05^b	28.94 ± 0.12^c	49.04 ± 2.09^a	0.231 ± 0.01^a
	20°C	23.73 ± 0.84^b	7.55 ± 0.79^b	31.29 ± 0.06^b	29.22 ± 0.16^b	36.47 ± 2.64^b	0.239 ± 0.01^a
	30°C	30.10 ± 0.08^a	3.18 ± 0.04^a	33.44 ± 0.05^a	31.79 ± 0.04^a	10.79 ± 0.57^c	0.134 0.001^b

***** Within parameters and chocolate-type, values with similar letters are not significantly different at $p \leq 0.05$.

Within chocolate-type, significant differences were noted between values of ΔH_{melt} for all storage temperature treatments ($p \leq 0.05$). Storage at 0°C increased the ΔH_{melt} and that at 30°C decreased ΔH_{melt} for both chocolates, with the change being much greater for storage at 30°C for both chocolates, and for milk chocolate as compared to dark. Also, a significant decrease in peak height was noted for storage at 30°C for both chocolates ($p \leq 0.05$). These observations suggested majorly SFC related changes for both chocolates due to temperature treatments. An increase in SFC due to storage at 0°C can be attributed to crystallisation of liquid fat present in chocolates at ambient temperatures before storage treatment, and a decrease in SFC due to storage at 30°C to melting of solid fat present in chocolates at ambient temperatures before storage treatment. As both chocolates were well-tempered and had β Form V crystal morphology in solid state before storage treatments, majority of the fat was

melted at temperatures ranging from 31.0 to 33.5°C. Hence for the 30°C storage treatment, the major change in ΔH_{melt} from that at ambient temperature was significantly greater with a large quantity of fat melted at this temperature. It also must be noted that unlike chocolates stored at 0°C and 20°C, T_{onset} and T_{index} values for chocolates stored at 30°C are very relative to the starting temperature of the heating ramp initiated at 30°C. This is again due to the fact that as the β Form V fat crystals present in remaining solid fat of chocolates stored at 30°C have a melting onset and T_{peak} similar to that recorded for DC0, DC20, MC0 and MC20. Although, it can be postulated that due to the significantly decreased SFC resulting from storage at 30°C, the energy and time required to melt the remaining solid fat crystals in the DC30 and MC30 samples was much lower than that of the other treatments for the respective chocolate types.

Figure 5-9 shows the change in solid fat index (SFI) for DC0 and MC0, and DC30 and MC30 with increasing temperature. Areas under the curves (AUCs) and their relative fractional change for specific temperatures in the ramp are shown in Table 5-4 and Figure 5-10, respectively.

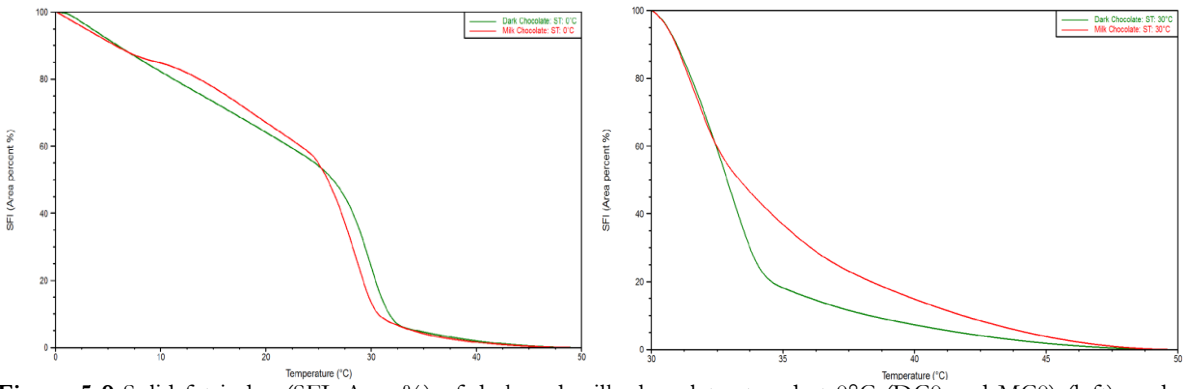


Figure 5-9 Solid fat index (SFI; Area %) of dark and milk chocolate stored at 0°C (DC0 and MC0) (left), and 30°C (DC30 and MC30) for DSC temperature ramp: 0 - 50°C deduced from their respective heat flow endotherms.

For chocolates subjected to 0°C storage treatment, ~ 99.4% and 99.5% of the initial solid fat (at 0°C) present in dark and milk chocolates respectively, was melted between 0°C and 45°C. The first 23°C increase in temperature resulted in ~ 35.7 % decrease in solid fat content of the dark chocolate ($AUC_{23°C}$: 64.21 ± 14.91 %) and ~ 37.5 % in milk chocolate ($AUC_{23°C}$: 62.60 ± 11.18 %), suggesting very marginal differences in rate of melting until this temperature. For both chocolates, a steep melting curve was observed between 23 - 32°C, after which ~ 91.7 % and 92.9 % of the initial solid fat was liquefied for dark and milk chocolate, respectively. The largest relative step change was recorded between 26°C and 32°C for both chocolates, over which the milk chocolate melted faster than the dark chocolate. Based on the melting points of all possible polymorphic forms of pure CB (in the case of dark

chocolate) and from the isosolid (phase change behaviours) diagrams of 'acceptable' blend concentrations of native/fractionated milkfat and CB (in the case of milk chocolate) [33] [34], majority of the fat phase can be in a solid state when subjected to controlled storage at 0°C for a period of 14 days. Considering this, it can be indicated that the SFC values of the dark and milk chocolates at 20°C were around 68-70%, and that at around 30°C were 22.34 ± 2.61% (MC0: $AUC_{29°C}$) and 37.68 ± 8.41% (DC0: $AUC_{29°C}$) for milk and dark chocolate respectively. Hence, reiterating differences previously discussed between DC20 and MC20 (cf. Section 3.4.5), it is possible to argue that, in the case of both dark and milk chocolate subjected to 0°C storage, the products may not have returned back to a similar fat phase character (as that of DC20 and MC20 respectively) upon raising the temperature to 20°C.

(A)

Temperature (°C)	Dark Chocolate, AUC (%)	Milk Chocolate, AUC (%)
0	99.99 ± 0.0	99.99 ± 0.0
5	94.31 ± 0.9	91.60 ± 3.3
10	86.72 ± 0.9	85.66 ± 6.7
15	78.54 ± 1.6	79.06 ± 9.5
20	69.93 ± 13.4	68.89 ± 10.7
23	64.21 ± 14.1	62.61 ± 11.2
26	56.43 ± 13.4	50.31 ± 9.3
29	37.68 ± 8.4	22.34 ± 2.6
32	8.26 ± 2.4	7.08 ± 1.5
35	3.59 ± 2.7	3.87 ± 1.4
40	1.41 ± 1.2	1.58 ± 0.7
45	0.36 ± 0.3	0.37 ± 0.1

(B)

Temperature (°C)	Dark Chocolate, AUC (%)	Milk Chocolate, AUC (%)
30	99.99 ± 0.0	99.99 ± 0.0
33	44.60 ± 1.2	46.94 ± 0.6
36	11.40 ± 2.2	24.18 ± 0.6
39	6.43 ± 1.5	13.76 ± 0.3
42	3.39 ± 0.8	7.35 ± 0.1
45	1.32 ± 0.3	2.89 ± 0.05
48	0.27 ± 0.07	0.45 ± 0.03

Table 5-4: Percentage areas under the curves (AUC %) for dark and milk chocolate representing their respective SFI at specific temperatures from 0°C to 45°C; DC0 and MC0 (A), and from 30°C to 48°C DC30 and MC30 (B).

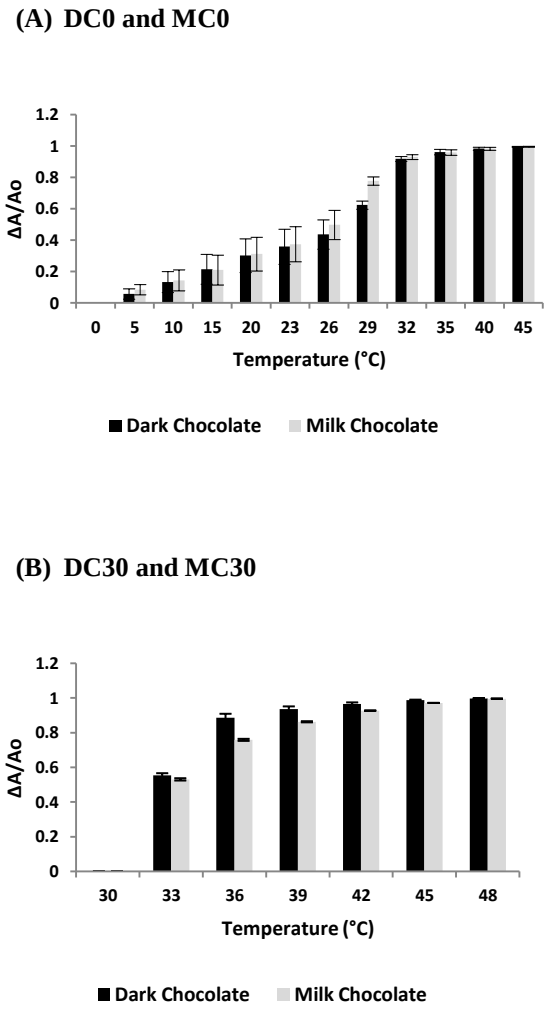


Figure 5-10 Fractional change in AUCs of dark and milk chocolate at specific temperatures from 0°C to 45°C; DC0 and MC0 (A), and from 30°C to 48°C DC30 and MC30 (B).

For chocolates subjected to 30°C storage treatment, the milk chocolate consistently showed higher SFI values and a slower rate of melting as compared to the dark chocolate. As explained previously, this could be related to high melting fraction (HMF) contributed by a specific concentration of native milk fat or externally added to the milk chocolate. ~ 55.3 % and 53 % of the solid fat present at 30°C was melted just over a 3°C rise in temperature for the dark and milk chocolate respectively. Again, considering the possibility of only a low quantity of solid fat present at 30°C in both products, the amount of energy and time required for the complete phase change during this transition was very low (DC30: ΔH_{melt} 31.57 ± 0.085 J/g, MC30: ΔH_{melt} 10.79 ± 0.578 J/g, and DC30: T_{index} 4.786 ± 0.025°C, MC30: T_{index} 3.18 ± 0.04°C).

5.3.4.2 Change in Hardness

Hardness of dark and milk chocolates stored at 0°C, 20°C and 30°C, and relative-change in hardness for both chocolates after storage temperature treatments are shown in Figure 5-11. For both chocolates the changes in hardness were related to the change in SFC caused by the storage temperature treatments.

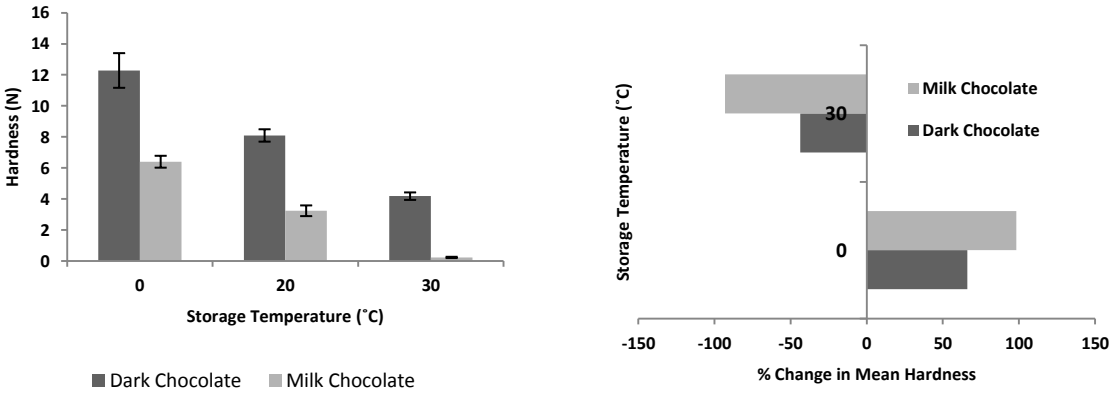


Figure 5-11 Hardness of dark and milk chocolates after storage at 0°C, 20°C and 30°C of 2 weeks measured by TA penetration test (left), and percentage change in hardness (relative to hardness at 20°C) as a result of storage at 0°C and 30°C.

There was an increase in hardness of both dark and milk chocolate stored at 0°C resulting from increased SFC, and a decrease in hardness of both chocolate types stored at 30°C resulting from decreased SFC, as compared to chocolates stored at 20°C. The percentage change in hardness (relative to hardness at 20°C) for milk chocolate caused by storage at 0°C (98.31%) and 30°C (- 93.01%) was much greater than that caused in dark chocolate (DC0: 66.03% and DC30: - 43.54%). This again indicated towards a greater change in SFC relative to that present at ambient temperature in milk chocolate as compared to dark chocolate resulting from both depressed and elevated storage temperatures.

In summary, storage-temperature treatments affected the continuous phase physical character of both chocolates. Investigation suggested mainly SFC-related changes as opposed to any significant changes associated with polymorphic form as a result of storage treatments. Firstly, enthalpy of melting (ΔH_{melt}) significantly differed between all storage treatments for both chocolates, although for chocolates stored at 30˚C the effect was relatively greater. Secondly, for either of the chocolates, all other thermal parameters did not differ significantly between 0˚C and 20˚C treatments, while those of chocolates stored at 30˚C were significantly different. Thirdly, significant change in hardness of chocolates was observed after all storage treatments. Storage at 0˚C caused further crystallisation of fat in the chocolate matrices, increasing the overall SFC and in turn resulting in greater energy requirements for complete liquefaction and significantly increased hardness. Conversely, storage at 30˚C caused relative decrease in SFC resulting from melting of solid chocolate fat set in the stable polymorphic form (β Form V; melting range: 29.0-33.5˚C) which led to significant softening of chocolates.

5.3.5 Adaptation of Chocolate Eating Strategies and Saliva Incorporation

Eating strategies and saliva incorporation exercised by subjects in formation of ready-to-swallow boluses towards changes in the physical character of the dark and milk chocolate was investigated. Three chocolate eating parameters of S1, S2 and S3, characteristic of their mastication strategies until the first point of swallow are compared in Figure 5-12, and moisture contents of ready to swallow boluses obtained from all subjects are compared in Figure 5-13. As expected, each subject demonstrated a characteristic eating strategy in bolus preparation and responded to changes in the physical character (melting behaviour, solid fat content and hardness) of chocolates induced by storage treatments. As discussed previously, hardness of chocolates and ΔH_{melt} after storage treatments were in the order – DC0>DC20>DC30 and MC0>MC20>MC30. Each subject adapted their eating strategies for either chocolates to this order as reflected by the mastication parameters (Figure 5-12). For each subject, number of chews and oral processing time were in the similar order for both chocolates. Subjects conserved their characteristic eating pattern, and maintained similar masticatory frequencies which did not differ significantly between-treatments for both chocolates ($p>0.05$). While, such adaptation of the masticatory sequence have been documented for differences in food physical properties [3] [35] [36], subjects maintaining their general nature of overall eating behaviour as fast chewers, thorough chewers, and suckers through masticatory frequencies regardless of differences in texture for products with similar compositions has also been reported specifically in the case of eating chocolate [5].

Concerning differences in eating strategies between subjects, in the case of both chocolates and all storage treatments, S1 stood-out amongst the subjects, investing significantly greater number of chewing cycles and longer masticatory time for bolus preparation as compared to S2 and S3, regardless of chocolate-type and storage temperature treatment.

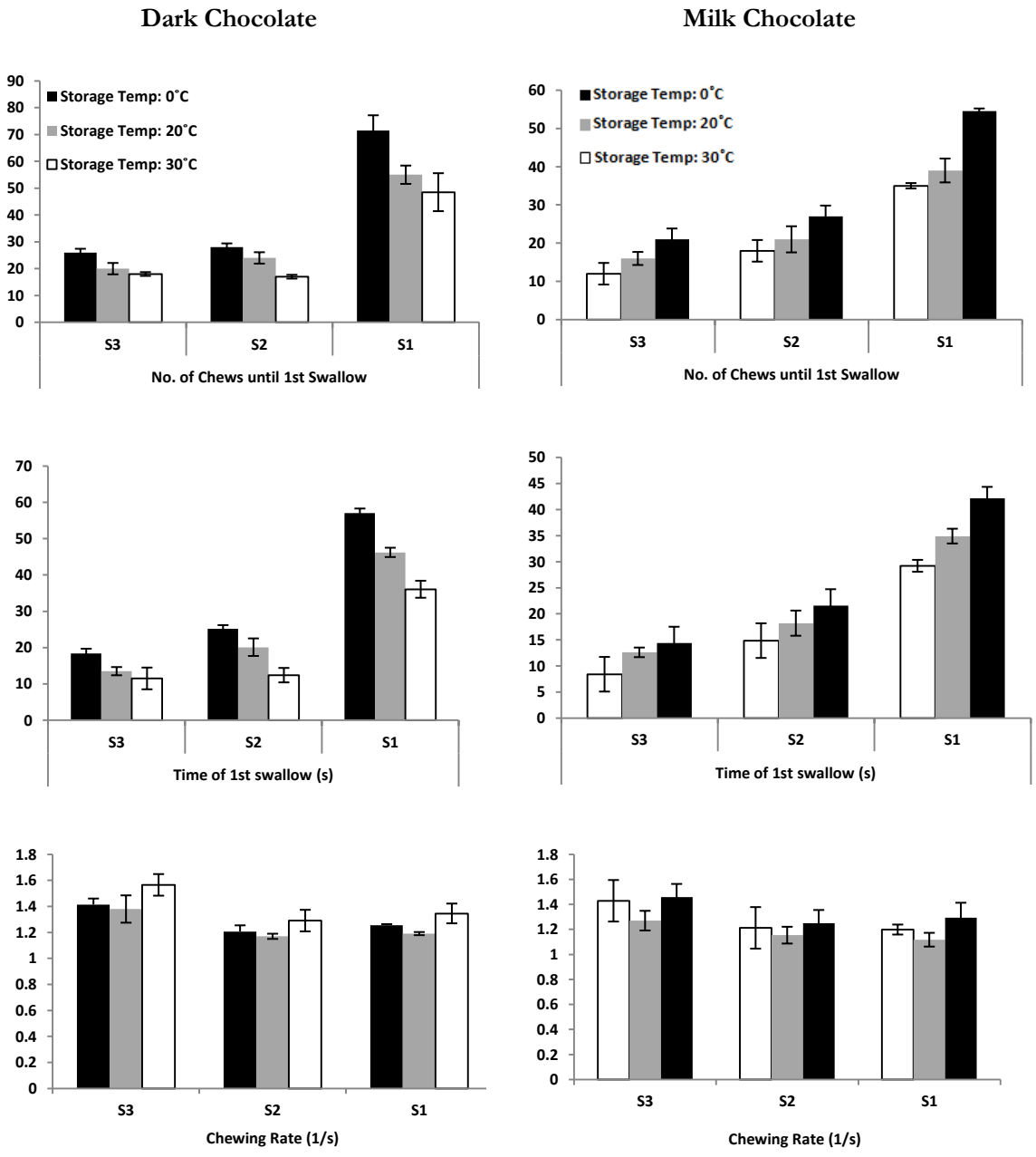


Figure 5-12 Eating strategies exercised by subjects S1, S2 and S3 until the first point of swallow for dark (left) and milk (right) chocolates after storage at 0°C, 20°C and 30°C for 2 weeks.

Clearly, hardness and energy requirement in liquefaction are physical factors related to solid fat content of chocolates which influenced individual eating strategies in an expected manner. In the case of both chocolates, an increase in hardness and energy requirement for

liquefaction resulting from an increase in SFC, resulted in an consequent increase in overall oral effort during mastication for bolus preparation. Subjects invested successively greater number of chews and oral processing time in response to increasing hardness and greater quantity of solid fat to liquefy to form a bolus suitable for swallowing.

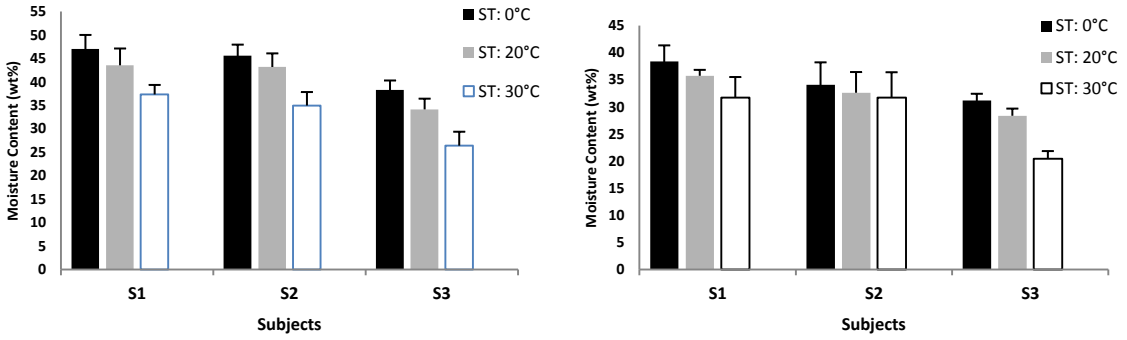


Figure 5-13 Moisture contents of ready-to-swallow dark (left) and milk (right) chocolate boluses for subjects – S1, S2 and S3 (Mean ± S.E.M). Chocolates were stored at 0°C, 20°C and 30°C for 2 weeks. ST = Storage temperature.

Comparing storage treatments within-chocolate type, bolus saliva contents for chocolates stored at 0˚C and 20˚C were relatively similar, while chocolates stored at 30˚C resulted in boluses with significantly less saliva content ($p \leq 0.05$; Figure 5-13 and Table 5-5). Contrasting inter-individual saliva incorporation in formation of ready-to-swallow boluses within chocolate-type, moisture contents of boluses formed by S1 and S2 were not significantly different, while those of boluses formed by S3 was significantly less ($p \leq 0.05$).

Once again, low intra-subject variability observed in the results suggested that subjects incorporated a characteristic level of saliva in formation of ready-to-swallow boluses, demonstrating good repeatability in the case of both chocolates and within-treatments (textures). While differences in hardness and energy requirements for liquefaction were observed between chocolates stored at 0˚C and 20˚C, the relatively greater reduction in ΔH_{melt}, and softening of chocolates caused by storage at 30˚C seems to have had a more pronounced effect on saliva incorporation during their oral processing. Significantly lower saliva incorporation in boluses of chocolates stored at 30˚C as compared to those stored at 0˚C and 20˚C seems to be related to their significantly lower melting properties (T_{onset}, T_{end}, T_{peak}, and ΔH_{melt}), requirement of lesser oral effort in bolus preparation, and consequently lower oral processing times in response to significantly reduced hardness and SFC. Differences in melting behaviour, SFC, and hardness of chocolates are physical factors linked with consequent differences in chocolate texture and related to a characteristic dynamic nature of

oral transformation encompassing events like phase transformation, particle size reduction, ingredient dissolution and bolus dilution. These can be thought to affect final moisture levels of a ready-to-swallow bolus mainly by influencing factors like overall oral-energy expenditure and oral residence/processing time which are also indicators to adaption of eating strategies demonstrated by individuals in response to changes in these physical properties. As bolus saliva content has been shown to influence the physical properties of ready-to-swallow boluses for various semi-solid, shear-thinning foods [12] [32], results suggest that regardless of adaptation in eating strategies demonstrated by individuals, differences in physical properties (textural differences) for a similar chocolate-type (similar composition) may result in modulation of saliva incorporation strategies, resulting in boluses of different and/or individual specific moisture contents at the point of swallow and may consequently relate to different bolus properties at the swallowing threshold.

Finally, two observations from the subject-effect for bolus moisture incorporation are noteworthy. Comparing bolus moisture contents recorded for S1, S2 and S3 within chocolate- and treatment-type clearly indicated that greater oral processing time invested in bolus preparation may be a factor related to higher moisture content in the ready-to-swallow boluses. Secondly, an effect of differences in salivary flow rate between subjects is also possible, as even though S1 and S2 had very different mastication strategies, moisture contents of their respective boluses were not significantly different; while S2 and S3 demonstrated relatively similar oral processing times and number of chews, their boluses had significantly different moisture incorporation at the point of swallow.

Table 5-5 Overall means and analysis of storage temperature treatment- and subject-effect for moisture contents, mechanical and rheological properties of ready-to-swallow dark and milk chocolate boluses for subjects S1, S2 and S3.

Variable	Storage Temperature Treatment Effect (Overall Means)								Subject Effect (Overall Means)							
	Dark Chocolate				*Milk Chocolate*				*Dark Chocolate*				*Milk Chocolate*			
	DC0	*DC20*	*DC30*	*P*	MC0	*MC20*	*MC30*	*P*	S1	S2	S3	*P*	S1	S2	S3	*P*
Bolus Moisture Content (wt%)	43.59a	40.25a	32.86b	**<0.0001**	34.53a	32.20a	27.93b	**0.0008**	42.59a	41.21a	32.11b	**<0.0001**	35.25a	32.79a	26.62b	**<0.0001**
Bolus Mechanical Properties																
- Firmness (N)	25.75a	24.30a	14.75b	**0.01**	17.49a	14.94a	6.39b	**0.01**	8.46b	25.40a	30.67a	**<0.0001**	6.07b	15.58a	17.18a	**0.001**
- Work of Spreading (N.mm)	52.62a	46.75a	26.98b	**0.01**	34.01a	38.54a	16.37b	**0.04**	18.08c	42.97b	62.31a	**<0.0001**	12.29c	27.01b	49.62a	**<0.0001**
- Adhesiveness (N.s)	1.69	2.049	2.38	0.21	3.71	4.27	5.06	0.27	2.09ab	1.36b	2.68a	**0.01**	4.35	3.34	5.14	0.08
- Cohesiveness	0.33	0.28	0.30	0.67	0.27b	0.23b	0.57a	**0.0005**	0.387a	0.346a	0.193b	**0.01**	0.46a	0.37a	0.22b	**0.004**
Rheological Parameters																
Power Law Consistency K (Pa.s)	2.15b	3.13a	2.85ab	**0.02**	1.56c	2.89b	3.85a	**<0.0001**	2.29b	1.89b	3.95a	**<0.0001**	2.44b	1.98b	3.67a	**0.0005**

Values in bold are for $p \leq 0.05$; values with no letter assignment are not significantly different by Fischer's LSD at $p \leq 0.05$.

5.3.6 Physical Properties of Ready-to-Swallow Chocolate Boluses

To evaluate the effect of storage temperature-induced physical changes in dark and milk chocolate, saliva incorporation and individual mastication strategies on overall physical-status of ready-to-swallow boluses of S1, S2 and S3, mechanical characteristics of the bolus cohesive lumps and rheological character of the liquid phase of chocolate boluses were quantified and compared.

5.3.6.1 Mechanical Characteristics

Figure 5-14 and Table 5-5 shows a comparative account of the mechanical properties of cohesive lumps in dark and milk chocolate boluses obtained from subjects – S1, S2 and S3 at the point of swallow. Firmness and work of spreading of bolus lumps obtained from each subject did not differ significantly between chocolates stored at 0°C and 20°C in the case of both chocolate types, while bolus lumps obtained for chocolates stored at 30°C were significantly less firm and required less work for spreading. Interestingly, these results suggest that adaption of mastication strategies demonstrated by subjects in response to changes in hardness and SFC, may not be necessarily sufficient to process the chocolate bolus lumps to a similar state of firmness and work of spreading at the first point of swallow. Also considering the saliva incorporation carried out by subjects in response to altered chocolate texture, results once again highlight that greater saliva incorporation may not relate to reduced firmness and work in deformation of the lumps.

Furthermore, results not only highlight the heterogeneity associated with the structure and formation process of the bolus lumps, but also that their firmness and work of spreading are inter-related properties which may be mainly related to initial chocolate hardness, and extent of melting which influences quantity of molten fat in the lumps and inter-particle friction upon deformation. Chocolates stored at 30°C were softer and required significantly lesser effort in liquefaction resulting from lower SFC as compared to their counterparts stored at 20°C and 0°C. These attributes not only relate to the consequent presence of softer comminuted particles which make-up their bolus lumps, but also to the fact that relatively higher molten fat content in the bolus lumps results in significantly reduced overall firmness and lower resistance to compression.

Interestingly, no significant difference was noted for adhesiveness of bolus lumps, between-treatments for both chocolates, i.e. regardless of differences in physical properties

induced by storage treatments, each subject produced boluses wherein the cohesive lumps were equally adhesive.

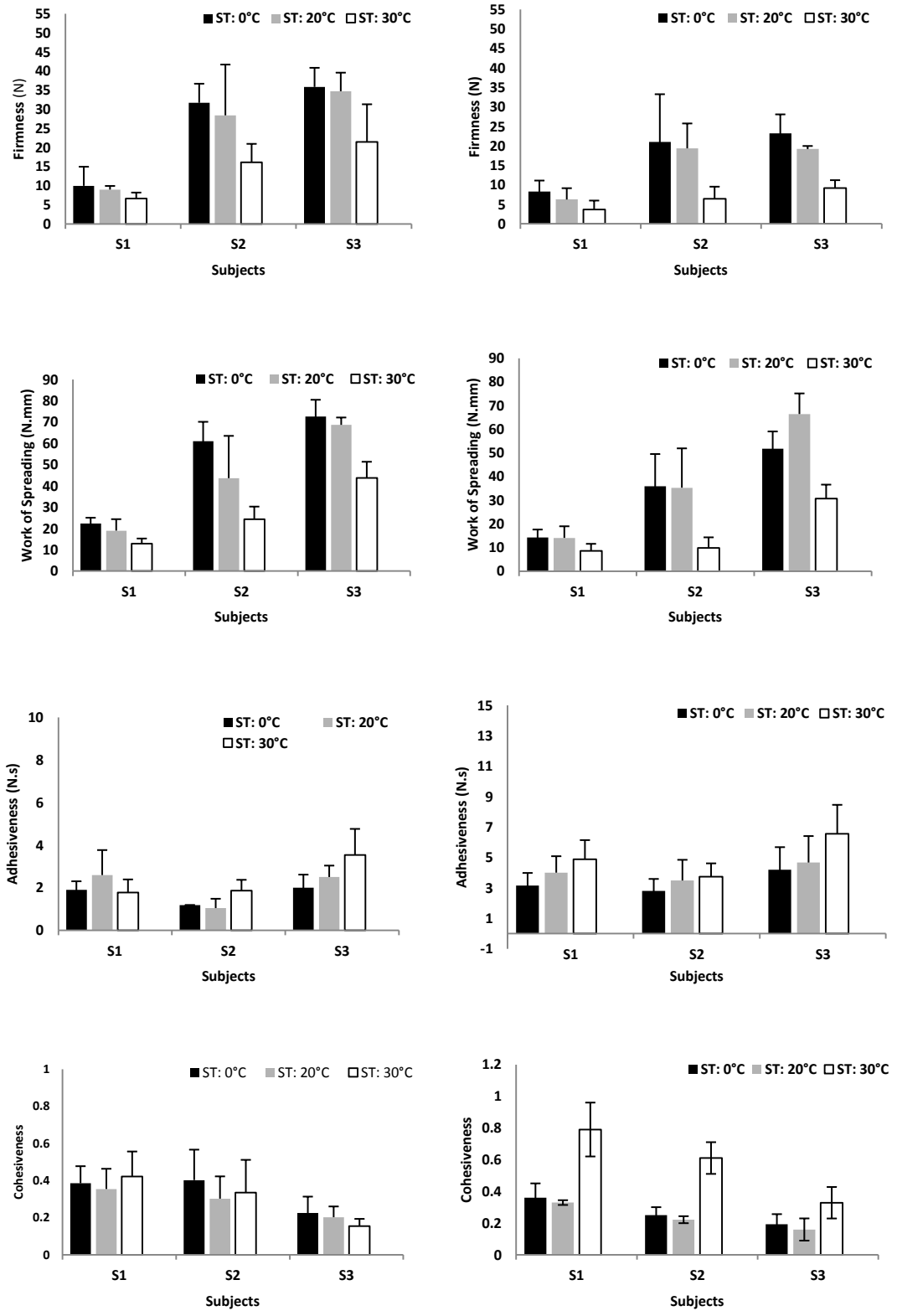


Figure 5-14 Mechanical characteristics of cohesive lumps in the ready-to-swallow dark (left) and milk (right) chocolate boluses (ST = storage temperature) (Mean ± S.D).

As highlighted previously, this once gain suggests that chocolate composition, and hence, the characteristic ingredient-integrations with saliva and oral/instrumental surfaces may be the chief factor governing adhesion properties of the bolus lumps as opposed to extent of oral processing, saliva incorporation, initial chocolate hardness and SFC, all of which varied to influence dynamics of oral transformation and other properties of the ready-to-swallow chocolate boluses.

A similar trend as to adhesiveness was observed for the property of bolus cohesiveness in the case of dark chocolate, wherein bolus lumps obtained from the ready-to-swallow bolus were equally cohesive regardless of treatment-type. Milk chocolate stored at 30˚C (MC30) consistently resulted in bolus lumps with relatively greater cohesiveness as compared to MC0 and MC20. Moreover for both chocolates, S1 and S2 who incorporated similar amounts of saliva in preparation of ready-to-swallow boluses, but exercised different mastication strategies, produced bolus lumps with similar cohesiveness. Opposed to this, cohesiveness of bolus lumps obtained from boluses of S3 was significantly lower as compared to S1 and S2, while this subject demonstrated the lowest oral processing time and saliva incorporation in bolus formation for both chocolate types.

5.3.6.2 Rheological Character of Ready-to-Swallow Chocolate Boluses

Concerning the rheological character of ready-to-swallow chocolate boluses the hypothesis tested was - for a particular chocolate-type, the adaptation of eating strategies and saliva incorporation demonstrated by each subject in response to variations in SFC, melting properties and hardness induced by storage treatments would result in boluses of characteristic (subject-specific) and similar swallowable consistency. Results revealed that for of both chocolates a treatment-effect was observed for the rheological consistencies of ready-to-swallow boluses i.e. differences in physical properties induced by storage temperature treatments resulted in a similar chocolate-type being processed to different end-points in terms of bolus viscosity by each subject (Figure 5-15, Table 5-5). Once again taking in to account that each subject adapted their mastication and saliva incorporation strategy to changes in chocolate hardness and SFC, the results suggest that differences in dynamics of oral transformation caused by storage treatments may have played a role resulting in different bolus consistencies at the point of swallow.

Considering a common chocolate-type which underwent a specific storage temperature treatment, major factors explaining differences in final bolus viscosity could be –

differences in bolus moisture content (saliva volume fraction), differences in molten chocolate content in the bolus liquid phase (molten chocolate volume fraction) related to extent of chocolate melting, and eating parameters of the subjects i.e. masticatory effort (number of chewing cycles, masticatory frequencies and oral muscle-work input) and oral processing time. Moreover, factors like inter-individual variation in saliva composition and viscosity, and extent of bolus mixing are also important.

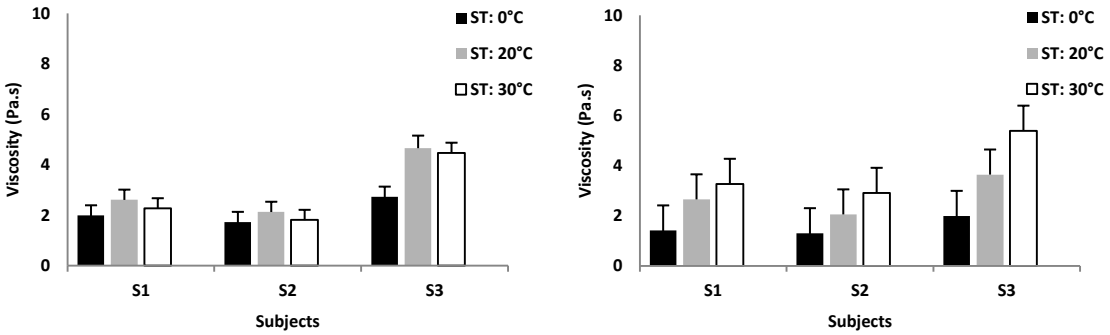


Figure 5-15 Mean viscosities of ready-to-swallow dark (left) and milk (right) chocolate boluses for subjects – S1, S2 and S3 obtained from Power Law fitting to bolus rheograms (ST=storage temperature) (Mean ± S.E.M).

In particular, very few consistent trends could be inferred which could indicate relation of specific physical changes induced in chocolates due to storage treatments to results of bolus viscosity. In the case of dark chocolate, viscosities of ready-to-swallow boluses ranged from 1.73 ± 0.29 Pa.s to 4.66 ± 0.82 Pa.s, wherein chocolates stored at 0°C (DC0) consistently resulted in the least viscous bolus as compared to those stored at 20°C and 30°C. For milk chocolate, boluses with viscosities ranging from 1.31 ± 0.41 Pa.s to 5.39 ± 1.06 Pa.s were obtained from subjects. Once again, all subject processed the chocolate stored at 0°C (MC0) to the thinnest consistency, while boluses of chocolates stored at 30°C (DC30) resulted in significantly more viscous boluses as compared to MC0 and MC20.

The degree of dilution of the bolus liquid phase and its relation with extent of chocolate melting seems largely explanatory of these results. Chocolates stored at 0°C had higher SFC as compared to their counterparts stored at 20°C and 30°C. Even though subjects adapted their mastication strategies in response to this physical factor by investing longer oral processing time and number of chewing cycles, lower viscosities of boluses of chocolates stored at 0°C seems to result from the fact that their boluses were excessively diluted by the higher saliva incorporation which took place during their oral processing, along with the presence of relatively lower quantities of molten chocolate in the bolus liquid phase resulting

from a lesser extent of melting. This was supported by the fact that the cohesive lumps at the point of swallow for DC0 and MC0 were also relatively firmer, resulting from the presence of greater quantity of unmelted chocolate particles. While on the other hand, chocolates stored at 30˚C had significantly low solid fat, contributing to high bolus viscosities resulting from a greater molten chocolate volume fraction in bolus liquid phase at point of swallow, especially as subjects invested low oral processing time and incorporated less saliva in bolus formation.

In summary, for a similar chocolate-type physical changes induced in solid fat content due to storage treatments resulted in different bolus viscosities at the point of swallow for subjects varying in eating strategies. While alterations in chocolate properties (within chocolate-type) may result in different ready-to-swallow bolus viscosities within subjects, it seems there is good within-subject repeatability to process a particular texture-type to similar bolus consistency. Although, eating (oral processing time and masticatory effort) and saliva incorporation strategies during the masticatory sequence may be adapted by subjects in response to the changes induced in chocolate texture related to SFC, the extent of chocolate melting which governs the molten chocolate ingredient volume fraction in the bolus liquid phase can be considered an important factor influencing consistency of boluses at the point of swallow.

CHAPTER 6

Summary and Concluding Remarks

The thesis ventures into complexities of chocolate oral processing with the aim of investigating how composition-structure-property relationships in chocolate, along with individual oral processing strategies influence chocolate bolus formation and define the physical character of ready-to-swallow chocolate boluses. This work has generated novel knowledge concerning fundamentals of chocolate oral processing, with a view to establish an approach which unravels links between specific composition- and microstructure-related physical properties of dark and milk chocolate and bolus formation as a result of human oral processing. To the best of the author's knowledge, quantitative characterisation of microstructure and physical properties which eating-chocolate boluses attain as a result of various mastication and saliva incorporation strategies has been achieved for the first time via this work.

The first objective (Chapter 3) of this thesis was to select a dark chocolate and a milk chocolate as models, which would allow for the investigation of oral processing and bolus characteristics of two widely consumed but contrasting chocolate matrices with different composition and physical (and related-textural) attributes; and furthermore, to carry out detailed characterisation of the microstructure and specific physical properties of these chocolate models which may play a role differentiating their oral behaviour and bolus properties. Secondly, the aim (Chapter 4) was to investigate the eating (mastication and swallowing) strategies exercised by a population of human subjects for the selected chocolate models, and devise a strategy of population segregation based on chocolate eating behaviour and screening-out of specific test candidates who demonstrated different overall chocolate eating strategies. Thirdly, a set of studies involving the selected test subjects were carried out with the aim to – a) understand the occurrences of specific dynamic features of chocolate bolus formation during oral processing (Chapter 4); - b) investigate the effect of chocolate-type (differences in composition-related physical properties of the chocolate models) and saliva incorporation and eating strategies on the physical properties of ready-to-swallow chocolate boluses (Chapter 5A); and – c) characterise the effect of storage temperature

(structure-related changes induced in physical attributes of chocolates by various storage temperature treatments) on eating and saliva incorporation strategies and the physical properties of ready-to-swallow chocolate boluses (Chapter 5B).

To address the first objective of research, a detailed investigation was undertaken with the goal to establish how specific compositional and microstructural attributes of the selected model chocolates relate to their characteristic physical/material properties of importance to oral processing. This study successfully described the important roles which the compositional and structural elements of solid particle phase, continuous fat phase, and the surface-active emulsifier phase in chocolate play in defining chocolate microstructure and governing physical and textural properties. In particular, chocolate hardness; mechanical properties of chocolate melts - firmness, consistency, cohesiveness, index of viscosity; rheological properties - yield stress and plastic viscosity; thermal properties – melting behaviour; solid fat content (SFC); and chocolate microstructure were characterised and compared for the two chocolate models. Differences in particle size distribution between the chocolates, and presence of milk ingredients (milkfat and milkfat fractions, milk powder, lactose) and surface-active agent (soy lecithin) in the milk chocolate were recognised and discussed as the primary factors contributing to underlying differences in physical properties between the chocolate models.

More specifically, the presence of free milkfat and milk solids in combination with cocoa butter present in the milk chocolate result in a eutectic effect related to lowered solid fat content. This consequently results in the milk chocolate being significantly softer than the dark chocolate. Analysis of thermal behaviour of chocolate models also demonstrated significantly lower values of enthalpy of melting (ΔH_{melt}) for milk chocolate as compared to dark chocolate, again confirming that the lesser energy requirement for complete liquefaction was associated with the relatively lower SFC of the milk chocolate as compared to the dark chocolate. The underlying source of this effect can be attributed to the differences in molecular composition of milkfat and cocoa butter TAGs. The thermodynamic and stearic incompatibility associated with the coexistence of these two different lipid types leads to alterations in the kinetics of blend co-crystallisation and melting. This factor also clearly reflected on other melting behaviour parameters (T_{onset}, T_{end}, T_{index}, T_{peak}, and peak height) of the two chocolates. While both chocolates were found to have the desired β Form-V crystal homogeneity, the dark chocolate demonstrated an overall slower rate of melting and was found to have higher T_{onset}, T_{end}, T_{peak}, and peak height as compared to the milk chocolate. All

of these factors indicated towards the effect of milk components in differentiating the crystallinity and melting behaviour of milk chocolate from that of the dark chocolate.

Different melt mechanical and rheological behaviour was also revealed for the two chocolate models. At a specific melt temperature (40°C), the dark chocolate melt was more cohesive, consistent, and firm as compared to the milk chocolate, while it also demonstrated greater plastic viscosity and yield stress. Once again these differences in flow/deformation behaviour were attributed to arise from specific compositional and structural characteristics differentiating the two chocolates. In particular, these differences were explained through the factors of - a) particle size distribution parameters which are related to the availability of total particle surface area for fat coating, degree of particle-particle contact/interaction, and structural packing; b) contribution of milkfat to the free-fat pool and presence of lactose in the case of milk chocolate; and c) presence of surface-active agent in milk chocolate which in effect modulates frictional forces, particle-particle and particle-fat interactions, and contributes towards deagglomeration and dispersibility of the particle phase in suspension. Optical and confocal laser scanning microscopy (CLSM) were also successfully utilised in unravelling unique features of the characteristic solid particles-in-suspension and crystalline microstructure of the chocolate models. Novel characterisation of autofluroscence properties of cocoa particles was achieved through CLSM imaging.

To address the next objective of research, a human panel study with a population of 24 healthy subjects was implemented to investigate eating strategies exercised in oral processing and bolus formation of the two chocolate models. Four chewing parameters – total chewing time, total number of chews, number of chews until first swallow and chewing rate, and three swallowing parameters (total number of swallowing events, time of first swallow and time of last swallow) were identified and assessed as a measure of overall eating strategies of subjects. Several different eating strategies were witnessed for both chocolates in this study which clearly indicated that chocolate eating behaviour varied considerably across consumers. In the case of either chocolate, different subjects invested different oral processing time and masticatory effort to prepare boluses suitable for swallowing for the first time and until complete oral clearance. When differences between chocolates were evaluated, subjects seemed to demonstrate an adaptive response towards differences in chocolate texture (physical properties) as reflected by the parameters - total number chews in the masticatory sequence from oral acquisition (and/or first bite) to complete clearance, total oral residence time, number of chews and oral processing time invested in preparation of chocolate bolus to

be ready-to-swallow for the first time. Higher values of each of these parameters were noted for dark chocolate as compared to milk chocolate, for all subjects. However on the other hand, results also suggested that subjects conserved their general eating pattern between chocolates and maintained their overall masticatory frequency.

It was postulated that both food (chocolate)-related physical attributes and human-related behavioural factors were explanatory of these findings. Concerning the chocolate-related factors in particular, these characteristic differences in eating strategies between chocolates are likely to be related to differences in chocolate hardness, melting behaviour, and melt mechanical and rheological characteristics which influence the dynamics of oral transformation, and hence the associated sensory cues derived from the continuously evolving bolus properties during the masticatory sequence. All subjects were successful in clearly differentiating the two chocolates in terms of sensory descriptors associated with chocolate physical attributes perceived during oral processing. Sensory attributes of chocolate hardness, onset and speed of melting, and perceived thickness of melt were in excellent agreement with the results of related physical properties of the chocolates quantified instrumentally. Furthermore, cohesiveness, stickiness, mouth-coating and ease of swallowing were other attributes in question during sensory analysis, for which a significant majority of subjects reported that the dark chocolate was more cohesive and mouth-coating, but was perceived less sticky and harder to swallow as compared to the milk chocolate.

Hierarchical cluster analysis, analysis of variance (ANOVA) and principal component analysis were implemented for population segregation (clustering) according to traits of eating strategies, and for the selection of 3 test subjects (S1, S2 and S3) differing in their overall chocolate eating behaviour. With respect to the overall eating strategies of the complete population, selected subjects represented those with very high (S1), moderate (S2), or very low (S3) oral processing time, total number of chews in the overall masticatory sequence, time of first swallow, number of chews until first swallow and total chewing rate; parameters which were also significantly different between these subjects, for both chocolates.

Utilising the selected test subjects, firstly, a unique qualitative assessment of the process of oral transformation of the dark and milk chocolate was achieved. This study allowed for the understanding of some dynamic features of chocolate bolus formation. As an outcome, an empirical scale termed as – "Window of Chocolate Mastication" was developed for both chocolates through specific oral perceptions described by subjects and visual observation of their boluses expectorated at defined times during the masticatory sequence.

This diagrammatic depiction of sequential as well as concurrent attributes of physical transformation of chocolates in the mouth, and specific time-span/s of their occurrence clearly highlighted the complex nature of chocolate oral processing. Several commonalities relating to physico-sensory events during the overall masticatory sequence for both chocolates were perceived by all subjects regardless of their different eating strategies. All subjects successfully reported – a) fracture at first-bite and subsequent comminution of size reduced particles during the initial masticatory sequence as indicative of chocolate hardness perception, b) continuous and concurrent perceptions of fat melting, saliva incorporation, and coating and deposition of molten chocolate in oral cavity, and c) formation of cohesive bolus lumps during the initial masticatory sequence and their presence at the first perception to swallow. Size-reduced solid/partially-melted chocolate particles adhering together by the action of molten fat and saliva in the bolus lumps was confirmed by observation of expectorates. Subjects could also identify predominant regions for the textural perceptions of viscosity, creaminess, fattiness, stickiness and cohesiveness, which were depicted in the window, and interestingly shared comparable positions.

Regardless of eating strategy, occurrence of several voluntary swallowing events was noted before complete oral clearance of chocolates which indicated that only a part of the bolus was ready-to-swallow when the first perception to swallow was generated. Observation of expectorates confirmed that at the first point of swallow, chocolate boluses constituted a pool of liquid bolus phase as well as cohesive bolus lumps. While the liquid phase was swallowed by subjects, cohesive lumps underwent further oral processing to be transformed into a swallowable consistency. The selection of subjects who represented different extremities of chocolate eating strategies amongst a larger population facilitated qualitative mapping of the wide expanses over which the above noted attributes derived from chocolate bolus transformation were perceived. A shorter spread of these dynamic attributes at their respective positions in the window was observed in the case of milk chocolate as compared to dark chocolate. This was a clear indicator of differences in oral transformation behaviour between chocolates and the adaptive response of subjects to differences in chocolate texture.

The next set of studies aimed at quantitative characterisation of microstructural and rheological properties of ready-to-swallow dark and milk chocolate boluses produced by the 3 selected test subjects at the first point of swallow. Firstly, these studies facilitated the understanding of competing effects of differences in chocolate composition and physical properties, and mastication and saliva incorporation strategies of subjects on microstructure

and physical properties of ready-to-swallow chocolate boluses. Mechanical shearing by teeth and tongue, and saliva incorporation during oral processing resulted in a bolus liquid phase comprising of coarse oil-in-water emulsion microstructure in the case of both chocolates. Flocculation and coalescence of fat globules, suspended cocoa, and dissolving sugar crystals were witnessed in continuous pockets of saliva and molten chocolate fat. Distinct differences between subjects for bolus microstructure were difficult to interpret, although between chocolates, boluses of dark chocolate had a denser microstructure with extensive flocculation. These characteristic structuring features observed in ready-to-swallow chocolate boluses may have implications on ingredient-oral surface interaction, mouth-coating and other textural attributes, rheological behaviour of the chocolate bolus, and swallowing.

Saliva incorporation in ready-to-swallow boluses was a chocolate- and subject-dependent factor. Firstly, greater saliva content of dark chocolate boluses as compared to that in milk chocolate boluses could be related to greater oral processing time and effort required to transform it to a suitable-to-swallow state. These factors indicated towards their agreement with differences in hardness, melt mechanical and rheological properties, and melting behaviour between the two chocolates. Secondly, findings proved that ready-to-swallow boluses of a similar chocolate-type could contain a different amount of saliva for different subjects. Results suggested that this finding may not only be related to oral processing time and number of chews invested in bolus preparation, but also to saliva flow rates of subjects.

When the rheological behaviour of boluses was studied, the liquid phase of the ready-to-swallow bolus for either chocolate showed a shear-thinning response. Viscosity of chocolate boluses was found to be a subject-dependent property, which seems not only related to saliva incorporation, oral processing time and number of chews invested in bolus preparation, but also to salivary flow rates of subjects. Subjects incorporating less saliva over a very short oral processing time, and investing lesser number of chews in bolus preparation may produce relatively viscous boluses for swallowing. While for subjects with different eating strategies, differences in salivary flow-rate may relate to achieving similar bolus viscosities. Interestingly, results also showed that regardless of differences in composition and physical properties between chocolates, each subject achieved a similar viscosity for dark and milk chocolate boluses at the point of swallow. This supports the idea that regardless of eating strategy, there might be a characteristic swallowing threshold for different subjects in terms of chocolate bolus viscosity, which may be related to their inter-individual perception of readiness and/or safeness to swallow. Clearly, adaptation of eating and saliva incorporation

demonstrated by each subject, and different concentration of soluble ingredients in the chocolates, leveraged the fact that chocolates with contrasting textures were transformed to similar ready-to-swallow bolus viscosities.

Concerning mechanical properties of ready-to-swallow chocolate boluses, results suggest that firmness and work of spreading of cohesive-lumps in the boluses were both chocolate- and subject-dependent properties. Hardness of chocolates and melting behaviour dominantly influenced these properties, in that, regardless of adaptation for mastication and saliva incorporation strategies by subjects, harder and slower-melting dark chocolate resulted in firmer cohesive lumps which also required higher work for compression as compared to milk chocolate. Subject-dependency of these properties was also evident as an increase in oral processing time and number of chewing cycles resulted in a decrease of bolus firmness and work of spreading for both chocolates. On the other hand, adhesiveness of bolus lumps seemed largely dependent on chocolate composition, while mastication strategies and saliva content did not affect this attribute. Greater adhesiveness of milk chocolate boluses was explained through the presence of milk ingredients in this chocolate. Considering that the chocolates significantly differed in composition and physical properties, and the subjects exercised wide ranging saliva and eating strategies, the findings of no subject-dependency in the case of adhesiveness, and no chocolate-dependency in the case of bolus cohesiveness are interesting especially from a perspective of markers of a swallowing threshold. Studies involving a larger population would be necessary to further test these results and gain definite conclusions.

Finally, the study conducted to assess whether storage temperature of chocolates affected their oral processing confirmed the importance of the fat phase-related physical properties in influencing dynamics of chocolate bolus formation and its physical properties at the point of swallow. When both chocolate models were subjected to storage treatments at 0°C, 20°C and 30°C, mainly SFC-related changes in physical properties were noted. Investigation of hardness and melting behaviour suggested that relative to chocolates stored at 20°C, storage at 0°C resulted in further crystallisation of fat leading to an increase in SFC, and consequently in hardness and ΔH_{melt} for both chocolate types. Conversely, storage at 30°C resulted in decrease in hardness and ΔH_{melt} related to a decrease in SFC.

Once again subjects adapted their eating and saliva incorporation strategies to these changes to form ready-to-swallow boluses. While an increase in hardness and ΔH_{melt} for both chocolates resulted in longer oral processing times and number of chews, subjects maintained

their chewing rate to prepare chocolate boluses suitable for swallowing. Results of bolus saliva content once again confirmed its positive relation to hardness and energy requirement for liquefaction (ΔH_{melt}), especially as the significant softening and reduction in ΔH_{melt} for chocolates stored at 30°C resulted in boluses with significantly low saliva content. Subject-dependency of this factor was again encountered as bolus saliva contents seemed related to inter-individual oral processing time for bolus preparation and saliva flow rate.

Results suggested that significant difference in chocolate hardness and melting behaviour induced by storage temperature may result in a subject achieving different ready-to-swallow bolus properties for a similar chocolate-type. Furthermore, adaptation in eating and saliva incorporation demonstrated by subjects to these alterations in chocolate texture may not affect this finding. For both chocolates, significant reduction in ΔH_{melt} due to storage at 30°C resulted in very low firmness and work of spreading of bolus cohesive-lumps. This could be attributed to the presence of a greater quantity of molten fat in the lumps at the point of swallow. For either chocolate, each subject produced cohesive lumps with similar adhesiveness regardless of storage treatment-type. Again, this corroborated that adhesiveness of chocolate boluses is very likely to be a composition-related attribute. Chocolate storage treatments also resulted in each subject processing a particular chocolate-type to different bolus liquid phase viscosities at their respective points of swallow. Prominent traits indicating relation of specific physical changes induced in chocolates due to storage treatments to bolus viscosities were not distinguished. Although, a subset of results suggested that different SFCs which governed the relative extent of melting that a chocolate underwent until the point of swallow, may have influenced the degree of bolus dilution, and hence its viscosity. Nevertheless, good within-subject repeatability to process a particular texture-type to similar ready-to-swallow bolus rheological consistency was once again witnessed.

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CHAPTER-1

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