Delivering functional ingredients in chocolate – the effect of oral preference on chocolate sensory perception

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Abstract

Chocolate has specific rheological behaviour during oral processing that delivers its distinct sensory characteristics. When incorporating functional or flavouring ingredients into chocolate, these properties must be maintained to meet consumer expectation. Water-soluble and fat-soluble ingredients have a potential effect on the properties of chocolate; therefore, successfully adding functional supplements in this medium can have challenges. Functional foods are a new product category that offers improvements in targeted physiological functions to consumers. However, modern functional foods (low sugar and fat) need modifications in recipe formulation which impacts on the product's texture and flavour release. What is more, flavour perception may itself be significantly influenced by the consumer's individual oral preference (OP).

The first aim of this thesis is to investigate the effect of flavouring ingredients on chocolate microstructure, mouthfeel (texture) and flavour release, during oral processing. In this research, three groups of subjects were classified by their oral processing "pattern", namely those with a chewing preference (CP), a sucking preference (SP), and a mixed group of those who have a preference for chewing and sucking (MP). Chocolate samples (72% dark chocolate) were prepared with different flavour ingredients (water-soluble: ginger powder and fat-soluble: peppermint crystal). Instrumental testing of chocolate viscosity and hardness showed no significant differences in chocolate with low concentrations of added ingredients (0.5% ginger and 0.1% menthol), while chocolate with higher concentration (2.5% ginger and 0.5% menthol) showed a significant difference compared with standard chocolate. Modified Qualitative Descriptive Analysis (MQDA) tests showed no significant differences in sensory perception of texture between the formulations, or the oral processing behaviour groups.

There was an impact on flavour perception both from composition and from oral processing behaviour. The CP group rated the chocolate with the lowest flavouring concentration as also having the lowest cocoa flavour intensity. Moreover, the MP and SP groups showed a similar perception of cocoa flavour intensity. These researches indicated that extra ingredients can influence the original properties of chocolate, and OP is a key parameter determining the flavour perception of subjects.

The second aim of this thesis is to investigate the features and differences between three OPs (chewing preference: CP, sucking preference: SP and mixed preference: MP) on eating behaviour and flavour perception. In this study, chocolate samples (72% dark chocolate) were prepared with different flavour ingredients (ginger and menthol). The oral behaviour tests showed subjects with a CP had the shortest consumption time and the highest chewing rate; subjects with a SP presented the longest sample consumption time and the lowest chewing rate, and, the subjects with the MP sate in between the other OPs. However, with an increase in off-flavour intensity (either ginger or menthol) a change in oral behaviour occurred for each OP, resulting in an extension of consumption time and a decrease in chewing rate. During Temporal Dominance of Sensation (TDS) testing, subjects with the CP more frequently indicated a singular dominant flavour perception and had the lowest frequency of dominant flavour changes, while subjects with the SP indicated multiple dominant flavours and the highest frequency of dominant flavour changes during sample consumption. The results of the TDS testing also indicate that OP contributed to perception of dominant flavour or frequency of dominant change when consuming samples with a low concentration of flavour. However, stronger flavour intensity in the sample became the main factor influencing subjects' perception of dominant flavour or frequency of dominant change. These studies displayed the features of each OP, and reflected the difference on dominant flavour perception by each OP during oral processing.

The third aim of this thesis is to use alternative sensory and physical methods to prove or supplement the previous studies (Chapters 3 and 4). An analysis of the bolus structure was employed, and it was related to the Time-intensity (TI) test to discover the relationship between flavour perception and the restructuring of food through oral processing. In this study, chocolate samples (72% dark chocolate) were prepared some with added flavours in different concentrations (0.5% and 2.5% ginger powder; 0.1% and 0.5% menthol). In terms of the TI test, three different OPs (chewing, sucking, and mixed) resulted in significant differences in Imax (maximum intensity perception by subject), T_{imax} (time at which maximum intensity was perceived by subject), T_{tot} (total duration of flavour perception) and Area (total intensity of flavour) as a result of the differences in oral processing behaviour (consumption time and chewing rate). The results from the TI curves indicate that all subjects were influenced by the intensity of the added flavour, with the T_{imax} and T_{tot} being significantly extended when consuming samples with the highest added flavour intensity. Interestingly similar results in I_{max} between subjects with the MP (mixed preference i.e. chewing and sucking) and the SP (sucking preference) when consuming samples with the highest added flavour intensity indicate that subjects with the MP seem to switch to SP at such times. In terms of the bolus structure of the chocolate samples, the distribution of cocoa butter clearly relates to the oral processing of the chocolate at different consumption times. Combining the results of the TI curve and the bolus microstructure, it was found that a large number of cocoa butter particles with the smallest average area corresponded with the highest flavour intensity perception (subjects with the CP), and the smallest number of cocoa butter particles with the largest average area corresponded with the lowest flavour intensity perception (subjects with SP).

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Abbreviations

ANOVA	Analysis of Variance
CB	Cocoa butter
CF	Chewing frequency
CP	Chewing preference
CLSM	Confocal Laser Scanning Microscope
DSC	Differential Scanning Calorimetry
EMG	Electromyography
GC	Ginger chocolate
GC-MS	Gas Chromatography – Mass Spectrometry
HG	High ginger concentration
HM	High mint concentration
ICA	International Confectionary Association
IOCCC	International Office of Cocoa, Chocolate and Confectionary
LG	Low ginger concentration
LM	Low mint concentration
MC	Mint chocolate
MMA	Masseter muscle activity
MP	Mixed preference
MF	Masticating force
PGPR	Polyglycerol polyricinoleate

PSD	Particle Size Distribution
QDA	Quantitative Descriptive Analysis
RATD	Retronasal Aroma Trapping Device
SP	Sucking preference
SC	Standard chocolate
SFC	Solid Fat Content
TDS	Temporal Dominance of Sensations
TI	Time Intensity
VOCs	Volatile Organic Compounds
3D	Three-dimensional

Symbols

Chapter 3

τ_0	yield stress
η_p	plastic viscosity
γ	shear rate
η	index of flow viscosity
I_{max}	maximum intensity by participant's perception
Ti_{max}	time at which maximum intensity was perceived
T_{tot}	total duration time of the flavour perception
Area	total intensity of flavour
T_{onset}	onset temperature (^oC)
T_{peak}	peak temperature (^oC)

Chapter 4

φ	maximum solid fraction in the suspension
B	Einstein coefficient

1 Introduction

1.1 Background

Chocolate is the one of most popular types of confectionary in the world due to its unique flavour and texture. In the current market, there are many chocolate products marketed as being healthier, and some are marketed as a kind of functional food due to modification of the traditional formulation. However, these new ingredients added to chocolate could greatly influence the sensory profile of chocolate during oral processing. In the consumption of chocolate, the breakdown of the food's structure and the release of flavour compounds from suspension through oral processing provide the consumer with the taste profile of the product (flavour and texture) (Afoakwa et al., 2008).

Chocolate has multiple flavour compounds, both non-volatile compounds (sweetness, bitterness and sourness) and volatile compounds (cocoa flavour). Chocolate's flavour release is a complex interrelation between melting, flow properties, salivation and mastication. There have a lots factor that influence the release of flavour during oral processing and these include a change in food matrix structure and oral physiology. The flavour perception of chocolate is time dependent as its structure changes from a semi-solid to a liquid phase during the oral process. Van Ruth and Roozen (2000) reported that a food matrix which undergoes continuous change during oral

processing has an impact on flavour release. The perception of non-volatile flavours mainly depends on the sequence of compounds in contact with the tongue, and the release rate is dependent on rheological behaviour (Afoakwa et al., 2009; Gonçalves & Lannes, 2010; Beckett, 2009). For volatile flavour release, Kinsella (1990) found it mainly depends on flavour concentration in the air phase, and it was affected by chocolate's flavour retention because most flavour release occurs during the melting stage. In addition, Beckett et al., 2017 also reported that a chocolate's composition, ingredients and particle size would influence flavour release during consumption.

In the last two decades, many studies (Afoakwa et al., 2009; Feron et al., 2014; Overjo-Lopez et al. 2004) have investigated the mechanisms of flavour release in various food models such as chocolate, chewing gum and cheese. However, few studies have carried out research on the impact of oral preference (OP) by people on flavour release during oral processing. The difference in flavour perception by different OPs could lead to the difference of food product acceptability when incorporating functional ingredients with strong flavours into current food products. A better understanding of the effect of oral behaviour on flavour release is very important to make successful functional food products.

1.2 Objectives:

There are two main objectives in this study:

1) The first objective is to investigate the effect of functional or flavouring ingredients on sensory perception by subjects with different oral preferences (OP)

2) The second objective is to gain a better understanding of features of each OP, and determine the difference that OP has on flavour perception during oral processing

1.3 Thesis Outline:

Chapter 2 reviews the background of chocolate in terms of composition, properties and manufacturing. In addition, this chapter also introduces human oral behaviour and its effect on flavour perception.

Chapter 3 describes chocolate making with additional flavouring ingredients and introduces the methods used to research the effect of extra ingredients on chocolate properties. Also, this chapter describes the development of the sensory methodology to identify the features of each OP and their effect on flavour perception.

Chapter 4 describes the effects of extra ingredients on chocolate properties as studied using three main instrumental tests (rheology, hardness and melting point), and the

impact on flavour perception as found by Modified Quantitative Descriptive Analysis (MQDA).

Chapter 5 identifies the features of each OP during oral processing, and describes the difference of each OP in terms of dominant flavour perception as determined using Temporal Dominance of Sensations (TDS).

Chapter 6 describes the effect of the different OPs on flavour perception clearly displayed during oral processing and describes the connection between bolus structure and OP in terms of flavour perception.

Chapter 7 discusses the main findings of this study. The limitation in sensory techniques in the methodology and possible future research directions are also considered in this chapter.

Chapter 8 summary of all the findings of this study.

2 Literature review

Section 2.1 introduces chocolate composition and structure. Section 2.2 introduces the manufacturing of chocolate. Section 2.3 focuses on the key factors affecting the perception of chocolate texture and flavour, and briefly introduces some functional foods and potential ingredients (flavour additives) added to chocolate, especially mint and ginger which are used to investigate flavour release in this research. Section 2.4 describes the mechanism of oral processing and its effect on food consumption. Section 2.5 focuses on the crucial factors for flavour release or perception during food oral processing.

Section 2.1: Chocolate composition and structure

Section 2.2: Chocolate manufacturing

Section 2.3: Chocolate sensory evaluation

Section 2.4: Introduction of oral processing

Section 2.5: Flavour release and perception during oral processing

2.1 Chocolate composition and structure

2.1.1 Chocolate composition

Chocolate is a very common food worldwide, for example, 7.2 million tons of chocolate was consumed worldwide in 2009 (Statista, 2015). Chocolate is a very popular confectionary product that can bring feelings of pleasure when eaten. In addition to sensual pleasures, chocolate also contains polyphenols which have an antioxidant function, and many micro-nutrients, such as carotene, folic acid and some vitamins that are good for our health (Statista, 2015; Afoakwa et al., 2008). In the current market, the main chocolate types are dark, white and milk chocolate. Chocolate consists of carbohydrates, fats and proteins, and the different types have a different mix of proteins, fats and carbohydrates (Table 2.1). In addition, depending on culture, available raw materials and consumer preference, some chocolate may also have other flavours added, such as liquor and nuts.

Table 2.1 Major components of three chocolate types (Source: CIQUAL, 2020)

Chocolate products	Fat (%)	Carbohydrate (%)	Protein (%)
White chocolate	34.2	57.1	6.16
Dark chocolate	46.3	26.2	10.4
Milk chocolate	30.8	55.6	7.5

2.1.2 Cocoa

The various flavours of cocoa, such as cocoa, bitterness, astringency, and sourness, are caused by the fermentation and roasting of the beans (Rohan, 1969). Cocoa has the highest amount of flavonoids of any food, even more than tea and wine (Chong et

al., 2009). Compared with milk chocolate, dark chocolate has more flavonoids due to its higher cocoa content (Barrett, 1994). The major subgroup of flavonoids in cocoa is flavanols, especially the flavanol monomeric epicatechins, catechins and procyanidins which are beneficial to health (Wood & Lass, 2001; Luna et al., 2002; Manach et al., 2004).

2.1.2.1 Cocoa butter

Cocoa butter (CB) is a vital element widely applied in production within the confectionery industry. The special composition of CB, the lattice of CB, endows products with unique physical properties, such as snap, lustre, and melting point. Most fats, especially animal, are composed of numerous and complex triacylglycerols (TAG), while, the CB composition is simpler (Wood & Lass, 2001). CB triglycerides have monounsaturated oleic acid at 2–position and saturated lauric and stearic at 1, and 3–positions. The simple composition of the CB glyceride can make chocolate melt at temperatures ranging from 23 to 37 degrees centigrade. The crystallization of cocoa butter has six different forms (Section 2.2.4). Cocoa butter with the crystal form V (β_2) dominates well-tempered chocolate, and is considered to be the ideal form in chocolate production (Whitefield, 2005). In general, chocolate contains about 25 to 35% fat. The final concentration of fat in chocolate product will be determined by its processing, and the amount of fat affects the texture of the final product (Afoakwa et al., 2007).

2.1.2.2 Sugar

Sugar is another of the vital elements in chocolate's sensory perception, giving the chocolate an attractive taste and neutralizing the cocoa's bitter flavour. In chocolate confectionery, sucrose is added as the main sweetener (higher than 50%) (Krüger, 1999). In order to eliminate consumer concerns about the high sugar content in chocolate (especially in milk and white chocolate), sugar-free chocolate production using sucrose replacers (such as inulin and polydextrose) has gained much attention (Aidoo et al., 2017; Saputro et al., 2017). However, some sugar substitutes (such as mannitol, lactose and xylitol) display a significant influence on the chocolate's rheology properties, thereby affecting the chocolate manufacturing conditions and product quality. For example, glucose and fructose are difficult to dry. When adding these two sugars to chocolate, more moisture will be present in the chocolate and lead to an increase in viscosity due to the increase in the interaction of sugar particles.

2.1.2.3 Emulsifier

Chocolate has a continuous fat phase. However, hydrophilic and lipophobic sugar cannot be dissolved into it. The surface of the sugar must be coated by the fat in order to maintain a good flow property in molten chocolate during oral processing (Whitefield, 2005), requiring the use of emulsifiers. Polyglycerol polyricinoleate (PGPR) and soy lecithin are the commonest and most traditional emulsifiers used to obtain a desirable plastic viscosity and yield value in chocolate (Afoakwa et al., 2007).

They are surface-active ingredients capable of lowering the interfacial tension between the dispersed and continuous phases of liquid chocolate (Schantz & Rohm, 2005). The addition of lecithin noticeably alters the plastic viscosity and yield value, while it decreases viscosity of chocolate, and improves toleration of moisture content in chocolate when added at 0.1 to 0.3%. PGPR does not significantly impact plastic viscosity, but it can significantly decrease yield stress up to 50% when added at 0.2% (Schantz & Rohm, 2005). Therefore, chocolate manufacturers usually combine these two emulsifiers to balance out viscosity reduction.

2.1.2.4 Milk solids

Milk contains approximately 0.7% minerals, 3.5% protein, 5% milk fat and 5% lactose. Milk fat triglycerides, mainly saturated fatty acids, display a different crystalline structure to cocoa butter. Milk fat at room temperature is 80–85% liquid. The high content of free fat and fat in a dairy flavour additive (milk powder) results in a softening of the chocolate's texture, a decrease in the viscosity of molten chocolate and a lower melting point (German & Dillard, 1998; Liang & Hartel, 2004).

2.2 Chocolate manufacturing

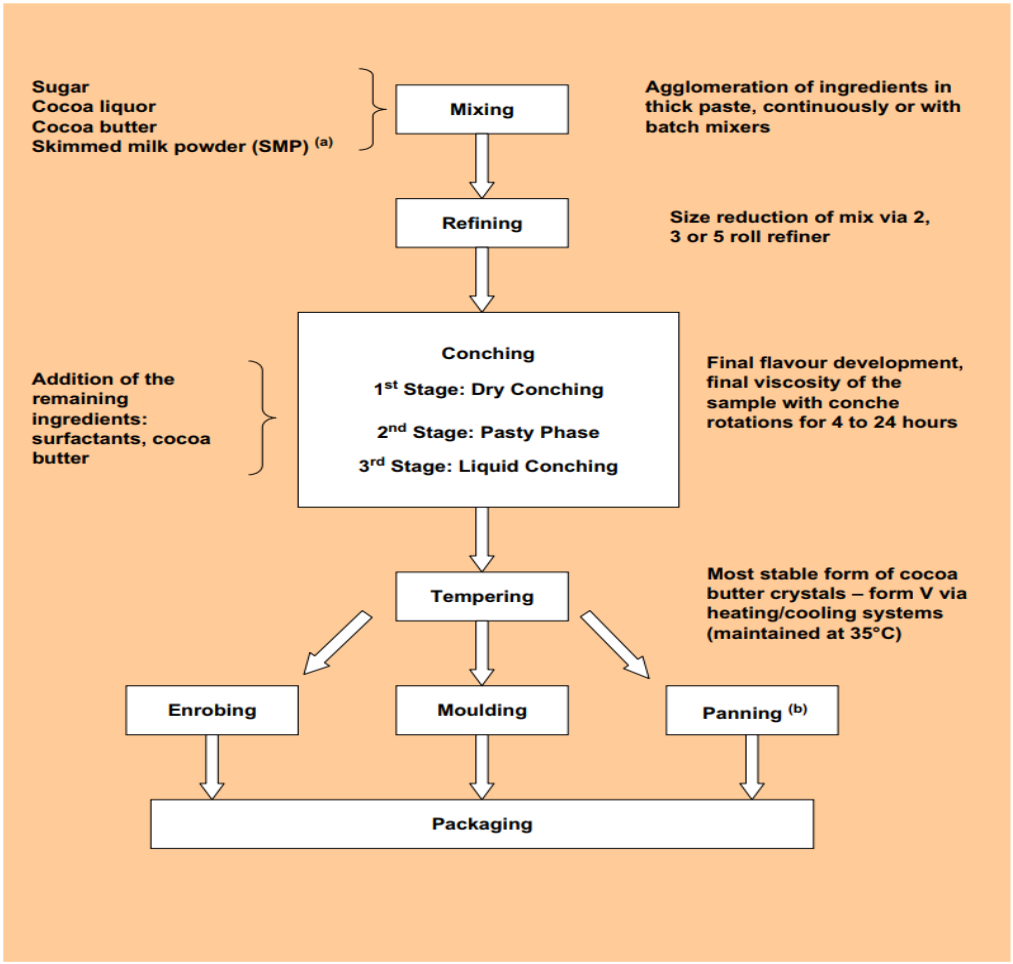


Figure 2.1 Flow chart of chocolate production (Source: Afoakwa et al., 2007)

displays the general manufacturing process of chocolate. To produce chocolate; cocoa liquor, cocoa butter, sugar and emulsifiers constitute the basic ingredients. These ingredients are firstly added and mixed with other ingredients such as milk, and the mixture is refined to reduce solid particle-size. The conching process is subsequently performed, i.e. agitating the chocolate mass at a high temperature (usually over 50 °C), and this is followed by the tempering treatment, i.e. the process

of heating, cooling and mixing. These operations contribute to the development of the final flavour and texture (Afoakwa et al., 2007; Mattia et al., 2017).

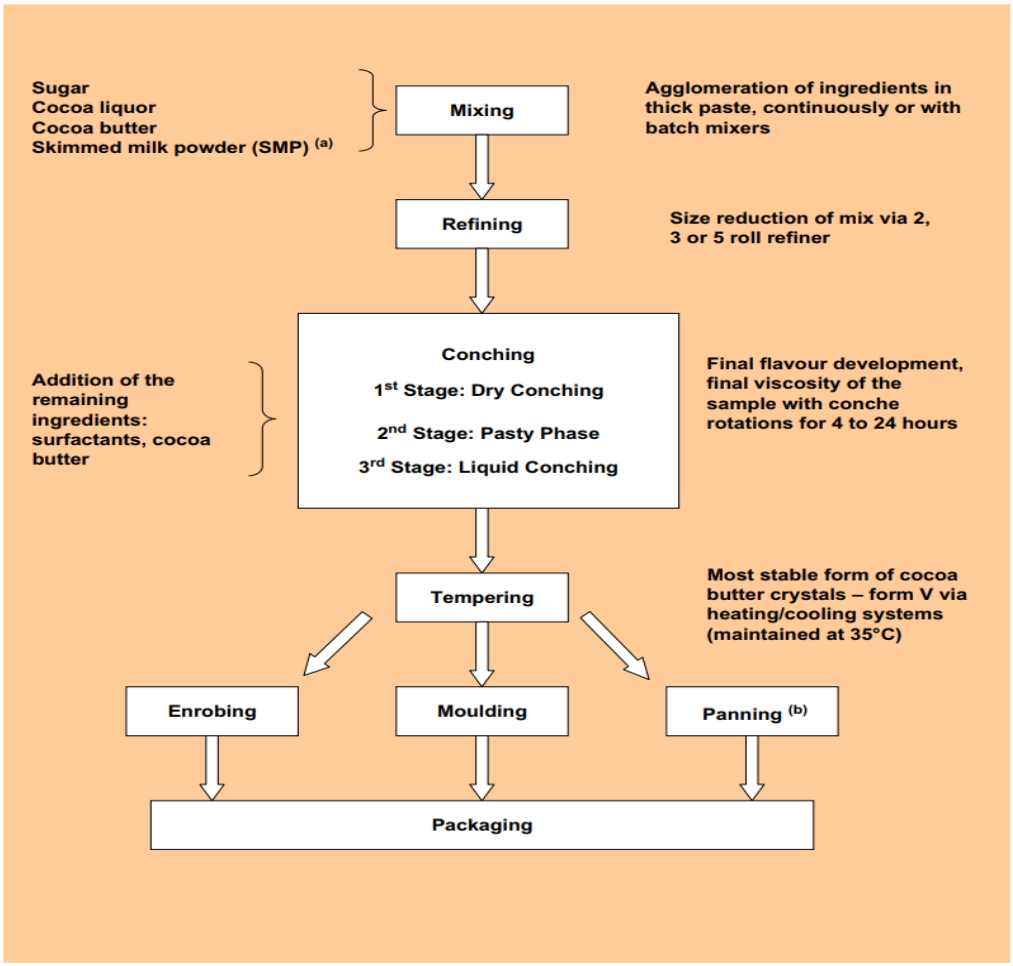


Figure 2.1 Flow chart of chocolate production (Source: Afoakwa et al., 2007)

2.2.1 Mixing

The mixing step is a foundational operation in the process of chocolate production which, by virtue of time-temperature combinations, adopting continuous or batch mixing ensures a constant consistency of the formulation. An example of batch mixing, chocolate that contains cocoa butter (CB), cocoa liquor, milk power, milk fat

and sugar (which is dependent on the category of product) receives 12 – 15 minutes of complete mixing at 40 –50 ˚C (Mattia et al., 2017).

2.2.2 Refining

Chocolate refining is vitally important for producing a smooth texture which is what is desired by the modern chocolate confectionery industry. The mixture of cocoa liquor and sugar (sometimes milk solids or other flavour ingredients are added depending on the chocolate type) at 8 –24% fat content is refined to a particle size < 30 μm by combining two-roll and five-roll refiners (Beckett, 2000). The final size of the particle has critical impact on the sensory and rheological property of the chocolate.

2.2.3 Conching

Conching acts as a unit operation which agitates the chocolate mass at a high temperature (usually over 50 °C). It is important for the creation of the final viscosity of the molten chocolate, the texture and the flavour (Beckett, 2000; Mattia et al., 2017; Afoakwa et al., 2007). The selection of different time-temperature combinations is conducted based on the final products to be produced. For dark chocolate, the temperature is required to be in the range of 70–82 °C, and for milk chocolate, the temperature ranges between 49–60 °C. The processing of the two types of chocolate

needs to be performed for 16–24 hours. Differences in the conching period and temperature combination can also change the texture and flavour of the chocolate (Konar, 2013; Owusu et al., 2013).

2.2.4 Tempering

Tempering is the final process of chocolate manufacturing. The difference in cocoa butter polymorphs leads to the difference in melting and crystallization temperatures (Stapley et al., 1999). In uncontrolled crystallization of cocoa butter, the crystals formed have different sizes, and some larger sized crystals can be seen clearly by the naked eye (Shafi et al., 2018).

The crystallization of fats in cocoa can take six different forms (i.e. polymorphous crystallization) (Talbot, 1999). During chocolate manufacturing, tempering is undertaken to ensure the presence of the appropriate form (V).

Table 2.2 lists the different properties of the six forms, and Form V is the best form for well-tempered chocolate, which has a glossy appearance, a good snap and high resistance to fat bloom (Beckett, 2000).

Table 2.2 Polymorphous crystallization and properties of cocoa butter (Source: Shafi et al. 2018)

Crystal form	Melting temperature	Description
I	17℃	Soft, easy to melt
II	21℃	Soft, easy to melt

III	26°C	Poor snap, easy to melt
IV	28°C	Good snap, easy to melt
V	34°C	Melts at body temperature
VI	36°C	Hard

Tempering involves pre-crystalizing a few triglycerides (1– 3% total) and using these crystals as seeds for the remaining lipids to solidify in the correct form. Figure 2.1 displays the temperature profile of the chocolate tempering process, which includes first heating the chocolate to 50 °C to melt the six forms of crystal, and then cooling the chocolate to a crystallization point (at 27 °C), for the formation of Types IV and V crystals, and finally heating the chocolate to 30 °C to removal of any type IV crystals (Talbot, 1999).

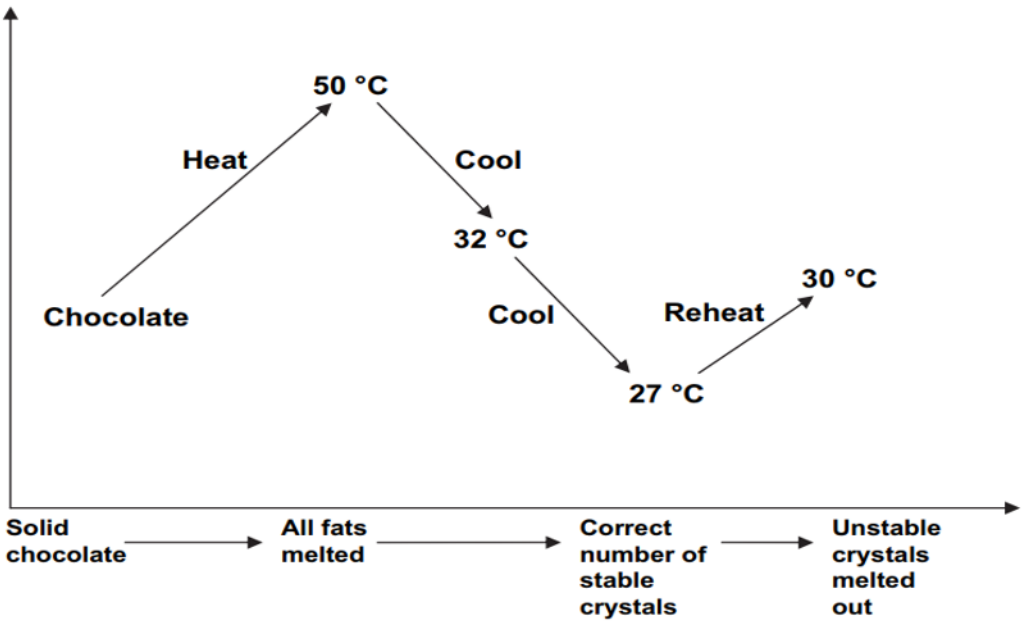


Figure 2.1 Tempering sequence during lipid crystallization in chocolate (Source: Afoakwa et al., 2007)

Seeding can also be used for chocolate tempering. Researchers have applied various seeding methods, adopting different operating temperatures, different numbers of seed crystals and different instruments for providing shear (Bolliger et al., 1999; Windhab et al., 2002). Below are the two classic methods to manually temper chocolate:

• Placing molten chocolate on an endothermic surface (a stone slab) until the chocolate thickens, indicating that there are enough crystal “seeds”; then warming the chocolate gently to the working temperature (Yaseda & Mochizuki, 1992).

• Stirring solid chocolate mass into molten chocolate for "inoculating" the liquid chocolate with the correct form of crystals (crystals from the solid chocolate that are already formed are applied as seed to the molten chocolate) (Debaste et al., 2008).

It has been suggested that the concentration of seed be within the range of 0.1 – 1.15 g/ 100 g of cocoa butter mass to achieve an excellent crystallization effect (Loisel et al., 1997). Concentrations of 2-5 g/ 100 g (Lonchampt & Hartel, 2004) and 0.027% (Kinta & Hartel, 2010) of cocoa butter mass have also been recommended.

2.3 Chocolate sensory evaluation

At room temperature, chocolate is generally solid and the cocoa butter in the chocolate largely determines its melting point which is close to 37 °C (body temperature). Gaokar et al. (2014) suggested that a chocolate’s sensory profile is

influenced by many factors which can be generalized into two main aspects: texture and flavour.

2.3.1 Texture perception of chocolate

Texture is important in the perception of chocolate, as it influences chocolate's physical behaviour during consumption, storage and processing (Gonçalves & Lannes, 2010). During oral processing, chocolate should melt in the mouth. Chocolate's texture mainly relies on suspension viscosity; the mixture of saliva and molten chocolate. When molten chocolate mixes with saliva in the mouth, the mouthfeel (mouth coating, thickness, smoothness etc.) can be perceived (Afoakwa et al., 2007). Beckett (2008) mentioned that the dispersion of the particulate phase and rheological behaviour dominates chocolate's mouthfeel.

The perception of texture mostly depends on the development and transformation of the chocolate's microstructure during oral processing (Silva et al., 2013). As a microstructure level, different compositions in chocolate significantly influence the texture of the final product. The lipid profile of the product in the continuous phase is the key factor for this sensory property. As Afoakwa et al. (2008) reported, chocolate usually contains about 25–35% fat (cocoa butter). Increasing the concentration of fat in chocolate, to up to 32%, significantly decreases the viscosity of the chocolate, as free fats will coat the surface of the particle and make them flow more easily.

Gonçalves & Lannes (2010) also proposed that different fats, having different profiles of fatty acid, can also affect the rheological properties.

In a chocolate product, the moisture content also affects the texture profile by influencing the apparent viscosity of molten chocolate. Higher moisture content (over 0.5 – 1.5%) in molten chocolate can affect the dispersion of sugar particles which can then accumulate to form gritty lumps of sugar. Beckett (2000) reported that manufacturers have to add an extra 1% fat to cover 0.3% additional moisture in chocolate. In addition, the emulsifiers (PGPR and soy lecithin) play an important role in the adjustment of chocolate texture. The function of emulsifiers is to decrease the surface tension between aqueous (sugar) and fat (cocoa butter) phases to maintain a stable flow property in the chocolate. As Beckett (2008) stated, only 0.1 to 0.3% emulsifiers can significantly decrease the viscosity and moisture tolerance of chocolate.

In addition, particle size distribution is also a key factor in chocolate's rheological behaviour. Afoakwa (2008) found that the mouthfeel (such as, cohesiveness, consistency and firmness) is related to the particle size of dark chocolate. Smaller particle size gives a creamy and smooth mouthfeel with good flow performance (low viscosity), while, larger-sized particles are linked to a rough mouthfeel with bad flow performance (Saeseaw et al., 2005). Gaonkar et al. (2014) suggested that a particle size greater than 25 μm can have a negative effect on chocolate texture, as particles of a greater size would be detected by the human palate. To be specific, chocolate having

smaller particle size (below 20 μm) will be perceived as having a smooth mouthfeel, while particles greater than 30 μm will deliver a gritty mouthfeel.

The reduction of the particle size can provide a desirable texture perception for some aspects (such as smooth mouth-feel), but the viscosity of the molten chocolate, through both yield value and plastic viscosity will increase due to an increase in the surface area of particles in contact with the cocoa butter (Ziegler et al., 2001). Therefore, more cocoa butter is required in order to coat the greater surface area of the particles (Beckett, 2008).

2.3.2 Flavour perception of chocolate

Chocolate has a unique character of flavour with various flavour compounds, having both volatile compounds and non-volatile compounds. In manufacturing, the types of cocoa bean used, the processing of the cocoa bean, and the chocolate processing are key factors in the formation of the chocolate's flavour (Whitefield, 2005). Flavour compounds in chocolate are formed by fermentation of the cocoa bean which is the first stage of chocolate processing. During this stage, the colour and flavour precursors of the cocoa bean are formed, and there is a reduction in bitterness. After this the flavour precursors are transformed into flavour compounds (such as cocoa flavour) by Maillard reactions. During chocolate processing, conching, as the key step in chocolate flavour development, can remove moisture and the chocolate's volatile acids, and serve the function of viscosity modification (Fowler, 1999; Saltini et al., 2013).

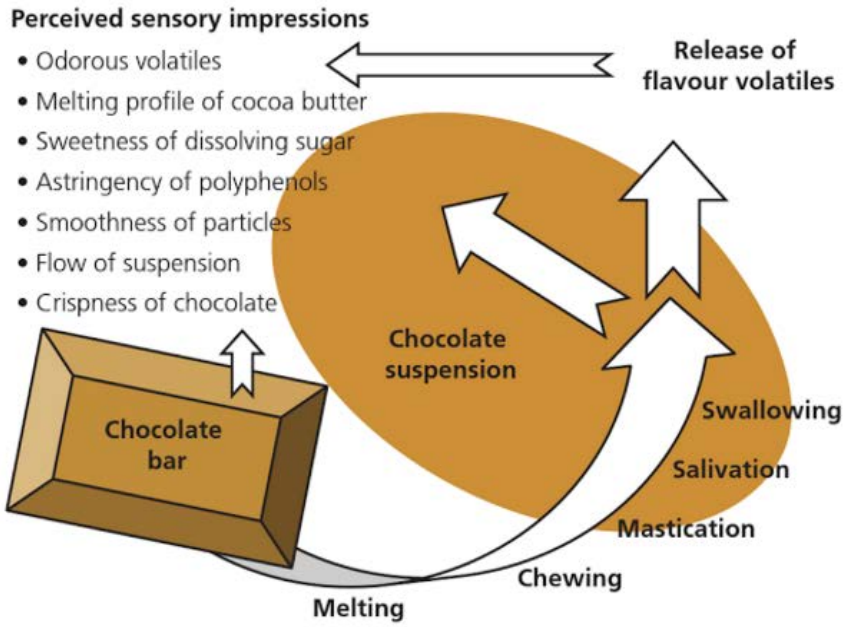


Figure 2. 2 Chocolate flavour release mechanisms (Source: Beckett et al. 2017)

The release of chocolate's flavours is intricately related to oral processing as Figure 2. 2 shows. Chocolate flavour perception is also influenced by the composition, intensity of the flavour ingredients and particle size. During oral processing, both volatile and non-volatile flavours are released into the saliva, a profile of texture and taste is then progressively developed in the mouth (Afoakwa et al., 2008). The perception of taste mainly relies on the sequence of the non-volatile flavours in contact with the tongue. In addition, Gonçalves and Lannes (2010) reported that the rate of flavour release and persistence of flavour in the mouth are relative to the rheological behaviour of the chocolate. They found that the persistence of flavour compounds in the mouth may be extended by an increase in the viscosity of the chocolate bolus. However, the extension of flavour persistence through increasing the viscosity may significantly influence the perception of texture during oral processing (Servais et al., 2003).

Recently, more flavour ingredients have been added to chocolate in order to form new chocolate products with attractive flavours or health benefits. Some of these flavours are volatile, water-soluble or sensitive to heat. These limitations could be problematic to flavour addition or delivery in chocolate. It is interesting to note that Estevinho et al. (2013) suggested that about 60% to 95% of flavour compounds are lost or damaged in food processing and storage. Thus, incorporating flavour ingredients into chocolate may be a challenge in new product development.

2.3.3 Functional food and ingredients

2.3.3.1 Functional food

Functional foods have become a new product category that offers improvements in targeted physiological functions to consumers (Young, 2000). In recent years, consumer requirements in regard to food products have changed noticeably. An increasing number of consumers think that food directly contributes to their health (over and above basic nutrition) (Mollet & Rowland, 2002). Nowadays, foods not only provide energy and necessary nutrients for people, but they can also improve the physical well-being of consumers and cure or prevent some diseases which are nutrition-related (Menrad, 2003). For functional food products, some components are often linked to health benefits (Laahteenmaaki, 2003).

Table 2.3 presents some examples of “functional” foods with a variety of added bioactive components and their claimed health benefits.

Table 2.3 Functional foods currently on the market (Source: Hasler, 2002; Aggarwal et al., 2008; De Moura et al., 2011; De Moura et al., 2011; Santos et al., 2014; Hinneburg et al., 2006; Figueroa-Perez et al., 2011)

Functional food	Bioactive component	Health claim	Reference
Fortified margarines	Plant sterol and stanol esters	Reduce total and LDL cholesterol	Hasler, 2002
Whole oat bread	β-Glucan	Reduce total and LDL cholesterol	De Moura et al., 2011
Cranberry juice	Proanthocyanidins	Reduce urinary tract infections	Kevin et al., 2011
Fermented dairy products	Probiotics	Boost immunity	Aggarwal et al., 2008
Mint products	phenolic compounds; β-carotene; ascorbic acid	Natural antioxidants; reduce cholesterol levels	Santos et al., 2014; Hinneburg et al., 2006
Ginger products	Gingerols	Prevent cancer	Figueroa-Perez et al., 2011

2.3.3.2 Commercial functional chocolate

An increasing number of chocolates with different flavours have been introduced to the current market, such as mint chocolate, chili chocolate and ginger chocolate. Most manufacturers pay most attention to incorporating different flavours into chocolate formulations to satisfy consumer demand in terms of sensory satisfaction. Although some chocolate manufacturers have introduced some functionally enhanced chocolate onto the market, incorporating functional ingredients into chocolate has the challenges of taking into account both the concentration of the functional ingredients (in order to be truly functional) and maintaining the characteristic properties of chocolate. Therefore, design tools for the successful formulation of functional chocolate would be desirable to chocolate manufacturers.

2.3.3.3 Potential ingredients

Polyphenols are strong antioxidants. They exist in many foods naturally, such as chocolate, coffee, olives, red wine, and certain types of fruit (Ackar, et al., 2013). Jalil and Ismail (2008) suggest that chocolate is one of the most polyphenol-rich foods. Green tea, matcha tea and other ingredients which have a high amount of polyphenolic compounds could be added to chocolate in order to further enhance chocolate's antioxidant functionality.

2.3.3.3.1 Ginger as a potential ingredient

Ginger is a subtropical monocotyledon herb. *Zingiber offcinale* is the plant from which the ginger spice is obtained (from the rhizome of the plant, which can be used either fresh or dried). Ginger is used as a spice extensively in food around the world, especially in Asia, due to its unique flavour characteristics and function (Zhao et al., 2009; Tapsell et al., 2006). Current studies have reported that ginger has considerable therapeutic characteristics which include being an antioxidant, having an antimicrobial and antibiotic influence, providing a direct anti-inflammatory effect, as well as having an ability to suppress the forming of inflammatory compounds (Dedov et al., 2002; Tang, 1992; Ali et al., 2008).

Ginger contains about 3–6% crude fibre (from dry matter), 3–6% ash, 6–8 % fatty acids and triglycerides, 9% protein and free amino acids, and 50% carbohydrates, as determined by climate, geography, and variety of ginger species (Leung, 1984; Tang,

1992). The main phytochemicals in ginger are shogoal, gingerone, gingirole and gingerbene; the chemical structures of these are shown in Figure 2.3. In addition, shogaols and gingerols as bioactive constituents of ginger also provide the major pungent sensation during consumption (Singh et al., 2008).

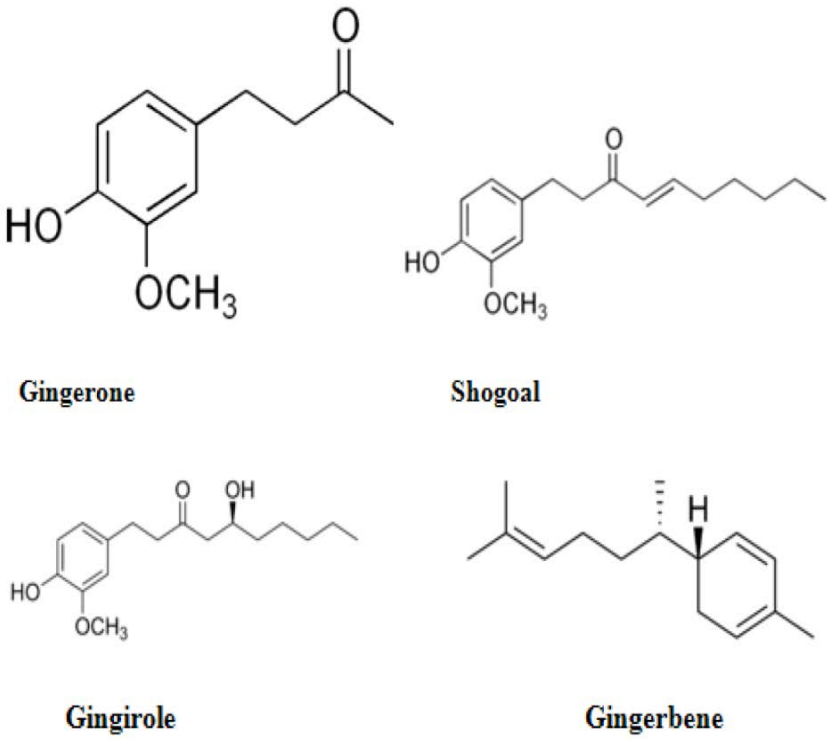


Figure 2.3 The chemical structure of the main phytochemicals in ginger (Bhatt, 2013)

Ginger products currently available on the market include ginger powder, fresh ginger and ginger oil(Vasala, 2001). In addition, oleoresin and essential oil with strong ginger flavour characteristics are widely used in many medicinal substances, and in the food industry as flavour additives (Singh et al., 2008). They can be extracted from fresh ginger through distillation, and ethanol or acetone extraction (Pushpa et al., 2015). Ginger is a very popular ingredient in food processing worldwide due to its

fresh and pleasant aroma and spicy characteristics (Balestra et al., 2011). In the West, ginger has been used extensively for culinary purposes in pickles, soups, puddings, cakes, confectionery (such as ginger chocolate), as well as for soft drink making (such as ginger beer). In Asian countries, fresh ginger is processed and added as a flavouring agent in everyday cuisine (Zhao et al., 2009; Vasala, 2001). In China, it is also used to treat diseases such as arthritis and muscular discomfort (Wang & Wang, 2005)

2.3.3.3.2 Mint as a potential ingredient

Peppermint (*Mentha x piperita*) refers to a well-known plant employed in various forms (i.e., leaf water, leaf extract, leaf, and oil) (Nair, 2001). It is a perennial herb native to Mediterranean Europe, but has subsequently been cultivated in numerous places around the globe. Peppermint oil is the primary product of peppermint and is usually distilled from the herb *Mentha x piperita L*. It is light in colour with a strong, refreshing aroma, and can be dissolved in essential base oil and ethanol (Kline et al., 2001). The elements of peppermint oil given in monographs of the International Pharmacopoeia cover carvone (max. 1.0%), pulegone (max. 4.0%), menthol (30.0–55.0%), isopulegol (max. 0.2%), menthyl acetate (2.8–10.0%), isomenthone (1.5–10.0%), menthofuran (1.0–9.0%), menthone (14.0–32.0%), cineole (3.5–14.0%) and limonene (1.0–5.0%) (Shrivastava, 2009; Verma et al., 2011).

Figure 2.4 shows the chemical structures of these elements.

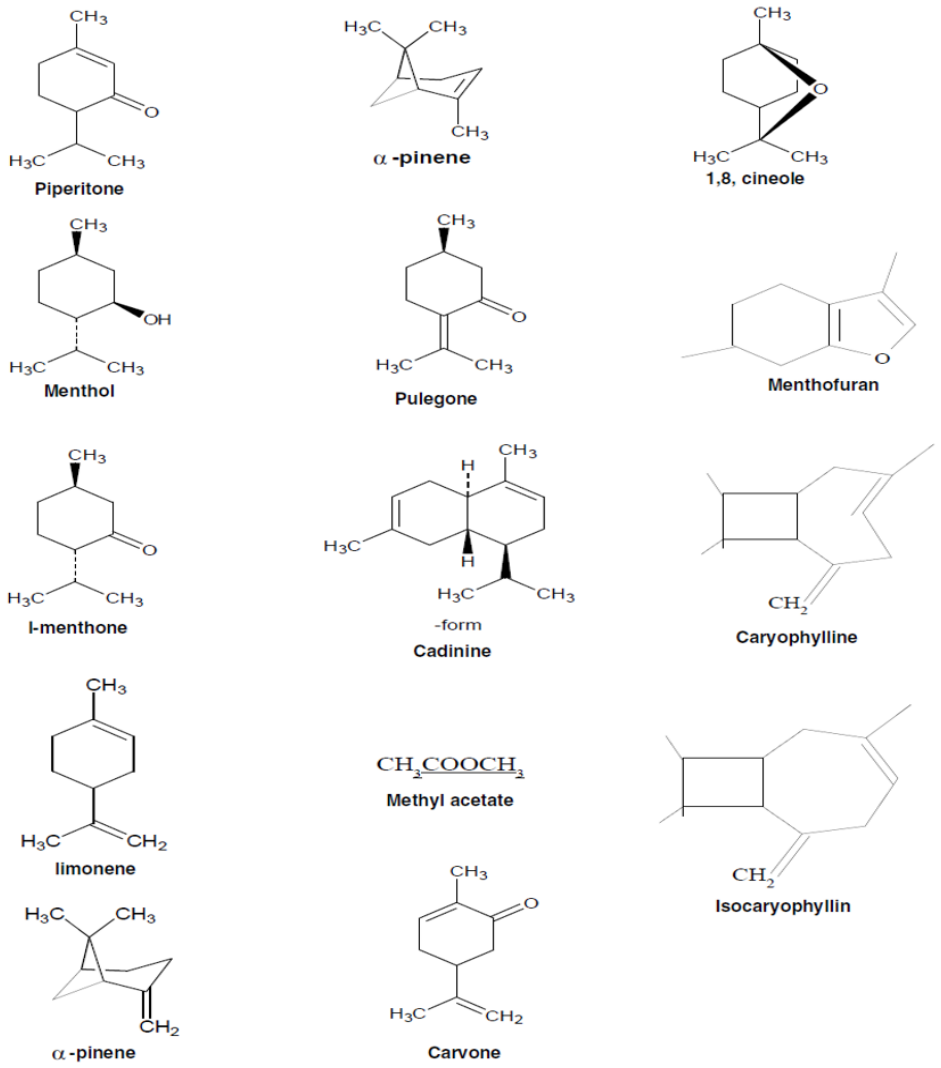


Figure 2.4 Chemical constituents of peppermint oil (Source: Shrivastava, 2009)

The dominant groups of the peppermint flavour's chemical components include quinones, volatile flavonoids and acid (Kline et al., 2001). Menthol is also an important flavour compound of peppermint (the minty flavour) (Galeotti et al., 2002). It refers to a natural plant origin element and is the main compound in peppermint oil. In general, menthol can be extracted from peppermint and corn mint oil by steam

distilling (Eccles, 1994). It can also be obtained or synthesized from other essential oils for example, Indian turpentine oil, eucalyptus oil and citronella oil, however, the yield from these is much lower than from peppermint and corn mint oil. Menthol refers to a cyclic terpene alcohol that covers three asymmetric carbon atoms. In the optical isomers, (2)-menthol appears extensively and naturally, and it can act as a fragrance and flavour compound (Dolzhenko et al., 2010). Thus, it has been extensively applied as flavouring for some food products (such as chewing gum) toothpaste and some oral hygiene products (Eccles, 1994).

Peppermint has antioxidant and antimicrobial functionalities. According to Lv et al.'s (2012) study of the effect of peppermint on physiological processes, on both human and animal subjects, it has antimicrobial, antitumor, immuno-modulating and beneficial digestive effects. In pharmacy, peppermint is included in topical antipruritic, antiseptic and cooling formulations. In addition, (2)-menthol is part of eutectic formulations in local anaesthetic agents (Jyvakorpi, 1996; Dolzhenko et al., 2010).

2.4 Oral processing

Oral processing is not simply the food consumption mechanism but also the process by which perception or appreciation of food flavour and texture is performed. Food consumption in the mouth includes oral operations such as initial biting, mastication, bolus formation, bolus or food mass transportation and swallowing (Figure 2.5).

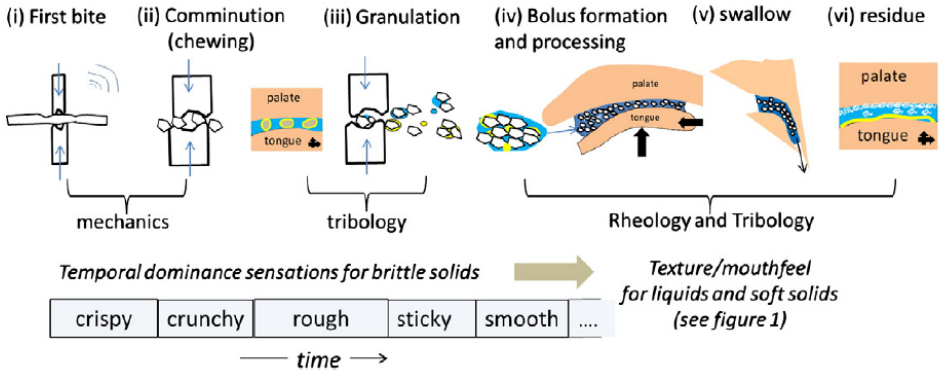


Figure 2.5 Flow chart of oral processing of solid food (Stokes et al., 2013)

2.4.1 Role of oral processing

Oral processing is the first step in transferring food to the digestive system. It involves jaw movements and muscle activity in the face and tongue (Figure 2.). All of these actions contribute to the swallowing of food. Before swallowing, one function of oral processing is to perceive the food's texture and flavour (Lenfant et al., 2009). The food is also transformed into a bolus (a mixture of food and saliva) during the mastication process. At this stage, flavour compounds will be released by mastication or naturally released into the saliva, and the taste buds on the tongue and the olfactory receptors in the nose would are stimulated (Linforth et al., 2002). In addition, Lucas (2004) and van der Glas et al. (2018) referred to food breakage during mastication being significantly influenced by tooth shape and total occlusal area. This can also result in a difference in flavour release and bolus formation.

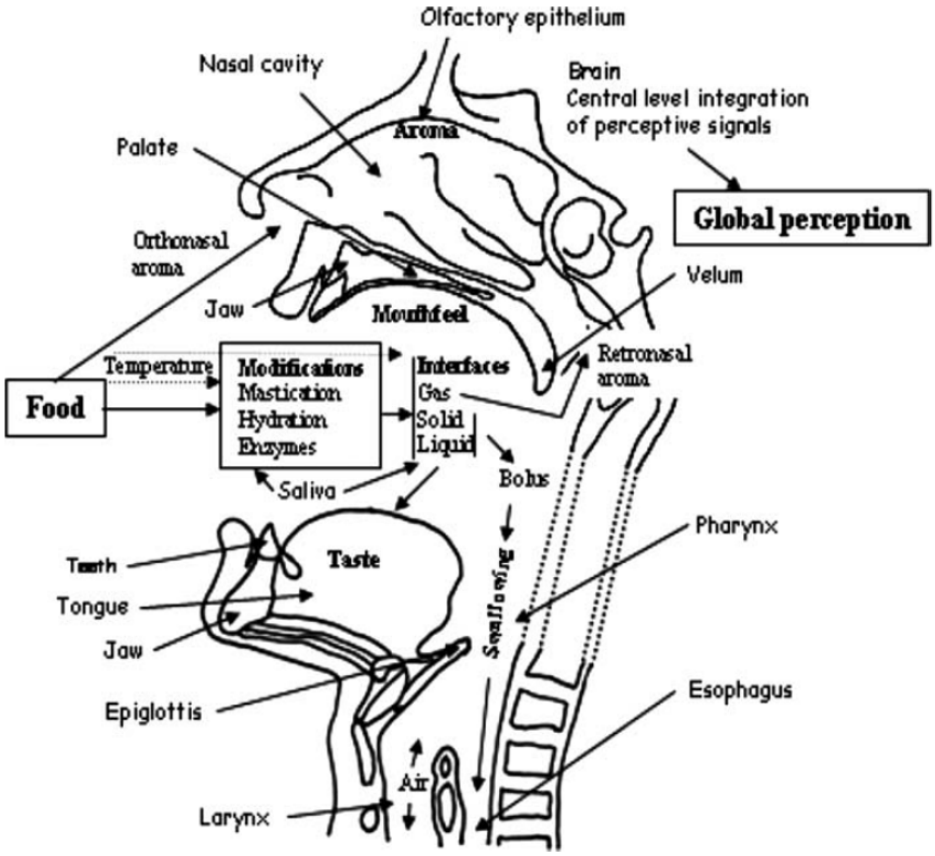


Figure 2.6 Oral organs cross sectional diagram (Salles et al., 2011)

Figure 2.8 provides a framework of the main food physics and physiological parameters during food oral processing. Mastication is a key step in oral processing as it breaks down the food matrix and forms the bolus with saliva for swallowing (Liu et al., 2017). Mastication is a complex process involving movements of the maxilla and mandible jaws, tongue and cheeks, and lips to a lesser extent.

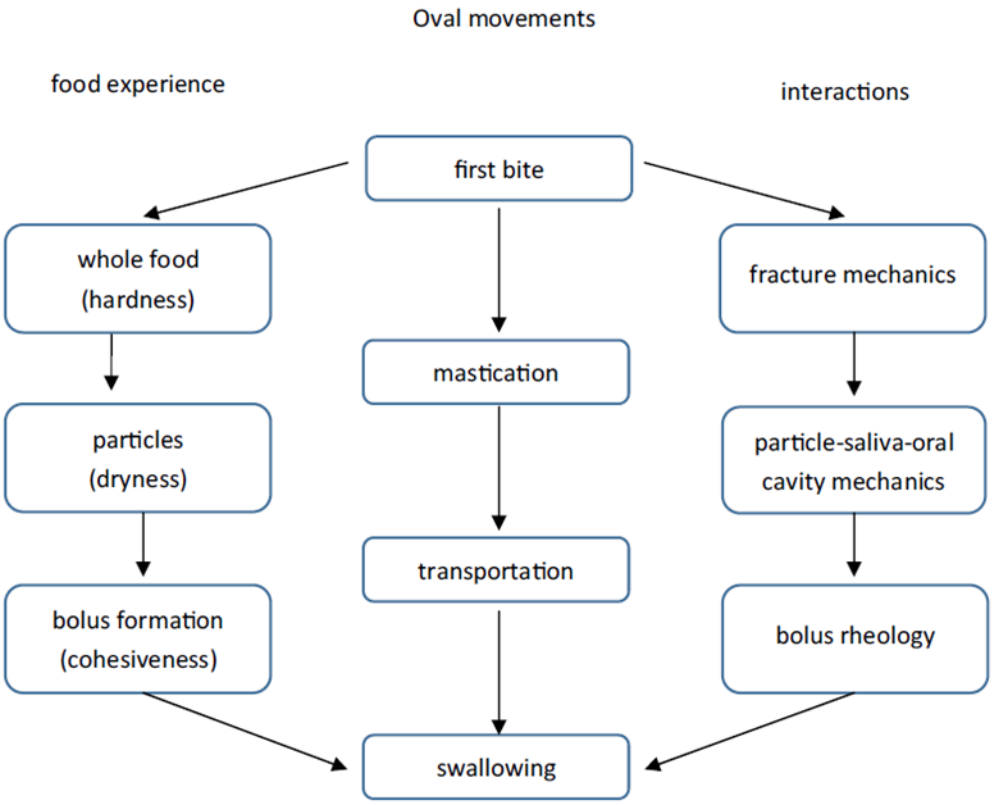


Figure 2.7 Oral processing framework (Liu et al., 2017)

Selway and Stokes (2014) posited that during oral processing, rheological properties play a more important role than fracture characteristics in transforming the food matrix into a bolus. In fact, both the rheological properties and fracture characteristics of the food matrix can affect sensory perception, especially texture perception that can have an impact on preference and acceptance by consumers (Foster et al. 2011).

2.4.2 The first bite

The first bite is the onset of food consumption, which includes part or full acquisition of a food sample. It could also be part of the first chewing cycle in which a subject is given a consistently sized sample in an experiment (Medicis & Hiiemae, 1998). People can perceive a lot about texture (such as cohesiveness, hardness and springiness) despite the first bite being a one bite act (Agrawal et al., 1997; Foegeding, 2007). According to the model of Schwartz et al. (1989), a first bite can fall into three phases which are: slow opening of the mandible, fast closing of the mandible, and slow closing of the mandible. Studies have found that bite size was determined by individual style and food type (de Wijk et al., 2008; Hutchings et al., 2009; Sharp & Jaquess, 2009). Hill and McCutcheon (1984) researched the influence of physical characteristics, gender, degree of hunger, and food preference on chewing rate and bite size. They found that larger bites were taken by people who are obese and have a high preference for that particular food. Gender was also an important factor for bite size. Their research found the bite size of men (7.4 g/bite) was significantly larger than of females (5.4 g/bite).

In addition, Wijk et al. (2008) discovered that the size of the first bite was also influenced by food texture. They found that the size of the first bite of semi-solid or solid food is comparatively larger than that of liquid foods. The sizes of subsequent bites of liquid food are increasingly large, while the opposite has been reported for semi-solid and solid foods. Hutchings et al. (2009) carried out a comparison of normal

bite sizes (length, volume, and weight) for forty-five subjects of six food bars. Compared to bite volume, bite length displayed higher consistency. This study suggested that a consistent bite amount is likely to indicate regular feeding behaviour more effectively and display a higher suitability compared to providing steady mass samples.

Food geometry also noticeably impacts biting behaviour. As Peyron et al. (1997) suggested, when sample thickness was increased, perception of hardness in food from the initial bite was increased. Kohyama, et al. (2005) measured biting force with the use of a sheet sensor on which there were multiple points and obtained biting stress measurements with the contact area as well as the applied force. They reported that the peak force, contact area and peak stress at the fracture point for foods that are brittle and hard (carrots) were larger in thicker samples. In terms of soft and tough foods (fish gels), the peak force and contact area increased with the rise in the thickness, but maximum stress was similar.

2.4.3 Mastication

Mastication is aimed to prepare foods for swallowing, and it is considered the initial part of the digestion process. During mastication, the sizes of the food is reduced, it is mixed with saliva and formed into a bolus which is a mixture of small food particles and moisture. Meanwhile, flavours are released from the food and more textural

perception (such as smoothness, viscosity, cohesiveness and adhesiveness) can occur (Lucas et al., 2002; Hutchings & Lillford, 1998; Cakir et al., 2012).

Engelen et al. (2005) studied the influence of oral behaviour and different food products on swallowing. They found that the mastication cycle has a negative relationship with the rate of saliva flow when subjects consumed cake and Melba toast. This suggested that less of the mastication cycle was required to form the bolus when subjects had more saliva. In addition, they also observed that subjects would maintain their chewing patterns (such as chewing rate) when they consumed different types of food, although there were still differences in the chewing behaviours among the subjects.

In addition, Hiiemae et al. (1996) showed food consistency also influenced chewing behaviours. They found that there is a significant difference in the bite size (weight and volume) when subjects consumed various foods (apple, banana and cookie). More specifically, the weight of one 'natural' bite of apple (7.75 g) is significantly lower than one 'natural' bite of banana (12.45 g). This phenomenon greatly affects the number of mastication cycles. In further research, Thexton and Hiiemae (1997) proposed that the number of mastication cycles needed for formation of the bolus (ready-to-swallow) is greatly influenced by the properties of food. Food properties (including hardness, viscosity and composition) have been reported to impact mastication. During oral processing, hardness of food is perceived in the masticating process (from first bite to before swallowing for solid food) and has an effect on

masticating force (MF), jaw muscle activity, as well as the mandibular jaw moving process (Kohyama et al., 2004; Peyron et al., 2002; Mathevon et al., 1995). The MF in the course of chewing silicon rubber samples presented a significant increase (100 to 150 N) with the increase of sample hardness (Kohyama et al., 2004). Similar results were reported by Foster et al. (2006). They found the duration of mastication, muscle activity and number of chewing cycles greatly increased with the increase in sample hardness. Yven et al. (2012) also stated that subjects changed their oral strategies to adapt to products with different characteristics. According to their research, most subjects changed their oral strategies by changing chewing time and muscular contraction amplitude (total muscle work) when they consumed cheeses with different texture and fat content.

2.4.4 Swallowing

Swallowing is considered the last stage of oral processing. Okada et al. (2007) adopted the video-fluoroscopy technique to study this, summarizing that people need at least two swallows, even when swallowing a small amount of food, solid or semi-solid food. Swallowing in the oral phase may mould food or food particles and saliva into boluses and force these boluses to the back of the oral cavity by retraction of the tongue base (Matsuo et al., 2008). Pressure generated through putting teeth into centric occlusion, as well as forming a lip seal, is required for moving these boluses.

The measurement of particle size distribution (PSD), moisture content as well as the slipperiness established recently, helps to investigate the properties of the bolus at the point of swallowing (Gaviao & Engelen, 2004). As Peyron et al. (2006) reported, the PSD of the bolus (when ready to swallow) was determined mainly by food type when subjects consumed them. They studied the PSD of food boluses after masticatory processing by subjects of various types of nuts (pistachio, peanut and almond) and raw vegetables (cauliflower, carrot and radish), finding a significant difference in the PSD of ready-to-swallow boluses between the different types of food, but a similar PSD for a certain food type for all subjects. Malbos et al., (2007) made further studies using six natural foods (gherkins, cheese, coconut, chicken breast, ham, egg white, mushrooms, and green olives). They also found there was no significant difference in the PSD of the bolus among subjects; however, the difference in PSD of the bolus could be predicted by food type. Based on the texture of each food in this research, it seems that there is an association between food hardness and the average particle size of ready-to-swallow boluses, i.e. generally hard and brittle food boluses exhibit particle size and soft and ductile foods exhibit larger-sized particles. Similar results were presented by Chen et al. (2013). They used seven foods of varying hardness to research the correlation between PSD of the bolus (ready to swallow) and food hardness. According to their results, the PSD of the bolus (ready to swallow) decreased with an increase in the hardness of the food.

The food bolus's particle size is considered a key element in generating the stimuli for the end of mastication and the start of swallowing, however, the decision to swallow also depends on lubrication and cohesiveness of the bolus (Bornhorst & Singh, 2012; Chan & Stokes, 2012). Engelen et al. (2005) investigated the chewing behaviours (chewing cycles) and saliva incorporation in boluses of different foods. They found that the number of chewing cycles was greatly different, about 17 (cake) and 63 (carrots) before swallowing. It seems a larger number of chewing cycles was needed for hard and brittle foods before swallowing. Specific to cake and Melba toast, an obvious negative correlation could be found between saliva incorporation and the number of chewing cycles. Accordingly, the number of chewing cycles is higher and residence time in the mouth is longer for hard and dry foods to be sufficiently broken down and for the formation of a cohesive bolus through adding sufficient saliva. In addition, Hutchings and Lillford (1998) suggested that the process of swallowing has to satisfy two thresholds; food particle size and lubrication of the bolus. According to the research of Chan and Stokes (2012), the particle size and bulk of the food is large at the beginning of oral processing, and this stage is dominated by breaking down and deforming processes. The progress of chewing is in line with reduced particle size and the food being diluted fluid with saliva. Surface friction and lubrication can determine the mouthfeel and aftertaste in the later phase of oral processing.

2.5 Release and perception of flavour in oral processing

Flavour, as a significant food attribute, can determine product quality and the consumer acceptance of a food product. The key elements influencing flavour are not only intensity of flavourings in foods, but also the mechanism of flavour release during oral processing. At the beginning of oral processing, the flavours in food are released in the saliva phase, and the taste buds on the tongue can sense the non-volatile flavours, while volatile flavours need to be first transported to the air phase from the saliva in mouth, and then passed to the olfactory receptors in the nose through the throat to be perceived there (Taylor, 2002). This processing depends on the oral parameters of the individual, such as chewing rate, time of swallow, saliva flow rate and composition (Muñoz-González et al., 2019).

2.5.1 Effect of saliva on flavour release

Saliva has a lot of functions in oral processing: cleaning food, cooling hot food, providing lubrication, forming the food bolus (Bornhorst & Singh, 2012). During food consumption, saliva influences the release and perception of aroma by diluting flavour compounds, interacting with aroma compounds, as well as providing a buffering capacity and enzymatic activity (Pedersen et al. 2002; Spielman, 1990). These phenomena can all be affected by salivary composition and the flow rate which may have high variation, caused by the degree of hydration, composition of the body,

smoking, exposure to light, food smells, previous stimulation, as well as the climatological environment (Dawes, 1981). However, studies have found that salivary flow rate seems not be affected by gender (Watanabe & Dawes, 1988; Engelen et al., 2003).

Some researchers have focused on how salivary flow rate affects flavour compound release, especially for volatile compounds. When volatile flavours are released from food into the saliva by mastication, there may be an interaction between the volatile flavour compounds and saliva composition (such as enzymes and mucins). Feron et al. (2014) have shown the influence of saliva properties on the release of volatile flavour through cheese. They found that saliva flow has a negative influence on volatile flavour release. This can be explained by the fact that saliva dilutes the aroma compounds when flavour was transferred from the food into the saliva phase by mastication. In addition, the research of Guichard et al. (2017) with the same food model (cheese) found that the content of sodium and lipolytic activity in saliva had a significant influence on aroma perception. More specifically, subjects with low sodium content and high lipolytic activity in saliva can sense a more intense aroma. Some reports stated that the volume of saliva is also important to flavour release during oral processing (Haahr et al. 2004; Ruth & Roozen, 2000). Based on the research of chewing gum by Haahr et al. (2004), due to the increase in saliva volume, more flavour compounds in chewing gum would be retained in the aqueous phase, so as to diminish its transportation through the retronasal route.

2.5.2 Effect of mastication on flavour release

Mastication can significantly affect the momentary release of flavour, and swallowing does also as the nasal airflow responsible for the latter delivers volatile compounds to receptors on the tongue and in the nose. Generally, the number of non-volatile compounds in saliva delivers an uptrend of flavour perception when the chewing process begins, which then reaches a peak, followed by a quick decrease after mastication ends (Davidson et al., 2000). For example, the concentration of sweetness (non-volatile compound) that was released from chewing gum reached its peak in saliva during the first minute of mastication and the maximum concentration of menthol and menthone (volatile compounds) subjects perceived was also found in the early stage of oral processing (Haahr et al., 2004). All flavours both volatile and non-volatile decrease with continuous mastication. In addition, Tournier et al. (2014) studied the relationship between mastication and release of non-volatile flavour during bread consumption. They showed that the salt flavour release mainly depends on mastication and duration of mastication. More specifically, the increase of mastication cycles led to an increase in sodium release and the maximum sodium concentration was induced by a longer chewing time (before the swallowing point).

Pionnier et al. (2004) demonstrated the relationship between the kinetics exhibited by non-volatile compounds that were released from model cheeses and different chewing parameters such as chewing efficiency, duration time and masticatory rate. These

parameters correlated with each other while also exhibiting an association with the salivary flow rate, e.g. if the chewing rate was low, the chewing time was longer and the salivary flow rate was low, thus the time for flavour compounds to reach the highest concentration in the saliva was retarded. In addition, they also found that chewing behaviour was greatly determined by individual variation. As Phan et al. (2008) observed, sodium release and saltiness perception presented significant difference due to individual variations. This phenomenon can be explained by individuals with different chewing behaviours (chewing rate, duration, etc.). The experiment regarding mastication found the mastication rate was the parameter which most impacted aroma release, compared with other parameters such as saliva, mastication, and texture of food (Mestres et al., 2006). As mastication rate increases, the overall aroma release will increase accordingly (Mestres et al., 2006; van Ruth et al., 2003). The stronger the muscle activity in the chewing process, the greater the intensity of the aroma, and the slower the aroma decreased (Hansson et al., 2003).

2.5.3 Effect of food matrix on flavour release

Food processing in the mouth is relative to the breakdown of structures by mastication and the lubrication provided by saliva to aid swallowing (Figure 2 8). The breakdown or destruction of food during oral processing is strongly connected to sensory perception and liking. In terms of flavour perception, the rapid and massive increase of food surface area during oral processing result in quick flavour detection by taste

buds on the tongue and olfactory receptors in the nasal cavity (Salles et al., 2011; Chen, 2015). Bakker et al. (1996) and van Ruth and Roozen (2000) also stated that the increase in food surface area, airflow in the mouth and food matrix breakdown influence flavour release. For example, a low chewing rate that is accompanied by a long consumption time would mean more flavours in the food would be released into the saliva. As Hiiemae et al. (1996) and Foster et al. (2006) reported, the chewing rate and food consumption duration were significantly affected by food texture. In addition, Tarrega et al. (2007) found that food matrix composition interacts with chewing activity, which decides the release of aroma. According to their research, the difference in cheese composition impacts the texture of the final product that in turn affects the chewing activity among subjects, both influencing the release of aroma. In addition, Heenan et al. (2012) studied the relationship between sugar composition and flavour release of a strawberry cereal bar. They found that polydextrose can improve the release of menthol, esters, and acetaldehyde when compared with glucose syrup solids. This reflects the fact that flavour release may be driven by a ‘salting-out’ effect, which is the reduction of free water volume due to a change of polydextrose’s state from rubbery to glassy. Similar results were found by Mehinagic et al. (2004).

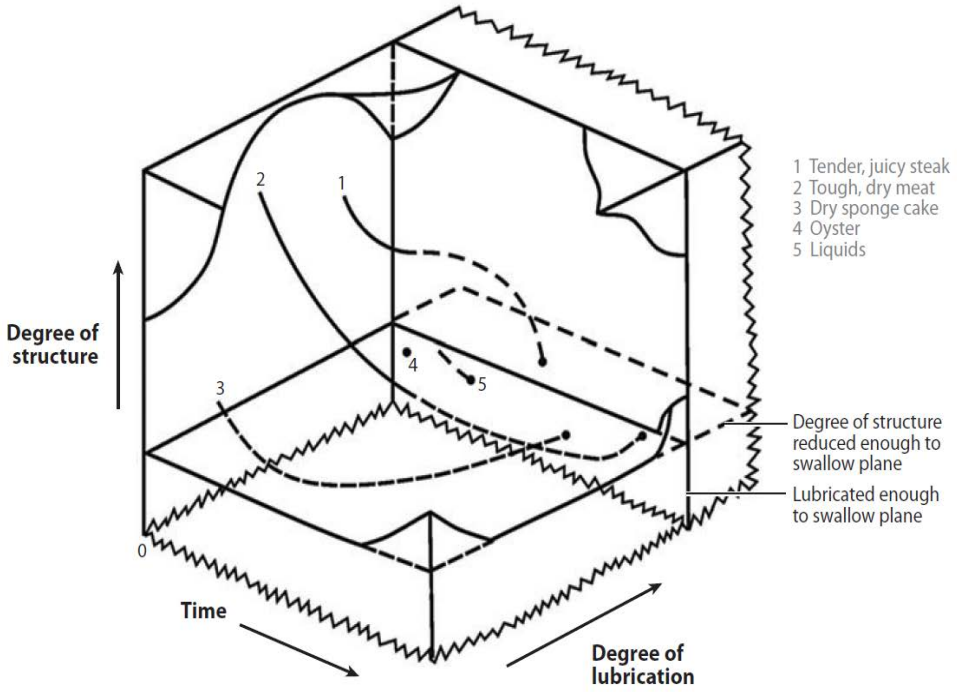


Figure 2 8 Model of food processing in the mouth (Hutchings & Lillford, 1998)

2.6 Difference in oral behaviour

The general function and features of oral processing are the same for each human, while several factors, such as age, health status, gender, race, oral behaviour preference etc. will lead to a significant difference in oral behaviour and sensory perception of food between consumers (Chen, 2009; Doyennette et al., 2014). In recent years, increasing attention has been paid to these differences in oral behaviour, owing to their effect on sensory perception (Chen & Engelen, 2012; Jeltema et al. 2015). According to the research of Brown and Braxton (2000), subjects can be classified by the efficiency of their food reduction (size reduction of solid food materials). They found that different subjects adopted different oral strategies during

oral breakdown of food. Engelen and de Wijk (2012) also stated that there were significant differences in oral patterns of individuals. Based on their research, four types of subjects were identified through self-description, being: simple, taster, manipulator and tonguer. In addition, Jeltema et al. (2014, 2015) identified four different groups of subjects (Crunchers, Chewers, Suckers and Smooshers) based on what the authors coined as "mouth behaviour" of individuals. According to their observation, the difference in mouth behaviour is driven by a subject's preference for different textures. De Wijk et al. (2003) found the degree of sensory perception depended on whether individuals consumed food product using their preferred (natural) oral processing style. These authors also found that specific oral behaviour by subjects was associated with enhancement of specific sensory perceptions (Engelen and de Wijk. 2012; de Wijk et al. 2008). For instance, chewing provides the best expectation of flavour perception.

2.7 Conclusion

Chocolate is a semi-solid confectionary product which consists of several ingredients in multiple phases. In the market today, there are many chocolate products that are modifications of the traditional chocolate formulation in regard to ingredients (cocoa butter, sugar and others flavour ingredients). However, changes to the chocolate formulation (such as particle size, cocoa butter content, emulsifiers and added ingredients) have a considerable impact on chocolate's texture and flavour, which in turn affects the sensory perception of the final product.

Sensory evaluation is an effective tool to determine or assess the quality or acceptability of a product by consumers. As the review above suggests, sensory evaluation of chocolate mainly encompasses texture and flavour perception. Changes in chocolate's texture and flavour, through adding or replacing ingredients, has been found to influence the sensory perception of the final products. Many consumers not only focus on the function of food products, but are also concerned with the sensory perception aspect. Maintaining good sensory characteristics while at the same time providing health enhancing functions (such as low energy (calorie) and high functional ingredient content) would be of great benefit to new product development.

To this end, research into oral processing helps researchers understand changes in the food matrix and their effect on sensory perception in the mouth. The oral process is a complex system which includes the first bite, mastication and swallowing. Most studies in this review reported that the hardness of food significantly impacts the perception of the food during initial oral processing (first bite and mastication). After this viscosity and lubrication of the bolus (a mixture of saliva and small food particles) dominate the later perception of food. Moreover, differences in subjects (chewing pattern, gender and preference) were also reported to have some impact on sensory perception, especially in terms of flavour release. However, some studies found individual eating pattern preference and a food's texture can influence each other. Researching this phenomenon would be a challenge due to oral processing's complexity.

3 Materials and Methods

This chapter will provide details of the development of the chocolate samples and the techniques and methodology applied in this research.

This chapter is split into the following three sections:

Section 3.1: Chocolate sample design and production processes

Section 3.2: The methods employed for chocolate properties testing and measurement, those used for testing and the bolus microstructure

Section 3.3: Design and procedure undertaken for the sensory evaluation for modified qualitative descriptive analysis (MQDA), temporal dominance of sensations (TDS), time intensity (TI), and the oral preference (OP) test.

3.1 Development of chocolate samples

3.1.1 Chocolate sample design

Chocolate samples were designed in order to discover the effect of functional ingredients on chocolate texture and the eating behaviour of participants. As discussed in the literature review, replacing or adding new ingredients to chocolate will significantly influence its texture and/or flavour, therefore as low a quantity of functional ingredients was added to the chocolate sample as possible (maximum 2.5% w/w). In terms of the selection of functional ingredients, menthol (crystals, 99%, fractionated from peppermint oil) and ginger (100% natural ginger, dried and ground from fresh ginger root) were chosen as being appropriate for their health benefits and would generally have an acceptable flavour. Both ingredients were obtained from NutriHerb BioTech Company (Nanjing, China). Dark chocolate (Table 3.1) having 72% cocoa solids (Chocolate Brown, Warkworth, New Zealand) would be the base chocolate used to develop the new formulations of chocolate. While ginger and menthol were chosen to represent functional ingredients with expected health benefits, we refer to them as flavouring ingredients added into chocolate henceforth in this thesis.

Table3.1 Nutritional information for 72% dark chocolate (per 100 g)

Energy	2247 kJ
Protein	9g
Fat-total	39g
-Saturated	24g
Carbohydrates-total	30g
-Sugars	26g
Sodium	10mg

In this research, five types of chocolate samples with different functional ingredients were produced using lab-scale facilities. These were standard chocolate (SC), chocolate samples with low ginger concentration (LG), chocolate samples with high ginger concentration (HG), chocolate samples with low menthol concentration (LM) and chocolate samples with high menthol concentration (HM), the specific formulations for each are show blow:

• Standard chocolate (SC): 72% dark chocolate

• Ginger chocolate (GC) w/w: 0.5% (LG), 2.5% (HG)

• Menthol chocolate (MC) w/w: 0.1% (LM), 0.5% (HM)

3.1.2 Sample preparation

The method to make the samples was adapted from Zhao et al., (2018) with some minor modifications. First dark chocolate with 72% cocoa solids (Chocolate Brown, Auckland, New Zealand) were melted using a water bath (WB-11, WiseBath, South Korea) at 60 °C for 1 hour to pre-crystallize the cocoa butter in the chocolate. Next, menthol crystals and ginger powder in different concentrations (as above) were gradually added to the molten liquor being stirred continuously at 200 rpm using a mechanical stirrer (RW 20 digital, IKA-works Inc. NC, USA) for 1 hour. The liquor mixture was then transferred to a tempering machine (Revolation 2B, ChocoVision, USA) with a temperature profile suitable for tempering dark chocolate as shown in Figure 3.1. During the tempering process, 0.5% of seed (cocoa butter) crystals were added to the molten chocolate at 42.22 °C to 32.2 °C, in order to promote

multiple crystal formation to Form V crystals at the continuous cooling stage at 32.2 °C to 29.4 °C. After that, molten chocolate was reheated to 31.5 °C to remove any unstable crystals.

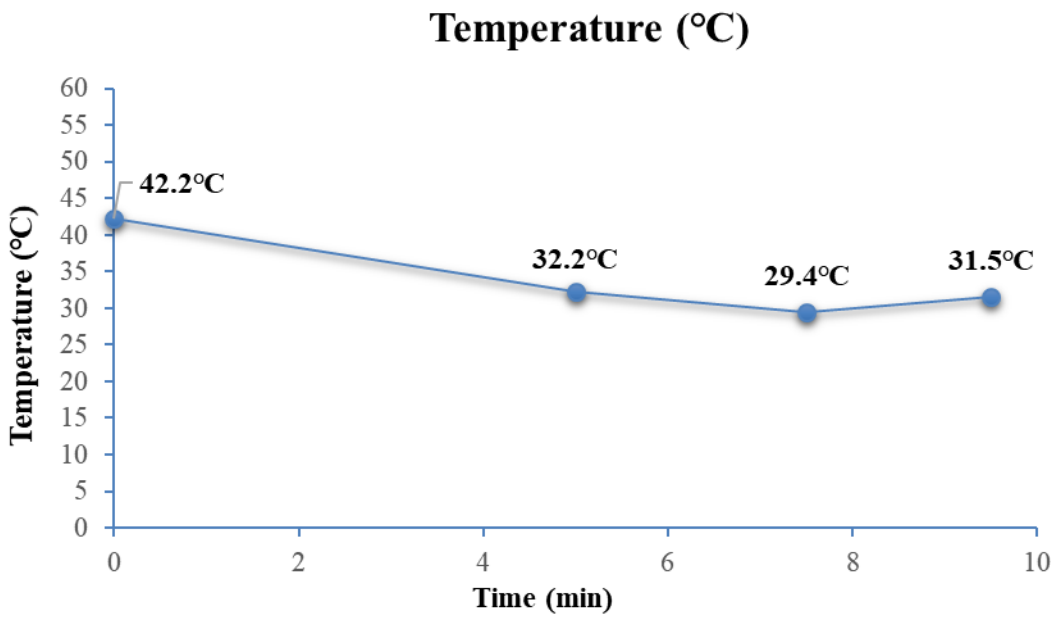


Figure 3.1 Temperature profile for chocolate tempering

After tempering, the chocolate liquor was transferred into three different moulds:

(1) Hemispherical mould (diameter: 26 mm): for the sensory test (MQDA, TDS, TI) and the eating behaviour analysis.

(2) Cylindrical mould (diameter 45 mm; depth 12 mm): for the chocolate hardness test.

(3) Rectangular mould (depth 60 mm, width 10 mm and length 70 mm): For the snap test.

The filled moulds were placed into a food-grade and constant temperature refrigerator (4 °C for 2 hours) before demoulding. All demoulded chocolate samples were transferred to a sealed container and stored at 20 °C for two weeks before being analysed.

3.2 Instrumental testing

The ingredients present in the cocoa dispersions affected the microstructure of the final chocolate matrix, which directly influenced its rheological behaviour and textural characteristics, such as viscosity (apparent viscosity and yield stress), melting point and hardness (Schantz & Rohm, 2005). With the formulation of these new chocolate samples made by adding different functional or flavouring ingredients in different concentrations, their effect on the chocolate's properties were measured using the instrumental tests below.

3.2.1 Hardness

Chocolate's hardness is one of its most important textural parameters, determining physical rigidity, and it directly affects sensory perception during consumption. The method employed for the chocolate hardness test was adapted from Afoakwa et al., (2008). A TA-XT2 texture analyser (Stable Micro Systems, Haslemere, England) was used to carry out the test. In this test, the five types of chocolate in the cylindrical shape (diameter 45 mm; depth 12 mm) were tested, and each sample underwent ten replications at 20 °C. For the equipment and system setting, a load cell of 50 N and a 2 mm stainless steel needle probe were used. 1 mm s^{-1} was set as a pre-test speed and a 2 mm s^{-1} speed was set as the test speed over a 5 mm depth. The maximum force to achieve penetration through the samples was indicative of hardness.

3.2.2 Differential Scanning Calorimetry (DSC)

The melting properties of chocolate differ depending on composition, type and quality of the chocolate (Shafi et al. 2018; Ostroska-Ligeza et al., 2019; Talbot, 1999; Afoakwa et al.,

2008). Differential scanning calorimetry (DSC) is usually applied to determine the chocolate melting characteristics, such as heat capacity, melting point, crystallization time and temperature (Tan & Kerr, 2017). The method of DSC measures phase transition heat during cooling (or heating) with a controlled temperature/time gradient applied to a small sample volume in order to provide near homogenous temperature conditions (Beckett et al., 2017). According to Cebula and Smith (1992), a lower scanning speed will produce more accurate results about peak height and resolution. However, Woda et al. (2006) suggested that excessively low scanning speed may lead to the recrystallization of the cocoa butter. In this study, the melting properties of the five types of chocolate were carried out using a Model 60 Differential Scanning Calorimeter (Shimadzu, Columbia, USA). The procedure was based on Aidoo's (2017) method with a heating rate of 2 °C/min. The onset temperature (T_{onset}: beginning of polymorphic form melting), end temperature (T_{endset}: end of polymorphic form melting) and peak temperature (T_{peak}: state of crystallization at peak temperature) values were calculated using an A-60 WS Thermal Analyser. Kinta and Hartel (2010) proposed that melting points can be determined by the temperature at which maximum energy is absorbed by the sample. Therefore, the value of T_{peak} would be the most important factor in comparing the difference in the chocolate samples.

3.2.3 Rheology

The rheological properties of molten chocolate are important parameters in industrial processing for evaluating the quality and texture of the product. Previous studies have reported that the rheological behavior of chocolate is determined by many factors, such as particle size distribution (PSD), fat content, presence of emulsifiers, moisture content, composition and processing (Schantz & Rohm, 2005; Fernandes, et al., 2013). Chocolate's

rheology can be quantified by two parameters which are apparent (plastic) viscosity and yield stress. Plastic viscosity is relative to chocolate's pumping characteristics, coating and sensory character. In addition, Ziegler et al., (2001) submitted that plastic viscosity also affects flavour perception during oral processing. Yield stress determines the transition between pseudo-solid and pseudo-liquid phases, which is relative to the minimum shear stress at the beginning of flow (Gonçalves et al., 2010). Molten chocolate is a non-Newtonian fluid, which can be described by three rheological models: Casson model (1), Herschel-Bulkley model (2) and Bingham model (3) (Chevalley, 1999; Sokmen & Gunes, 2006; Aderale, et al., 2017; Fernandes, Muller & Sandoval, 2013), as in the following equations:

Casson model:

$$\tau^{0.5} = \tau_0{}^{0.5} + \eta_{pl}{}^{0.5} * \gamma^{0.5} \quad (1)$$

Herschel-Bulkley:

$$\tau = \tau_0 + \eta_{pl} * (\gamma)^{n} \quad (2)$$

Bingham

$$\tau = \tau_0 + \eta_{pl} * \gamma \quad (3)$$

Where τ denotes shear stress, τ_0 is yield stress, η_{pl} is plastic viscosity and γ is shear rate, η means index of flow viscosity.

In terms of the Bingham model, the research of Fernandes et al. (2013) on rheological behavior of dark chocolate mentioned that the Bingham model provides a good description of cocoa butter rheology, while it lacks a comprehensive description of the flow properties of chocolate. The Herschel-Bulkley model provides a better description of non-Newtonian fluids, because it can accurately characterize the behaviour of non-Newtonian fluids at different shear rates. It improves the Bingham model by using power law expression to replace the plastic viscosity term (Herschel & Bulkley, 1926). However, the Herschel-Bulkley model has been challenged due to its assumption that the flow is homogeneous. Some studies suggest that fluid may exhibit the phenomena of shear localization at low shear rates. Comparing these three models, the Casson model is the most well-known and is recommended by the International Office of Cocoa, Chocolate and Confectionary (IOCCC) and the International Confectionary Association (ICA), and it widely used in research into the rheological behaviour of non-Newtonian fluids. The Casson model was designed for viscous suspensions of cylindrical particles, which provide a more appropriate description of fluids with a pseudoplastic character such as chocolate. For the method, the IOCCC recommends using a bob and cup geometry (Figure 3.2) to measure the viscosity of molten chocolate.

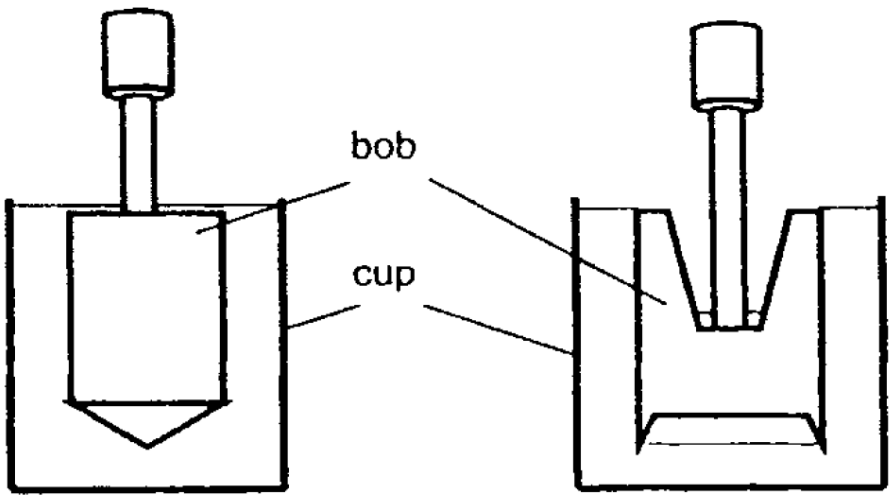


Figure 3.2: Geometry of bob and cup (Source: Aeschlimann & Beckett, 2000)

The ICA recommends the measurement setting for viscosity and shear stress at increased shear rates from 5 s^{-1} to 50 s^{-1} as the ramp-up stage and decreased shear rates from 50 s^{-1} to 5 s^{-1} as the ramp down stage (ICA, 2000). In this study, the Casson model with bob (25mm diameter) and cup (27.5 mm diameter) geometry (AR-G2 Rheometer, TA Instruments, Delaware, US) was used to investigate the effect of functional or flavouring ingredients on chocolate's rheological behaviour.

3.2.4 Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) is a technique for acquiring high resolution three-dimensional (3D) images of microstructure (Matsumoto & Halle, 1993). More specifically, CLSM provides a possible method for analysing images of complicated morphological structures and the capability of acquiring the optical equivalent of thin fluorescent sections from thick specimens or other appropriate morphological contexts (Fish & Davidson, 2009). A confocal microscope is a form of fluorescence microscope that sharpens the collected images by looking at light from only one focal plane. This allows for the collection of multiple focal planes in a so-called z-stack, which provide three-dimensional images (Collazo et al., 2005). Usually, the operation of a confocal microscope involves: (1) marking the target (2) mounting specimens for observation, (3) optimizing and adjusting images on the confocal plane (4) collecting images, (5) analysis of image data. In this study, the chocolate boluses were analysed using a confocal laser scanning microscope (CLSM-Olympus FV1000, USA)

3.2.4.1 Slide preparation for CLSM

Chocolate boluses were produced by 30 participants (three OP groups, each group having 10 participants according as outlined in Section 3.3.1) with different consumption times (10%, 30%, 50%, 80% and 100%). In this study, the chocolate bolus structure generated by different participants within the three OPs would be characterised by distribution of cocoa butter. For chocolate samples, the cocoa butter needs to be distinguished from other ingredients (such as sugar crystals and cocoa solids). Nile Red (Sigma-Aldrich, New Zealand) was chosen to stain the fat in the chocolate samples due to its simplicity, rapidity and ability to locate the hydrophobic phase (Halim & Webley, 2015). The procedure for this was adapted from Auty et al. (2001) with modifications. To stain the cocoa butter, Nile Red powder was added directly to the chocolate bolus a ratio of 0.0005:1. They were then sealed in containers covered with aluminium foil and placed in a cold dark place for staining (four hours). After staining, the containers were transferred to a water bath and heated for 15 minutes in order to fully melt the samples (bolus). The melted samples (bolus) were transferred to a single concave microscope slide and a coverslip was quickly placed on top.

3.2.4.2 CLSM Imaging

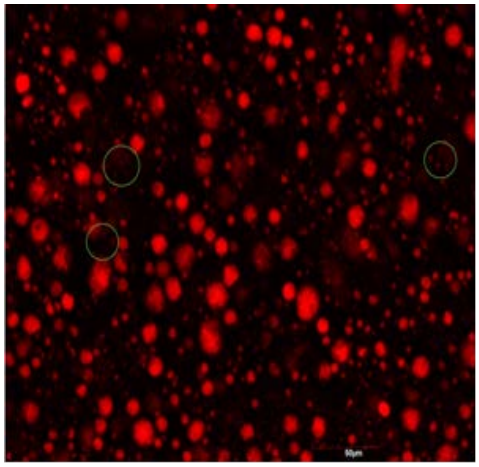
The lowest magnification lens (10-x) can obtain an overall image of the slide with the stained bolus sample; however, it only provides a low magnification image. High magnification lenses (40-100-x) provide higher magnification images with sufficient detail for data analysis, but they cannot obtain an overall image. Therefore, the slides of the samples were imaged using a 20-x lens (medium times lens) in order to gain a clear and global microstructure of the bolus. During imaging, three concurrent image channels with different excitation wavelengths (405, 473 and 559 nm, Table 3.2) were applied and layered to generate the final image. In addition, in order to collect an individual image from each

channel, the collection wavelength was set at 425–460 (blue), 499–561(green), and 655–755 nm (red). Each slide would have nine images taken at random locations.

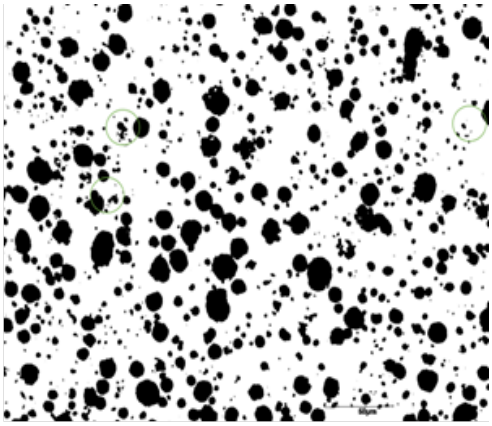
Table 3.2 Settings used for confocal laser scanning microscopy

	Image 1	**Image 2**	**Image 3**
Laser wavelength (nm)	405	473	559
Dye selection / filter	DAPI	Alexa Fluor 488	Alexa Fluor 568
Collection wavelength (nm)	425 – 460	499 – 561	655 – 755
Display colour / channel	Blue	Green	Red

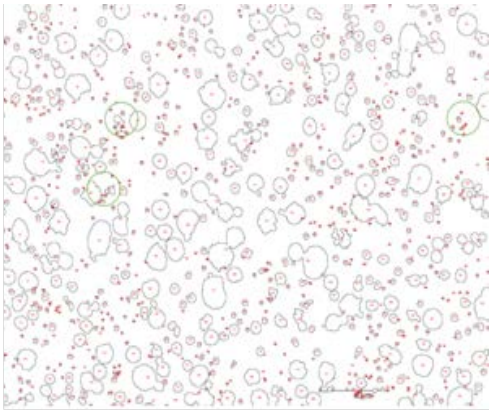
In terms of image analysis, the red channel image was selected, as the cocoa butter was most clear and distinguishable in this channel compared to the other channels. ImageJ Fiji software (National Institutes of Health, Bethesda, MD, USA) was applied for the analysis of particle size. The images produced by the CLSM were first converted to 8-bit greyscale and then the brightness scale was manually adjusted to outline the particles as a black and white image. In this analysis, particle count and total area of particle was the output of the software and the data were exported to Excel (Office 365, Microsoft, USA). Particles with an area of less than 1 μm^2/pixels and a roundness of less than 0.2 were removed as an artefact generated from undissolved Nile red crystals as Figure 3.3 shows.



(A)



(B)



(C)

Figure 3.3 Images (A) taken from the red channel of the CLSM; (B) 8-bit after thresholding; (C) outlined particles identified for measurements. Green circles indicate the presence of Nile Red crystals.

3.3 Sensory Test

3.3.1 Participant selection

For the sensory tests, one hundred participants (50 females, 50 males, age 20-30, BMI: 22.4±2.8) were recruited via advertisements for initial analysis of chewing behaviour and flavour perception. Participants had good general health and healthy dentition (based on the standard of normal dentition (Jiffry, 1981) without dentures, jaw and oral problems; complete dentition and no recent dental surgery). Test session time was 9:30-11:30 a.m., after breakfast. Participants arrived at the test location and completed a questionnaire recording their gender, age and Body Mass Index (BMI; refer Appendix A for questionnaire). Before and during sensory tests, participants were free to ask questions at any time about the operation or procedure of each sensory session in order to ensure there was no confusion. All sensory trials were conducted with approval from the University of Auckland Human Participant Ethics Committee, reference UAHPEC 021267.

Before the first sensory session, all participants underwent an oral preference test. Two pieces SC were served to all participants in order to classify their oral preference (OP) pattern whilst eating chocolate. All oral processing by participants was recorded on video. Two researchers conducted the observation-based video processing. If they had significant difference of assessment, the video was reviewed by a third researcher, and a final decision was made. The decision of OP classification followed the natural oral behaviour of each subject, which was established from the ratio of their time of chewing action:total consumption time or ratio of time of sucking action:total consumption time. By observation, participants were classified into one of three OP groups:

1. Chewing preference (CP): more than 80% of oral processing was chewing

2. Sucking preference (SP): more than 80% of oral processing was sucking

3. Mixed preference (MP): less than 80% of oral processing was either chewing or sucking

Note that the specific threshold of 80% was decided upon based on results collected from preliminary experiments. This panel was then reduced to smaller subsets for further sensory evaluation of flavoured and functional chocolate. The sensory analysis was conducted over four sessions as follows（Figure 3.4）:

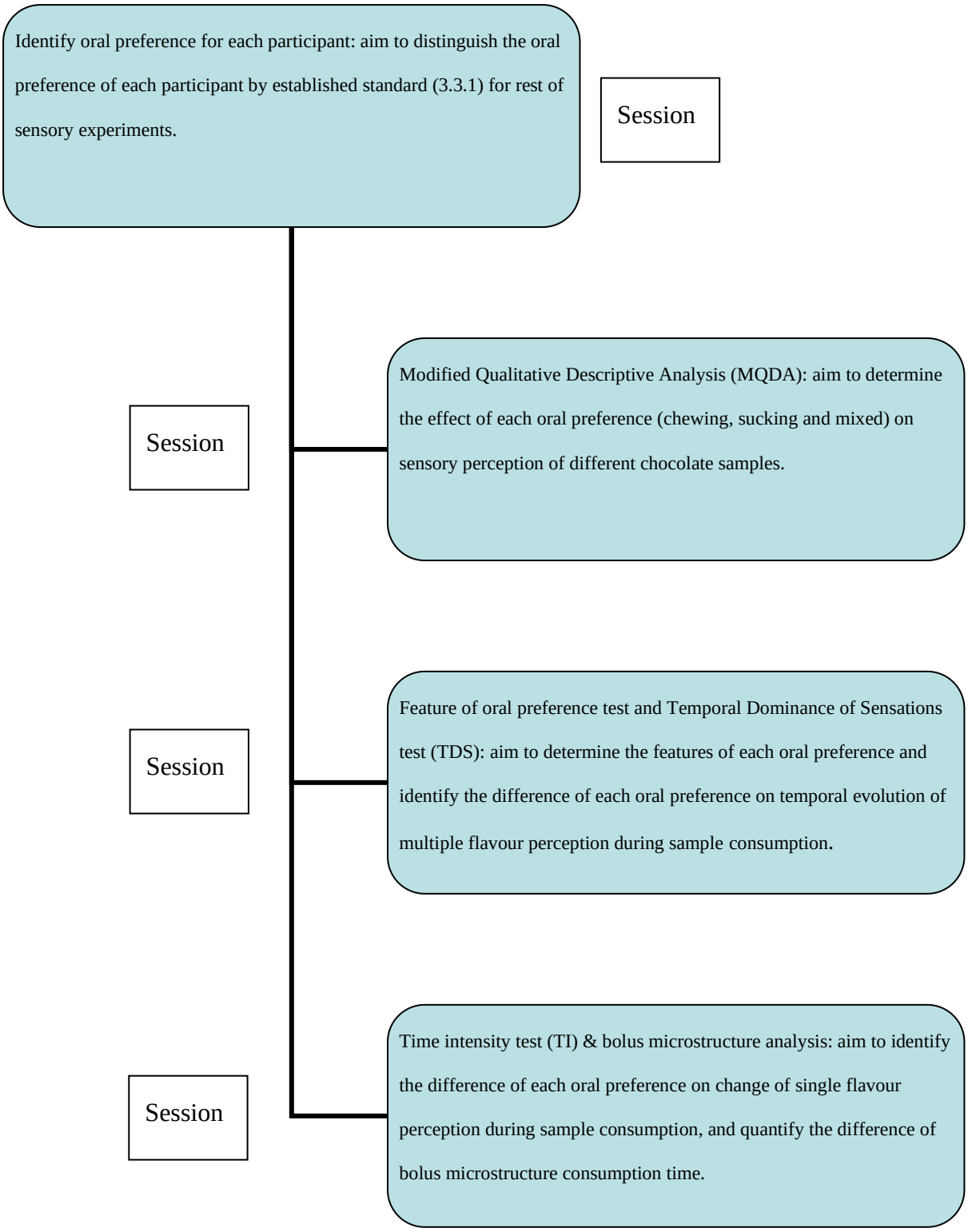


Figure 3.4: Experimental design for each sensory session.

• Session 1: 100 participants (50 females, 50 males, age 20-30, BMI: 22.4±2.8) for sensory analysis (initial analysis of chewing behaviour and flavour perception). The number in each

OP group was similar (33±1), any group with insufficient members would be filled by new participants, and any supernumerary members were not recorded during MQDA test (on session 2). • Session 2: 60 participants (20 of each OP group, 30 females, 30 males, age 23.8±3.6, BMI: 22.1±2.3), selected from the initial 100, based on chewing behaviour, for detailed analysis of chewing behaviour.

• Session 3: 60 participants (20 of each OP group, 30 females, 30 males, age 24.3±3.9, BMI: 22.5±2.6) for Temporal Dominance of Sensation (TDS) testing (Session 2 panel).

• Session 4: 60 participants (20 of each OP group, 30 females, 30 males, age 24.1±4.0, BMI: 22.6±2.2) for Time Intensity (TI) testing and 30 participants (10 of each OP group, 15 females, 15 males, age 24.6±4.1, BMI: 22.2±2.7) for bolus collection.

3.3.2 Modified Qualitative Descriptive Analysis (MQDA)

The MQDA test is a modified QDA test, which relies on untrained participants to carry out Qualitative Descriptive Analysis. In addition, a base-line (Figure 3.5) was given to participants in order to limit the diversity between individuals. A total of 100 untrained participants were recruited for the MQDA test (50 females, 50 males; age 25±5). Chocolate samples (weighing 5±0.3 g) either with or without flavouring (weighing 5±0.3 g) were served to participants in a randomised sequence. Samples were identified using 3-digit codes, offering no indication to the participants about their nature. A questionnaire was used to allow participants to rate intensity on a 10 cm, unstructured line scale with anchors from “low” to “high” for six attributes. A further line was used to rank preference from “dislike” to “like very much”, and this evaluation would be carried out after the six descriptive tests. The attributes in this session were:

Cocoa Flavour	Persistence	Off-flavour intensity
Smoothness	Thickness	Snapping

Preference (acceptability) of chocolate flavour (off-flavour chocolate)

The term “off-flavour” was used to represent any added flavour so as not to influence perception by specifying “ginger” or “menthol”. In addition, texture (Table 3.3) and flavouring or functional ingredients (ginger powder and menthol crystals) were provided to participants before MQDA test in order to give them a clear understanding of each parameter in the questionnaire.

Table 3.3 Description of texture attributes

Texture attributes	Description
Smoothness	The perception of fineness when chocolate sample rolling between the tongue and palate.
Thickness	The perception of chocolate viscosity when chocolate sample melted in the mouth.
Snapping	Usually described as the sound and feeling of chocolate breaking

The texture and flavour intensity sensations were significantly influenced by individual preference, and all participants were untrained (Taiti et al., 2017; Johansson, et al., 1999). In order to reduce the chance of inaccuracy in intensity sensation, and allow the participants to focus on an objective determination, establishing a mid-point (standard chocolate intensity sensation) was necessary. Standard chocolate (participants were aware of what it was) was

served to all participants, and they were then asked to mark a mid-point on a line scale before the MQDA test (The example below, Figure 3.5).

Smoothness

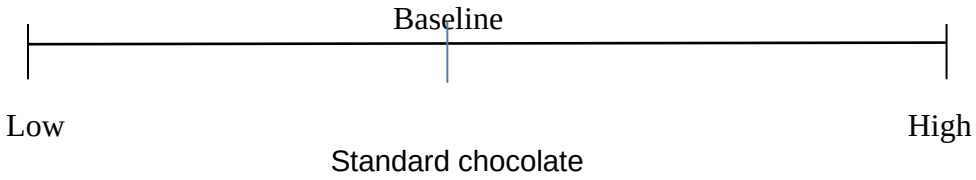


Figure 3.5 Establishment of a mid-point (base perception) on line scale

After this, five random samples (each piece weighing 5 ±0.3 g, either with or without added flavour) were served to participants. They were asked to eat the samples using their normal eating style and mark down the perceived intensities on the line scales. A two to three-minute break was given between samples, and a glass (250ml) of water was provided for rinsing their mouths prior to eating the next sample. Each sample was tested in duplicate. The total consumption time of a session was two hours.

3.4 Oral preference test and TDS (Temporal Dominance of Sensations) test

3.4.1 Participants and chocolate samples

60 participants (age: 25±5 years; 30 females and 30 males) were recruited from 100 untrained participants who attended a previous quantity descriptive analysis (MQDA) test session. The participants were grouped by OP into three categories (Chewing preference: CP, sucking preference: SP and mixed preference: MP; 20 of each OP group) in line with previous

research. The five types of chocolate samples (weighing 5 ±0.3 g; having a hemisphere a 26 mm diameter) tested in this study are presented, with or without added flavour ingredients.

3.4.2 Oral preference (OP) test

Five chocolate samples with or without flavouring ingredients were served to participants in a randomised order. Samples were identified by using 3-digit codes, and samples were stored at 20 ˚C before each session. For each participant, four parameters were recorded during this session, being; number of chews before first swallow, total eating time, total number of chews, time of first swallow, with chewing rate being calculated by number of chews over chewing time (s). Participants were asked to raise their hand when they had swallowed. The eating parameters were recorded on video, and data were collected through visual analysis of the video. During this research, all participants were asked to eat samples using their natural OP. A 2–3-minute break was given between samples and a glass (250 ml) of water was provided to rinse their mouths prior to the next sample. The session time was during a period between 10:00-11:30 a.m., after breakfast.

3.4.3 TDS (Temporal Dominance of Sensations) test

For this test, the same number of participants was recruited as in the oral preference test (Chapter 3). In the TDS test, five flavour parameters (sweetness, bitterness, sourness, cocoa flavour and off-flavour i.e. ginger or menthol) were selected. A training session was conducted with all participants in order to ensure they understood each flavour definition and the operation of the TDS software. In terms of TDS software training, they were told that the most intense flavour at a point in time during consumption was defined as the dominant

flavour, and that the dominant sensation may change over time. Each flavour parameter could be chosen more than once, and participants only needed to choose the flavour they perceived during sample consumption. Since chocolate samples with high flavour intensity (HM & HG) may bring a strong impression to participants, which might subsequently alter behaviour, only one training session was held before the formal test. In addition, there was a third repetition if there were significant differences on foregoing two repetitions. TDS software (Morgenstern©, The New Zealand Institute for Plant & Food Research Limited) was used to carry out the TDS test and data collection. The operation procedure followed the description by Pineau et al. (2009). During the test, participants placed the sample in their mouth and then clicked the start button on the computer screen. They could start to choose the dominant flavour when they perceived it and change it when other dominant flavours occurred. They clicked the end button when they had finished consumption of the sample. During this research, all participants were asked to eat the samples using their natural OP. Participants fasted for at least two hours prior to the TDS session and it took approximately 20 – 30 minutes to complete each session. A 2 – 3 min break was given between samples and a glass (250 ml) of water was provided to rinse their mouths prior to the next sample. The testing session was between 10:00-11:30 a.m., after breakfast.

3.4.4 TDS Measurement

The computation of TDS curves followed Pineau et al.'s (2009) method. The percentage dominance was according to selection of a single attribute (the flavour attribute in this study) of total number of times (participants x replications) divided by the panel level (participants x replications). All of the attributes are displayed on the same TDS graph, and the curve only represents selection of a particular attribute rather than intensity. Two lines are displayed in

the TDS graph, which are “chance level” and “significance level.” The “chance level” (Ps) represents the rate of dominance an attribute can be selected by chance. The “significance level” (P_0) represents the minimum value at which a value is considered significant (Souza et al. 2013).

3.5 Time Intensity (TI) test

The time-intensity technique allows the measurement of a single attribute's intensity, and any change in panellists’ perception of a specific attribute can be observed during consumption of the food product (Chung et al., 2003; Piggott, 2000). In this session, the “off-flavour” (ginger or menthol flavour) of each sample would be the specific attribute to measure the change in flavour intensity for different OPs. Sixty participants (age: 25 ±5 years; 30 females and 30 males;) were recruited from 100 untrained participants to attend the time intensity (TI) test, and 30 participants were recruited from 100 untrained participants to attend a test on the microstructure of the chocolate bolus. All participants were grouped by OP according to Section 3.3.1. Two different sensory sessions (TI and test of bolus structure) were carried out independently and on different days

3.5.1 Time intensity (TI) test

In the time-intensity (TI) test, 60 participants were recruited and grouped by OP into three categories (Chewing preference: CP, sucking preference: SP and mixed preference: MP) in line with previous research (3.3.1). SensoMaker sensory analysis software (Version 1.91, Nunes & Pinheiro, 2012) was used to carry out this test and for data collection. Two flavour parameters (ginger and menthol) with high and low intensity were selected for the TI test.

Before starting, a training session was conducted with all participants in order to ensure they understood the operation of the TI software (SensoMaker) and the session's procedure. Participants were allowed to ask any questions in relation to the test. As with the TDS testing only one training session was held before formal test, and an additional repetition was conducted if required..

During the test, four samples (each piece weighing 5 ±0.3 g, diameter of 26 mm and having different flavours and intensities: 0.5% or 2.5% ginger; 0.1% or 0.5% menthol) were served to the participants. They placed the sample in their mouth and then clicked the start button on the computer screen; they could then consume the sample using their natural OP. They clicked the end button when they had finished consumption of the sample.

During this research, all participants were asked to eat the samples using their natural OP. The TI software provided a flavour intensity scale from 0 to 10. Participants were able to continuously rate the flavour intensity as they perceived it using the arrow keys on the keyboard. Two hundred seconds of consumption time were provided to all participants, and participants clicked the stop button after their final swallow. In this test, the participants' flavour perception was recorded until the last swallow, the aftertaste was not considered. Four TI parameters were recorded:

- I_{max} (maximum intensity by participant's perception)
- Ti_{max} (time at which maximum intensity was perceived)
- T_{tot} (total duration time of the flavour perception)
- Area (total intensity of flavour)

Participants fasted for at least two hours prior to the TI session, and it took approximately 30 – 40 minutes to complete each session.

4 Effect of flavouring ingredients on sensory perception

Chocolate has specific rheological behaviour during oral processing that delivers its distinct sensory characteristics. When incorporating flavouring ingredients into chocolate, these properties must be maintained to meet consumer expectation. The results of this chapter reflected the influence of different ingredients (water soluble and fat soluble) on chocolate texture, and displayed the different in flavour perception by different oral preference.

4.1 Introduction

Functional foods have become a new product category that offers improvements in targeted physiological functions to consumers (Young, 2000). In recent years, consumer requirements for food products have changed noticeably. An increasing number of people believe that foods directly contribute to their health, over and above simple nutrition (Mollet & Rowland, 2002). Current market opportunities exist for food products that not only provide energy and essential nutrients for people, but offer improved mental or physical well-being.

Chocolate is the most popular candy product in the world due to its delightful mouthfeel and unique taste. In recent years, chocolate producers have been influenced by increased demands for healthy or functional products, especially low fat and low sugar chocolates (Belščˇak-Cvitanovic et al., 2015). Many manufacturers are considering the incorporation of functional ingredients into chocolate formulations (Table 4.1), to satisfy consumer demands from both a health and sensory perspective.

Table 4.1 Examples of functional chocolate in the current market

Name	Details	Claimed Functionality	Supplier
Slim chocolate	60% cocoa, no added sugar (inulin replacement)	Low glycaemic index; High proportion of dietary fibre as inulin	mcePharma
Probiotic chocolate	Active ingredient: Lactic acid bacteria	Maintain bowel health	Lotte
Omega-3 chocolate	Omega-3 added to chocolate	Reduce total and LDL; cholesterol and increase HDL cholesterol	Natra
Relaxation chocolate	herbal extracts from valerian and melissa	Valerian positively affects mental health;	mcePharma

Recent studies on the development of new chocolate formulations with a healthier profile (De Pelsmaeker et al., 2015) indicate some issues with these products often exhibiting inferior sensory properties, especially mouthfeel and flavour, which restrict their wider acceptance by consumers.

The sensory evaluation of chocolate comprises principally two aspects, which are taste (flavour) and texture (mouthfeel) (Gaonkar et al., 2014). In addition, the overall flavour perception was dominated by tastes (detect sensation of bitter, umami, sweet, salty and sour by taste bud on the tongue) and odors (perceived by olfactory receptor from air) (Salles et al., 2011). The quality of food texture is vital as it affects the physical behaviour of food during the processing, storage and consumption stages (Gonçalves & Lannes, 2010). Chocolate hardness is a distinct indicator of quality and predicts consumer acceptability in sensory evaluation (Beckett, 2003; Keogh et al., 2003). It is determined by particle dispersion (such as cocoa solids, sugar, milk solids and other added ingredient solids) and the crystallized fat

phase. Rheological properties of molten chocolate are also important attributes in sensory evaluation. The rheology of chocolate is usually denoted by two parameters which are apparent viscosity and yield stress. As Gonçalves and Lannes (2010) mentioned, chocolate's sensory character, is determined by plastic viscosity. Yield stress is a material property representing minimum shear stress at the beginning of flow, or the transition from elastic to viscous deformation (Afoakwa et al., 2008). When chocolate melts in the mouth, the texture relies heavily on the suspension viscosity, which is its resistance to flow (Beckett, 2008). Chocolate's mouthfeel is largely determined by the particulate phase, and its rheology. Silva et al., (2013) suggested that the perception of texture in chocolate depends mainly on the change of microstructure during oral processing. When chocolate melts and mixes with saliva in the mouth, the perception of textures including "smoothness" and "mouth-coating", will be sensed (Afoakwa et al., 2007).

Van Ruth and Roozen (2000) claimed that the continuous destruction of the food matrix in oral processing was an important factor in flavour release. Chocolate flavour discernment is time-dependent as its structure changes from a semi-solid to a liquid phase during oral process. Chocolate flavour release is influenced by composition, particle size and the intensity of other ingredients in the chocolate. When chocolate is consumed, the flavour compounds are released from the suspension and volatilise in the mouth. After this, the sensations of texture and taste will gradually develop (Beckett et al., 2017). In addition, the sensation of taste hinges on the sequence of flavour compounds in contact with the tongue, and the release rate of these compounds is related to rheological properties (Afoakwa et al., 2009; Gonçalves & Lannes, 2010; Beckett, 2009). Although an increase in chocolate viscosity will lengthen the persistence of flavour compounds in the mouth, an over-viscous chocolate will have an adverse effect on the final texture (Beckett et al., 2017; Servais et al., 2003). Moreover, Afoakwa et al., (2009) reported that different levels of fat content in

chocolate lead to flavour release differences depending on whether the flavour compounds are lipophobic or lipophilic.

As more and more consumers are interested in confectionery products with health claims, research into chocolate with special functions related to health benefits will be of interest. Chocolate has specific rheological behaviour and sensory properties which are determined by ingredients and manufacturing processes. Therefore, the delivery of various flavours and functional supplements are prone to limitations and challenges. The objective of this study was to explore how to develop newchocolate formulations and investigate the effect of flavouring ingredients on chocolate microstructure and flavour release when different eating patterns are employed during oral processing.

4.2 : Materials and Methods

4.2.1 Materials

Basic chocolate samples were made from 72% dark chocolate with added flavouring ingredients to represent lipophilic and hydrophilic flavouring ingredients. The chocolate sample were 72% cocoa (Chocolate Brown, Warkworth, New Zealand) and the nutritional details are given in Table 3.1. Ginger powder (100% natural ginger, water solubility of 87.3% as reported by manufacturer) was used to represent hydrophilic ingredients and peppermint (99% Menthol crystals, over 38% solubility in fatty oil and over 26% solubility in cocoa butter as reported by manufacturer) was used to represent lipophilic ingredients. Both ingredients were obtained from NutriHerb BioTech Company (Nanjing, China).

4.2.2 Sample preparation

All dark chocolate was melted in a 60 °C water bath for 1 hour (WB-11, WiseBath, South Korea). Ginger powder and menthol crystals in different concentrations (LG: 0.5%, HG: 2.5%, LG: 0.1%, HG: 0.5%) were added gradually to molten chocolate and the mixture was stirred continuously at 200 rpm using a mechanical stirrer (RW 20 digital, IKA-works Inc. NC, USA) for 1 hour to ensure all added ingredients were distributed uniformly in the molten chocolate. Then the mixture was transferred to a tempering machine (Revolation 2B, ChocoVision, USA) at 42.2°. Following the tempering machine process, the temperature was lowered to 32.2 °C and the seed mass (cocoa butter) was added. After that the molten liquor was continuously cooled to 29.4 °C. Finally, the molten liquor was reheated to 31.5 °C. The total time spent was 20 minutes. After this, the tempered chocolate liquor was decanted into a hemispherical mould for subsequent MQDA testing (diameter: 26 mm), a cylindrical mould for subsequent hardness testing (diameter 45 mm; depth 12 mm) and a rectangular mould for subsequent fracture testing (depth 60 mm, width 10 mm and length 70 mm). Moulds were placed into a food-grade and constant temperature refrigerator (4 °C for 2 hours) before demoulding. All samples were demoulded and transferred to a sealed container and stored at 20 °C for two weeks before being analysed.

4.2.3 Modified quantitative descriptive analysis (MQDA) test:

Before the sensory session, all subjects were be classified into three groups by different OP as referred to in Section 3.3.1. After that, 100 subjects were recruited for the MQDA test (50 females, 50 males; age 25±5 years) Standard chocolate (participants were aware of what it was) was served to all participants, and they were then asked to mark a mid-point on a line scale before the MQDA test. After that, five random samples (each piece weighing 5±0.3 g,

either with or without added flavour) were served to participants. Chocolate samples (weighing 5±0.3 g) either with or without flavouring (weighting 5±0.3 g) were served to subjects in a randomised sequence. Samples were identified using 3-digit codes offering no indication to the participants about their nature. Since all participants were untrained, fewer attributes (six for intensity and one for liking) were tested in order to avoid loss of concentration and confusion. A questionnaire (Appendix A) was used to allow participants to rate intensity on a 10 cm, unstructured line scale with anchors from "low" to "high" for six attributes, which are Cocoa flavour, Off-flavour intensity, Persistence of off-flavour, Smoothness, Thickness and Snapping. They were asked to eat the samples using their normal eating style and mark down the perceived intensities on line scales. A two to three-minute break was given between samples, and a glass (250 ml) of water was provided for rinsing their mouths prior to eating the next sample. Each sample was tested in duplicate. The total consumption time of a session was two hours. The testing time selected was between 10:00-11:30 a.m., after breakfast. All sensory trials were conducted with approval from the University of Auckland Human Participant Ethics Committee, reference UAHPEC 021267

4.2.4 Instrumental measurements

4.2.4.1 Hardness

The hardness of the five cylindrical moulded chocolates (diameter 45 mm and depth 12 mm) was measured with a "texture analyser" (TA-XT2, Stable Micro Systems, Haslemere, England) at room temperature (20 °C) with a load cell of 50 N and a 2 mm stainless steel needle probe. The cross-head speed was 1 mm s^{-1} as a pre-test speed and 2 mm s^{-1} as the test speed over a distance of 5 mm depth. The maximum penetration force (N) through the samples was indicative of hardness (ten replications were performed for each sample).

4.2.5 Differential Scanning Calorimetry (DSC)

The melting properties of the chocolates were analysed using a Differential Scanning Calorimeter (Model 60 DSC, Shimadzu, Columbia, USA). The DSC measurements were performed following Aidoo's (2017) procedure with minimal modification. Approximately 5mg samples, sliced from the chocolate's surface were sealed in an aluminium pan. The samples were heated from 10 °C to 40 °C at a rate of 2 °C/min. The onset temperature (T_{onset}), end temperature (T_{endset}) and peak temperature (T_{peak}) values were calculated using A-60WS Thermal Analyser software. Each test was conducted in triplicate.

4.2.6 Viscosity

The flow properties of the molten chocolate were measured using an bob (25 mm diameter) and cup (27.5 mm diameter) geometry (AR-G2 Rheometer, TA Instruments, Delaware, US). All chocolate samples were incubated at 40 °C for at least one hour for complete melting, and then approximately 30 g of molten sample was weighed into the cup and placed into the rheometer. According to the IOCCC (2000) method, the samples were pre-sheared at a rate of $5s^{-1}$ at 40 °C for 15 minutes before starting measurements. After that, shear stress was measured as a function of increasing shear rate from 5 s^{-1} to 50 s^{-1} for the ramp-up stage over 180 s, and holding the shear rate of 50 for 1 min. Thereafter, shear stress was decreased from 50 s^{-1} to 5 s^{-1} for the ramp-down stage. The Casson model is suggested by the IOCCC and is widely used to determine chocolate rheology behaviour (Bouzas & Brown, 1995). In this study, both the apparent viscosity and yield stress of molten chocolate were characterised by the Casson model as Equation (1) shows:

$$\tau^{0.5} = \tau_0^{0.5} + \eta_{pl}^{0.5} * \gamma^{0.5} \qquad (1)$$

Where τ denotes shear stress, τ_0 is yield stress, η_{pl} is plastic viscosity and γ is shear rate. Each sample was tested in triplicate.

4.2.7 Statistical analysis

Data analysis was performed using SPSS version 20 (IBM Corporation, USA). Analysis of variance (ANOVA) was undertaken to evaluate hardness, apparent viscosity, yield stress and melting point of each samples followed by Tukey's multiple comparison test (at a level of significance of $p < 0.05$). Each attribute of MQDA test was analysed using a fixed effect model, for testing the effect of subjects with different oral preference on sample perception (oral preference x sample) and differences between samples.

4.3 Results and discussion

4.3.1 Effect of flavouring ingredients on hardness of chocolate samples

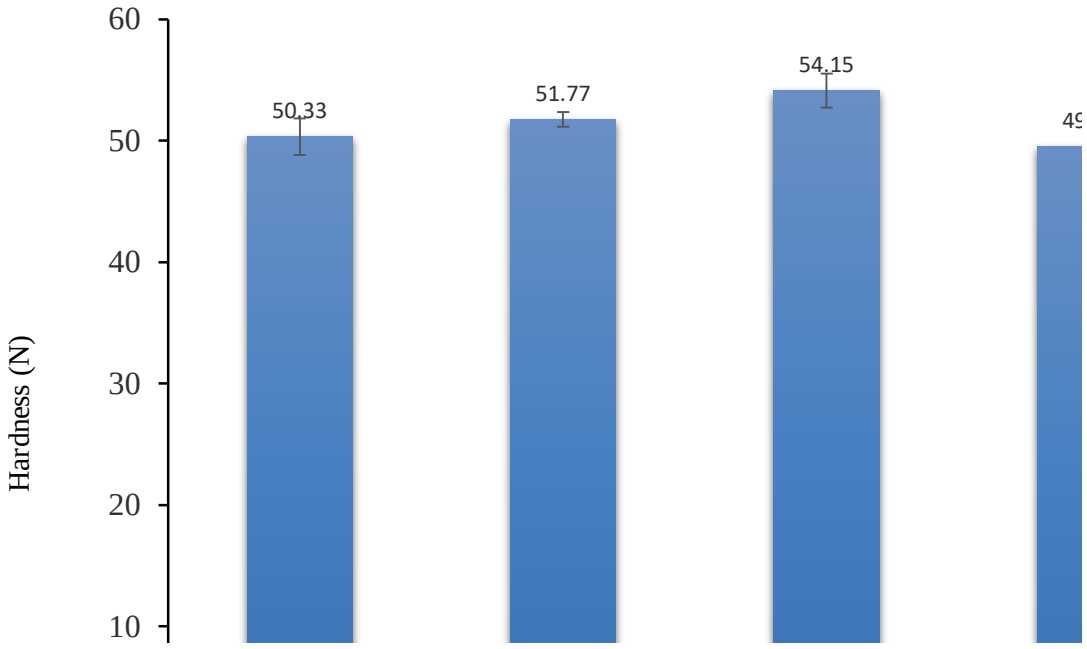


Figure 4.1: Effect on hardness of flavour ingredients in different concentrations

Table 4.2 Hardness of chocolate samples with different flavour ingredients

SC	LG	HG	LM	HM
50.33 ± 1.12^{b}	51.77 ± 0.97^{b}	54.15 ± 1.09^{a}	49.52 ± 0.92^{b}	45.77 ± 1.05^{c}

Note: Superscript letters in each row donate significant differences at $P < 0.05$

Figure 4.1 and Table 4.2 illustrates the influence of different flavour ingredients, in different concentrations, on the hardness of the chocolate samples in this study. Chocolate containing low ginger concentration (0.5%) exhibited no significant difference ($P>0.05$) to standard chocolate in regard to hardness. At a higher concentration (2.5%), ginger flavoured chocolate is harder than standard ($P<0.05$). According to Afoakwa et al., (2008), hardness of chocolate is relative to the strength of interparticle interaction. In this study, extra ginger powder added to chocolate lowered the fat content of the chocolate. As Do et al. (2007) noted, an increase in hardness mainly depends on the reduction of fat content in chocolate as a higher particle interaction would lead to higher resistance to breakage.

The chocolate containing low (0.1%) menthol concentration showed no significant difference ($P>0.05$) to standard chocolate in hardness, but exhibited lower hardness at higher (0.5%) concentration ($P<0.05$). This finding is consistent with Do et al.'s (2008) observations of the effect of added limonene on chocolate hardness, where chocolate containing limonene had lower hardness. The softening effect of menthol on chocolate can be explained by the change of solid fat content (SFC). The cocoa butter in chocolate is diluted when menthol (the main component in peppermint) is added to chocolate, which results in a decrease of solid fat content. Similar results were also reported by Liang and Hartel (2004); increased free milk fat

concentration caused a decrease in hardness of chocolate due to a reduction in the solid fat content. In addition, Keogh et al., (2003) and Lee et al. (2009) stated that cocoa butter substitution may affect the process of chocolate tempering, resulting in a change in hardness.

4.3.2 Effect of flavouring ingredients on viscosity of molten chocolate samples

Figure 4.2 illustrates the influence of flavouring ingredients with different concentrations on the rheology of the chocolate samples in this study. Chocolate containing low added ginger (0.5%) and low added menthol (0.1%) concentrations had no significant difference ($P>0.05$) to standard chocolate, in either Casson viscosity or yield stress (Table 4.3). Chocolate containing higher added menthol (0.5%) concentration showed lower Casson viscosity and yield stress than standard chocolate. This viscosity reduction of menthol chocolate can be explained by the change in the continuous phase. According to Krieger & Dougherty's (1959) model, the relative viscosity of a particulate suspension (ηr) mainly depends on the particle volume fraction φ, as Equation (2) shows. In the model, φ_{max} is the maximum solid fraction in the suspension and B is the Einstein coefficient (Pabst, 2004; Pabst et al., 2006).

$$\eta_r = (1 - \varphi/\varphi_m)^{-B\varphi m} \qquad (2)$$

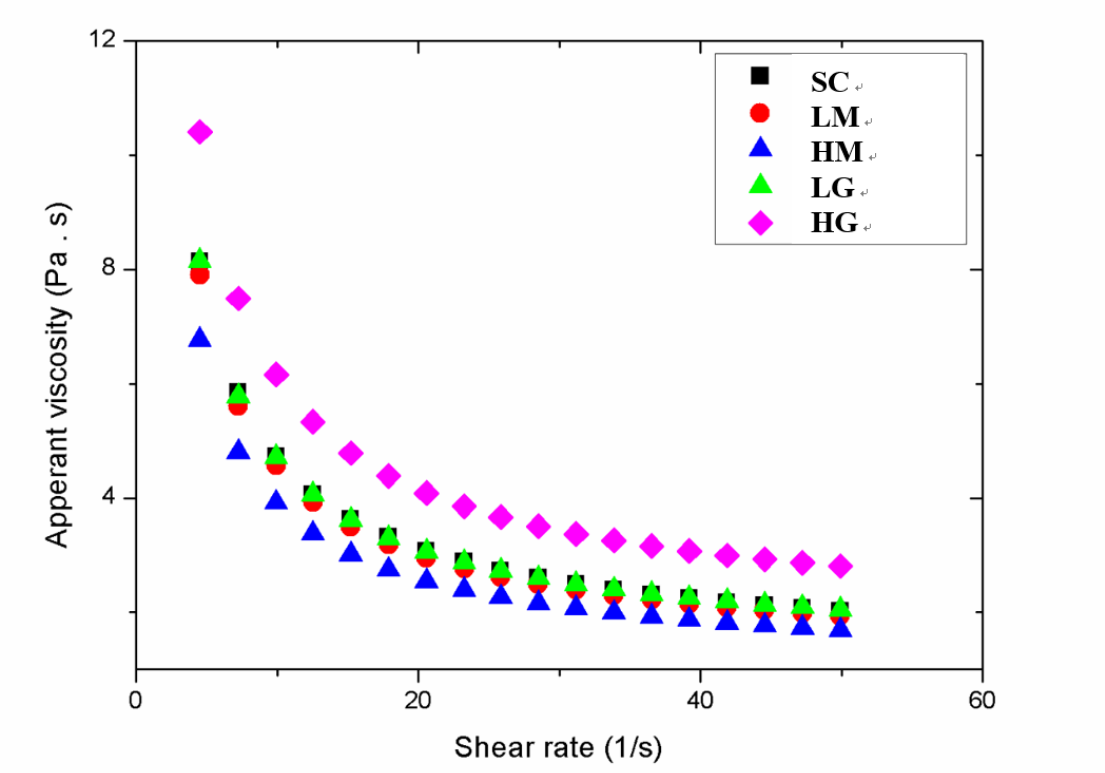


Figure 4.2 Apparent viscosity of molten chocolate with different concentrations of flavouring ingredients

In this study, the baseline chocolate had the same continuous volume fraction and particle volume fraction. Extra menthol added into the molten chocolate increased the continuous volume as menthol melted and mixed with cocoa butter at 40°C. The presence of menthol liquid would interact with the cocoa butter in molten chocolate, which would dilute the volume of the dispersed phase, resulting in a significant reduction of Casson viscosity and yield stress.

Table 4.3 Casson viscosity and yield stress of molten chocolate for different concentrations of flavouring ingredients

Formulation	Casson viscosity (Pa.s)	Casson yield stress (Pa)
Standard (SC)	0.62±0.02[b]	19.06±0.53[b]

Low ginger (LG)	0.63±0.04[b]	19.58±0.61[b]
High ginger (HG)	0.98±0.07[a]	22.95±0.78[a]
Low menthol (LM)	0.60±0.03[b]	18.79±0.47[b]
High menthol (HM)	0.51±0.03[c]	15.92±0.35[c]

Note: Superscript letters in each column denote significant differences at P < 0.05

Particle size distribution, high solid volume and interaction of particles have an influence on chocolate rheological properties (Bouzas and Brown, 1995). Servais et al. (2002) stated that increasing solids concentration led to higher viscosity of suspensions. In this study, extra ginger powder added to the molten chocolate led to higher solids volume (amount of small particles) and inter-particle interactions, resulting in a significant increase in Casson viscosity and yield stress (P > 0.05).

4.3.3 Effect of flavouring ingredients on melting properties of chocolate samples

Table 4.4: Average chocolate melting point by DSC

Formulation	Melting point (T_{peak})
Standard	33.27±0.23[a]
Low ginger (LG)	33.15±0.43[a]

High ginger (HG)	32.85±0.32[a]
Low menthol (LM)	33.41±0.46[a]
High menthol (HM)	33.02±0.15[a]

Note: Superscript letters in each column denote significant differences at P < 0.05

Melting point can be described as the temperature (T_{peak}) in a DSC test (Loisel et al., 1998; Talbot, 1999). The average melting point for each chocolate sample is shown in Table 4.4. The average melting point (T_{peak}) occurred between 32.85-33.41°C for each sample, and the observed difference in melting points have no significant difference (P< 0.05). According to the research of Beckett (2000), chocolate tempering is usually performed to obtain form V which has a melting temperature of around 32-34°C and provides good snap, the desired glossy appearance and a long shelf life. This indicates that all of the samples, notwithstanding the concertation of ingredients, are well-tempered chocolate, and the extra ingredients used in this research had no effect on dark chocolate's melting behaviour.

4.3.4 Effect of flavouring ingredients on sensory perception

4.3.4.1 Sensory properties

Table 4.5 The mean texture and flavour intensity score (1-10) of each chocolate sample by different OP groups

Oral preference groups	Cocoa flavour	Persistence of flavour	Off-flavour intensity	Smoothness	Thickness	Snapping
CP						
LG	2.6±0.8[b]	4.1±1.2[c]	3.7±1.7[c]	5.1±1.7[a]	4.8±1.0[a]	5.0±0.3[a]
HG	2.3±0.6[b]	6.3±1.8[b]	6.8±1.7[b]	4.8±1.7[a]	4.9±1.1[a]	5.1±0.4[a]

Oral preference groups	Cocoa flavour	Persistence of flavour	Off-flavour intensity	Smoothness	Thickness	Snapping
LM	2.4±0.6^{b}	6.5±1.2^{b}	6.2±1.8^{b}	5.2±1.5^{a}	5.0±1.4^{a}	4.9±0.2^{a}
HM	2.2±0.5^{b}	8.6±0.8^{a}	8.5±1^{a}	5.2±1.7^{a}	5.2±1.0^{a}	5.0±0.2^{a}
SC	5^{a}			5^{a}	5^{a}	5^{a}
MP						
LG	4.5±1.0^{b}	4.2±1.9^{c}	3.6±1.6^{c}	4.8±1.3^{a}	4.7±1.8^{a}	5.0±0.4^{a}
HG	3.5±0.9^{c}	6.4±1.0^{b}	6.3±1.3^{b}	4.6±1.1^{a}	4.5±1.0^{a}	5.1±0.5^{a}
LM	3.6±1.0^{c}	5.5±1.4^{b}	5.8±1.6^{b}	5.0±1.3^{a}	4.9±1.2^{a}	5.0±0.3^{a}
HM	2.3±1.5^{d}	7.8±1.4^{a}	8.2±0.8^{a}	5.0±1.6^{a}	4.9±1.9^{a}	5.0±0.2^{a}
SC	5^{a}			5^{a}	5^{a}	5^{a}
SP						
LG	4.2±0.6^{b}	4.4±1.6^{c}	3.7±1.4^{c}	5.1±1.7^{a}	5.2±1.4^{a}	5.0±0.2^{a}
HG	3.2±1.2^{c}	6.2±1.9^{b}	6.0±1.5^{b}	4.8±1.7^{a}	4.9±1.4^{a}	5.1±0.3^{a}
LM	3.3±1.2^{c}	6.1±1.5^{b}	5.5±1.8^{b}	5.2±1.0^{a}	5.3±1.2^{a}	5.0±0.3^{a}
HM	2.3±1.1^{d}	7.9±1.4^{a}	8.2±0.8^{a}	5.0±1.5^{a}	4.9±1.7^{a}	5.0±0.2^{a}
SC	5^{a}			5^{a}	5^{a}	5^{a}

Note: Superscript letters in each column denote significant differences (between samples for a specific OP) at P < 0.05

Table 4.5 shows the mean texture and flavour score for each chocolate sample for each eating style preference. It was clear that participants could not clearly distinguish the difference

between samples ($P<0.05$) in terms of *texture*. The influence of flavour variety and intensity on texture perception during oral processing has been referred to in many studies (Lethuaut et al., 2003; Saint-Eve et al., 2004; Tournier et al., 2009). Saint-Eve et al. (2004) found that yoghurts with butter and coconut aroma resulted in a higher intensity of thickness than yoghurt with apple aroma. However, Cayot et al. (1998) and Kälviäinen et al. (2000) observed that thickness and firmness perception were not significantly changed by adding different flavours to food. Tournier et al. (2007) also referred to the impact of flavour on texture perception, mainly in the context of thickness perception. In addition, according to the study of detectability of perceived texture in food gels by Santagiuliana et al. (2018), people were not sensitive to low-level changes in texture. Thereby, a small quantity of added ingredients (ginger and menthol) in this study did not affect the participants' perception of the samples' texture.

However, the results of the MQDA testing for *flavour* attributes, participants with different OP groups could clearly distinguish the intensity of the added flavour ingredients between low and high concentrations. It can be seen from Table 4.5, the HM chocolate presented the highest "off-flavour" intensity (in this case menthol) ($P<0.05$), and the LG chocolate presented the lowest "off-flavour" intensity (in this case ginger) and persistence time ($P<0.05$) for all OP groups. Compared to standard chocolate, chocolate made with low ginger, high ginger, low menthol and high menthol concentrations was perceived as having significantly different cocoa flavour intensity by all OP groups. In addition, chocolate made with the low ginger concentration presented the highest intensity of cocoa flavour, and chocolate made with a high menthol concentration presented the lowest cocoa flavour intensity for all OP groups. However, there was no significant ($P>0.05$) difference in cocoa flavour between chocolate samples for the CP group.

Table 4.6 Flavour preference for each sample

		Low Ginger (LG)	High Ginger (HG)	Low Menthol (LM)	High Menthol (HM)
Sample preference on flavour	**CP**	8.2 ± 1.3^{aA}	4.6 ± 0.8^{aB}	5.1 ± 1.1^{aB}	2.1 ± 0.4^{aC}
	SP	7.7 ± 0.8^{aA}	5.2 ± 1.2^{aB}	6.0 ± 1.7^{aB}	2.1 ± 0.6^{aC}
	MP	7.6 ± 1.1^{aA}	5.1 ± 1.0^{aB}	5.6 ± 1.4^{aB}	2.3 ± 0.3^{aC}

Note: Superscript letters (lower case) in each column denote significant differences (oral preference x *sample) at P < 0.05; Superscript letters (upper case) in each row donate significant differences (between samples for a specific OP) at P < 0.05.*

According to off-flavour intensity of each sample (Table 4.5), the flavour intensity of each sample they perceived can be clearly distinguished into three groups: low off-flavour perception (LG), medium off-flavour perception (HG and LM) and high off-flavour perception (HM). In addition, the preference of samples on flavour (Table 4.6) presented highest acceptability of low off-flavour perception and lowest acceptability of high off-flavour perception for all participants with different OP groups.

Table 4.7 Mean flavour score of each chocolate sample by different OP groups

Low Ginger (LG)	Cocoa flavour	Persistence of flavour	Off-flavour intensity
CP	2.6 ± 1.4^{b}	4.1 ± 1.2^{a}	3.7 ± 1.7^{a}
SP	4.2 ± 0.6^{a}	4.4 ± 1.6^{a}	3.7 ± 1.4^{a}
MP	4.5 ± 1.0^{a}	4.2 ± 1.9^{a}	3.6 ± 1.6^{a}

Low Ginger (LG)	Cocoa flavour	Persistence of flavour	Off-flavour intensity
High Ginger (HG)	**Cocoa flavour**	**Persistence of flavour**	**Off-flavour intensity**
CP	2.3±1.0[b]	6.3±1.8[a]	6.8±1.7[a]
SP	3.2±1.2[a]	6.2±1.9[a]	6.3±1.5[a]
MP	3.5±0.9[a]	6.4±1.0[a]	6.0±1.3[a]
Low menthol (LM)	**Cocoa flavour**	**Persistence of flavour**	**Off-flavour intensity**
CP	2.4±0.6[b]	6.5±1.2[a]	6.2±1.8[a]
SP	3.3±1.2[a]	6.1±1.5[a]	5.8±1.8[a]
MP	3.6±1.0[a]	5.5±1.4[a]	5.5±1.8[a]
High menthol (HM)	**Cocoa flavour**	**Persistence of flavour**	**Off-flavour intensity**
CP	2.2±1.2[a]	8.6±0.8[a]	8.5±1.0[a]
SP	2.3±1.1[a]	7.9±1.4[a]	8.2±0.8[a]
MP	2.3±1.5[a]	7.8±1.4[a]	8.2±0.8[a]

Note: Superscript letters in each column denote significant differences (oral preference x sample) at $P < 0.05$

In addition, participants in the different OP groups presented similar perception of flavour intensity (off-flavour) and persistence when they consumed same chocolate samples. However, the CP group presented a significant difference from SP and MP groups in cocoa flavour intensity. As Table 4.7 shows, the perception of cocoa flavour by the mixed and

sucking group showed no significant difference when they consumed LG, HG and LM chocolate, but the perception of the CP group in cocoa flavour intensity was significantly lower than the other OP groups. During oral processing, flavours in chocolate are released into the saliva. Non-volatile flavors (such as sweetness and bitterness) would be perceived by taste buds on the tongue, and volatile flavours (such as menthol, ginger and cocoa flavours) need to be transported by airflow in the mouth and then pass through the throat to the olfactory receptors in the nose, where they are perceived (Linforth et al., 2002). Mastication plays a vital role in nasal airflow due to jaw movement (closing and opening during oral processing). According to Hodgson et al. (2003), jaw movement (closure and opening the jaw) affects the volume of the mouth. When the jaw is closed, the volume of the mouth decreases and the air in the mouth is pumped into the pharynx. In this study, consumers with different oral processing preference displayed different oral behaviour during sample consumption. The difference in perception of flavour intensity (cocoa flavour) can be explained by many factors. González et al. (2019) stated that flavour release and perception depends on the oral parameters of the individual, such as chewing rate, time of swallow, saliva flow rate and composition. For instance, some studies indicated that the highest flavour intensity can be perceived when the border of velum-tongue is opening during oral processing, and its state (open or closed) highly depends on the rate of jaw and tongue movement (Mestres et al., 2006; Mishellany-Dutour et al. 2012). On the other hand, some studies stated that the main aroma/flavour release during oral processing occurred at the first exhalation after swallowing and decreased during mastication (Davidson et al., 2000; Linforth and Taylor, 2000). According to the study of Jeltema et al. (2016), different subjects will choose different oral strategies during oral processing, and the difference in oral strategies will influence the sensory perception of food. In this research, participants with different oral preference

presented significant differences in oral strategies when they were consuming samples. Thereby, the intensity of the cocoa flavour they perceived would be different.

4.4 Conclusion

When different concentrations of ingredients were added to dark chocolate, the rheology behaviour and texture of the chocolate changed. In this study, lower concentrations of menthol and ginger added to chocolate were similar to standard chocolate in regard to texture. The higher concentrations of menthol and ginger significantly affected rheology behaviour of the molten chocolate and texture of solid chocolate. In addition, none of the ingredients in different concentrations in this study influenced the melting property of the chocolate. Despite instrument tests suggesting a significant difference in chocolate properties when adding higher concentrations of extra ingredients, participants could not perceived any significant texture difference between each sample.

However, participants were very sensitive to changes in flavour and distinguished clearly between samples of different flavour concentrations. In addition, an interesting phenomenon was found in this study. Volatile flavour perception was influenced by OP style. Participants who chewed and sucked the chocolate and those who sucked the chocolate perceived the cocoa flavour similarly. However, the chewing group perceived a lower intensity of cocoa flavour when they was consuming samples than other groups with different oral preference.

5 Investigation &identification – effect of oral preference on flavour perception

Modern functional foods need modifications in recipe formulation which impacts on the product's texture and flavour release. What is more, flavour perception may itself be significantly influenced by the consumer's individual oral preference (OP). This chapter presented the features and differences between three OPs (chewing preference: CP, sucking preference: SP and mixed preference: MP) on oral behaviour and flavour perception.

5.1 Introduction

Food flavour is a vital part of a consumer's sensory perception. During oral processing, flavour components are released from the food matrix into the saliva or the headspace of the oral cavity and are transferred to the corresponding receptors (Taylor, 2002). Flavour can be categorized into two main types; volatile and non-volatile flavour. During food consumption, flavours in food are released to the saliva phase. Taste buds on the tongue will perceive non-volatile flavours, while volatile flavours must be transported first from the saliva to the air phase in the mouth and then pass through the throat to the olfactory receptors in the nose, where they are perceived.

Oral physiology is also an important factor in the flavour release of food. The oral processing of food includes three main steps: (1) breaking solid food into smaller particle sizes; (2) mixing the food with saliva and producing a bolus; (3) swallowing and transferring the bolus to the stomach (Selway & Stokes, 2014). Mastication is a key step in oral processing. Its function is to break down the food matrix and forma a bolus with saliva to facilitate

swallowing. Mastication is a complex system, which involves movements of the maxilla and mandible jaws, the tongue, the cheeks and the lips to a lesser extent. Haahr et al. (2004) studied the relationship between oral function and peppermint flavour release in chewing gum. They found that the concentration of the volatile compound in the air phase was directly related to chewing frequency (CF) and masseter muscle activity (MMA). In addition, the highest non-volatile flavour release occurs during the first minute of the chewing process. Some studies have investigated the effect of chewing patterns on flavour release during the oral process. Phan et al. 2008 posited that rapid flavour release was related to high bite force and slow flavour release was present during a longer chewing cycle. Similar results have beenreported in the research of Guichard et al. (2017) on the relationship between cheese flavour release and chewing activity. In their study, hydrophilic compounds released more quickly due to high amplitude of jaw movement during mastication, and higher rate of flavour release resulted in a quicker perception by subjects. However, Feron et al. (2014) and Guichard et al. (2017) also observed that higher chewing force can lead to a shorter chewing time during oral processing, and this resulted in a lower amount of volatile aroma in the oral cavity.

Oral preference is an individual habit. Different oral preferences (chewing, sucking and mixed) may have an influence on flavour release or flavour perception. The objectives of this study were to identify the features of different oral preferences and investigate whether the three OPs have an influence on both non-volatile and volatile flavour perception.

5.2 Method and materials

5.2.1 Oral behaviour test

60 participants were recruited from 100 untrained subjects (age: 25±5 years; 30 females and 30 males; predominantly students from the University of Auckland, New Zealand) who attended the test of OP, and they were grouped by different OP which are CP, SP and MP. Chocolate samples (weighing 5±0.3g; having a hemisphere a 26mm diameter) either with or without added flavour ingredients (Table 3-2) were served to participants in a randomised cross-over design. During this test, all participants were asked to eat samples using their natural OP. For each subject, four parameters were recorded in this test. These were; number of chews before first swallow, total eating time, total number of chews, time of first swallow, and chewing rate would be calculated by number of chews over chewing time (s). Subjects were asked to raise their hand when they swallowed. The eating parameters were recorded on video, and data were collected by visual analysis from the video.

5.2.2 TDS (Temporal Dominance of Sensations) test

For this test, the same number of subjects was recruited as in the previous session (test of oral preference, age: 25±5 years; 30 females and 30 males;). Subjects in this session were partly the same as the previous session (oral behaviour test). Five flavour parameters (sweetness, bitterness, sourness, cocoa flavour, off-flavour: ginger or menthol) were selected for this TDS study. The term “off-flavour” was used to represent any added flavour so as not to influence perception by specifying “ginger” or “menthol”. The meaning of “off-flavour” in this context was comprehensively explained to all participants in advance in case the term generated any negative emotion. Before test, a training session was conducted with all participants in order to ensure they understood the procedure and the operation of the TDS software. TDS

software (Morgenstern©, The New Zealand Institute for Plant & Food Research Limited) was used to conduct the TDS test and data collection. The procedure for TDS operation was as according to the description by Pineau et al. (2009) with minimal modification. The TDS data measurement is detailed in Section3.4. During this research, all participants were asked to eat the samples using their natural OP. Subjects fasted for at least two hours prior to the TDS session and it took approximately 20-30 minutes to complete each session. A 2-3 min break was given between samples and a glass (250ml) of water provided to rinse their mouths prior to the next sample. The testing session time was between 10:00-11:30 a.m., after breakfast.

5.2.3 Data Analysis

The chewing rate was calculated by chews divided by consumption time. The differences between samples on each eating parameter (such as chewing rate, time of first swallow and consumption time) and interaction between OP groups (oral preference x sample) were analysed using a fixed effect model by analysis of variance (ANOVA) with a Tukey's multiple comparison test at a level of significance of $p < 0.05$ (SPSS version 20, IBM Corporation, USA).

5.3 Results and Discussion

5.3.1 Oral preference test

In this study, six chewing and swallowing parameters were considered, to display the attributes of each OP and the effect of flavour perception on eating behaviour. The features of each OP group could be identified by total consumption time and total chewing rate. As

Table 2 shows, significant differences were found between the OP groups in relation to total consumption time. Subjects with the SP presented the highest (P<0.05) total consumption time (approximately 126s to 229s) for all samples. Subjects with the CP presented the lowest (P<0.05) consumption time (approximately 32–44 seconds) for all samples. For total chewing rate (Table 5.1), the CP group exhibited a significantly higher rate than the MP and SP (this group was deemed to have no chews as over half of the subjects in the SP group exhibited no chewing behaviour during sample consumption) on all samples. This shows that subjects with the CP had the fastest eating time of samples and the highest chewing rate; subjects with SP had the slowest sample consumption time and the lowest chewing rate and subjects with the MP were in between the other OP groups.

Table 5.1 Mean values (n=40 person-time) for total consumption time and chewing rate

Overall means	OP	Standard	Low ginger chocolate (LG)	High ginger chocolate (HG)	Low menthol chocolate (LM)	High menthol chocolate (HM)
Total eating time	SP	126.28 ± 32.06^{aA}	132.88 ± 31.01^{aA}	175.25 ± 36.52^{bA}	174.75 ± 39.37^{bA}	229.87 ± 42.16^{cA}
	MP	83.41 ± 14.98^{aB}	89.08 ± 16.97^{aB}	87.75 ± 19.11^{aB}	88.17 ± 19.89^{aB}	88.25 ± 21.15^{aB}
	CP	32.53 ± 5.26^{aC}	32.23 ± 4.03^{aC}	43.46 ± 5.31^{bC}	42.61 ± 5.57^{bC}	44.53 ± 6.64^{bC}
Total chewing rate	MP	0.47 ± 0.17^{aB}	0.42 ± 0.12^{abB}	0.40 ± 0.13^{abB}	0.40 ± 0.16^{abB}	0.32 ± 0.17^{bB}
	CP	1.06 ± 0.26^{aA}	1.05 ± 0.20^{aA}	0.72 ± 0.27^{bA}	0.72 ± 0.32^{bA}	0.67 ± 0.37^{bA}

Note: Superscript letters (upper case) in each column denote significant differences (oral preference x sample) at P < 0.05; Superscript letters (lower case) in each row denote significant differences (between samples for a specific OP) at P < 0.05.

Subjects' total eating time for SP and CP presented a significant increase (P<0.05) for samples with 2.5% ginger (HG), 0.1% (LM) and 0.5% menthol (HM). This indicates that a

subject's eating behaviour (preference) from the SP and CP groups could be influenced by off-flavour (ginger or menthol) intensity during oral processing. Flavour release had a significant correlation with chewing action which can accelerate flavour compounds passing to the nasal receptors (Haahr et al., 2004). In the study of flavour release on gum, Haahr et al., (2004) found higher flavour release was related to higher chewing rate. In addition, the increase of surface area by mastication also facilitated flavour release (Malone et al., 2003). For flavour perception, a massive and rapidly increasing food surface area resulting from mastication leads to a quick flavour detection and a more intense flavour perception (after swallowing) by both taste buds and olfactory receptors (Salles et al., 2011; Linforth and Taylor, 2000). For subjects with a CP, a higher rate of chewing may have resulted in higher flavour release through increasing the sample's surface area (breaking the sample down into smaller particles), and more flavour volatiles being pumped into the nose by the chewing action. Some research also found that subjects with specific oral behaviour were associated with enhancement of specific sensory perception (Engelen and de Wijk. 2012; de Wijk et al. 2008). For instance, compared with otheroral strategies, chewing provides a stronger experience of flavour perception. In addition, Jeltema et al. (2016) found that the choice of oral strategies by subjects are more associated with comfort of texture and flavour extraction. According to the previous study in this research using modified quantitative descriptive analysis (MQDA) test, subjects expressed lower acceptability of off-flavour intensity when they consumed samples with 2.5% ginger (HG), 0.1% (LM) and 0.5% menthol (HM). When the off-flavour concentration increased in the samples (HG, LM and HM), the stronger or unacceptable flavour released during oral processing had an influence on the eating behaviours of all subjects in this study. The same trend was also demonstrated in total chewing rate. The extension of consumption time also resulted in a decrease of total chewing rate in subjects with the CP when they consumed HG, LM and HM samples. However, the

lack of a significant difference between LM and HM in total chewing rate may be due to the difference in choice of oral strategies when subjects perceived stronger (unacceptable) flavour intensity. For instance, some participants increased their chewing rate, and some participants decreased their chewing rate to adapt to the stronger (unacceptable) flavour intensity during sample consumption.

Tongue movements during oral processing also play an important role in formatting and transporting the bolus (Benjamin et al., 2012). During oral processing, the velum-tongue border is either closed or opened, and the degree of openness depends on tongue and jaw movements (Buettner et al., 2002). A more intense flavour perception can be achieved by deliberately opening the velum-tongue border (Linforth & Taylor, 2000). As Zafar et al., (2000) have put forward, the tongue's movement away from the palate may form aerosols which would enhance flavour release. In addition, Mishellany-Dutour et al. (2012) also stated that subjects with high flavour release at the nostrils presented a constant in-mouth air cavity following empty deglutition, and the constant in-mouth air cavity with continuous aroma release relates to constant and slight opening of isthmus by tongue-velum praxis during mastication and swallowing. On the other hand, subjects with low flavour release at the nostril presented a large change of in-mouth air cavity which displayed a transient opening of isthmus with a steady fauces closing by tongue-velum praxis at swallowing. Compared to subjects with a CP, subjects with a SP mainly rely on tongue movements to facilitate bolus formation during semi-solid food consumption (Taylor & Roberts, 2004). The increased eating time of subject with a SP (at HG, LM and HM samples) reflected a lower frequency of jaw and tongue movements when they sensed stronger or unacceptable flavours.

For the subjects with an MP, they have two distinct eating behaviours, both mastication (chewing) and sucking (tongue movements) during sample consumption. This resulted in

them being easily able to transfer to a different OP when they perceived samples with different flavour concentrations. An interesting phenomenon was observed among subjects with MP, they would eat faster by increasing the number of chews or eat more slowly by decreasing the number of chews when they were eating samples with higher off-flavour concentrations in order to either reduce the flavour release from the sample or get rid of the flavour as soon as possible. This can be illustrated by the subjects with an MP maintaining a stable ($P>0.05$) eating time (Table 5.1). However, HM sample with highest flavour intensity still had a significant effect on the eating behaviour of subjects with an MP. Subjects with an MP showed a significant ($P<0.05$) decrease in total chewing rate when they ate HM samples. In addition, for total number of chews, subjects with an MP showed lower ($P<0.05$) total chews when eating HM chocolate when compared with the other samples, while there was no such difference ($P>0.05$) for the standard, LG, HG and LM samples (Table 5.2). Blissett et al. (2006) stated that volatile release from food during oral processing was significantly influenced by oral parameters of the individual (chewing rate, number of chews and chewing force). The change of oral behaviour observed in this study may indicate adaptation for samples with different flavour intensity.

Table 5.2 Mean values (n=40 person-time) for chews of first swallow and total chews

Overall means	OP	Standard	Low ginger chocolate (LG)	High ginger chocolate (HG)	Low menthol chocolate (LM)	High menthol chocolate (HM)
Chews until first	MP	13.5 ± 5.2^{aB}	14.5 ± 6.07^{aB}	16.75 ± 7.96^{aB}	15.25 ± 7.67^{aB}	14.41 ± 6.40^{aB}

swallow	CP	23.38±6.75^{aA}	24.61±6.47^{aA}	26.31±6.58^{aA}	27.77±7.74^{aA}	26.53±7.93^{aA}
Total chews	MP	38.83±6.05^{aA}	37.58±5.77^{aA}	34.75±3.95^{aA}	35.16±4.98^{aA}	27.83±5.33^{bA}
	CP	34.53±5.01^{aA}	33.77±5.60^{aA}	31.23±6.26^{aA}	30.61±7.39^{aA}	29.92±8.06^{aA}

Note: Superscript letters (upper case) in each column denote significant differences (oral preference x sample) at P < 0.05; Superscript letters (lower case) in each row denote significant differences (between samples) at P < 0.05.

In terms of time until first swallow, subjects with the SP and MP were similar (P>0.05) in terms of time until first swallow (Table 5.3). Subjects with the CP presented a significantly (P<0.05) lower time until first swallow compared to subjects with the SP and MP on all samples due to earlier formation of a small size bolus due to the higher chewing rate. For chews until first swallow and rate of first swallow (Tables 5.2 and 5.3), subjects with the MP presented significantly (P<0.05) lower chewing times and frequency of first swallow compare to subjects with the CP. It was also discovered that samples with different flavours and flavour concentrations did not influence subjects' eating behaviour within the three OPs before the first swallow. Some studies have stated that the highest aroma release was perceived from the first "swallow-breath" (Buetter et al., 2001; Land, 1994 and Linforth & Taylor, 2000). Therefore, it appears that the eating behaviour of all subjects largely relies on their preference rather than on the flavour intensity of the food before the first swallow.

5.3 Mean values (n=40: person-time) for time and chewing rate of first swallow

Overall means	**OP**	**Standard**	**Low ginger chocolate (LG)**	**High ginger chocolate (HG)**	**Low menthol chocolate (LM)**	**High menthol chocolate (HM)**

Time of first swallow	SP	24.38±4.5^{aA}	25.62±5.0^{aA}	26.76±5.6^{aA}	26.25±5.1^{aA}	27.88±7.26^{aA}
	MP	25.91±5.61^{aA}	24.66±4.03^{aA}	26.91±4.53^{aA}	27.16±5.03^{aA}	27.56±4.68^{aA}
	CP	18.15±5.51^{bA}	19.69±4.39^{bA}	21.23±4.43^{bA}	22.07±6.03^{bA}	22.08±6.06^{bA}
Chewing rate of first swallow	MP	0.52±0.17^{bA}	0.58±0.12^{bA}	0.62±0.16^{bA}	0.56±0.16^{bA}	0.52±0.15^{bA}
	CP	1.29±0.35^{aA}	1.25±0.23^{aA}	1.24±0.23^{aA}	1.26±0.20^{aA}	1.20±0.39^{aA}

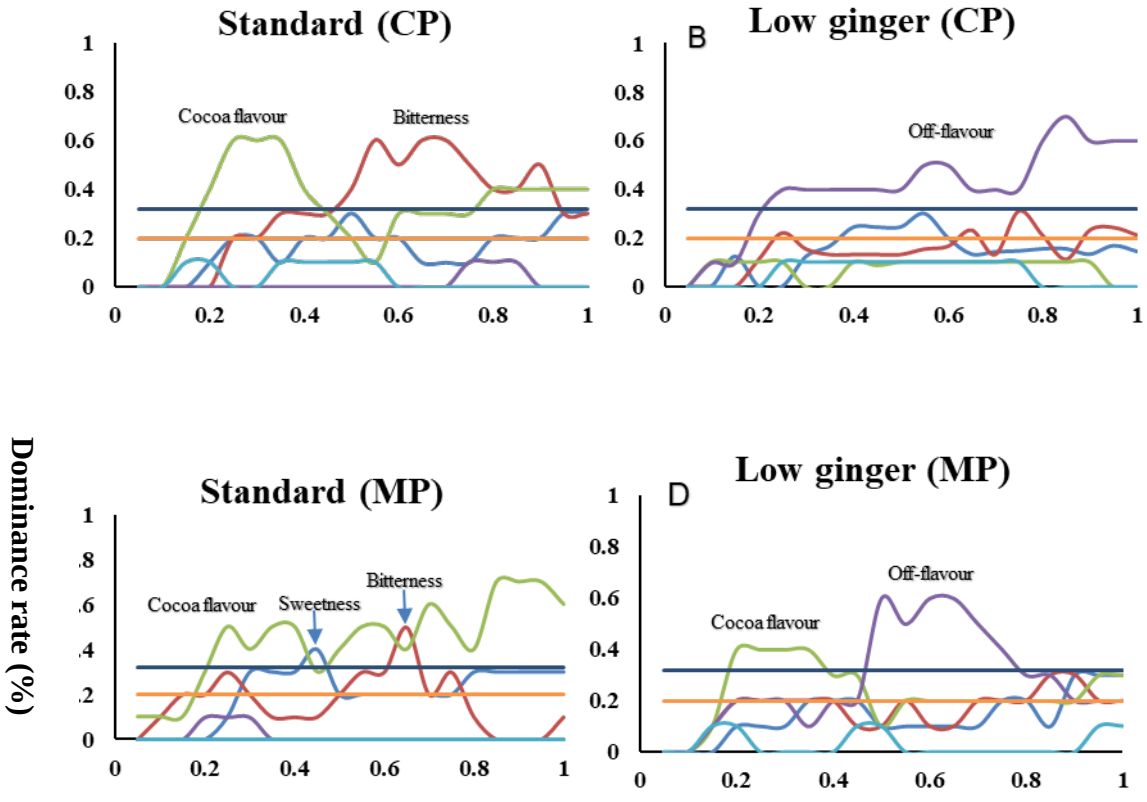
Note: Superscript letters upper case) in each row denote significant differences (oral preference x sample) at P < 0.05; Superscript letters (lower case) in each column denote significant differences (between samples) at P < 0.05.

5.3.2 Temporal dominance of sensations (TDS) test

The TDS curves (Figs. 5.1, 5.2 & 5.3) show the dominant flavour attributes over standardized time (%) for the chocolate samples either with or without added flavouring ingredients. A general overview of the standard sample without added flavouring (ginger and menthol) for the TDS curve shows a significant difference in dominant sensations by the three OPs. As the Fig 5.1 (C and E) shows, the MP and SP groups can sense more of the dominant flavour ("cocoa flavour", "bitterness" and "sweetness") than the CP group ("cocoa flavour" and "bitterness" only). Although the three OPs groups present the same dominate sensation, "cocoa flavour", at the beginning and end of sample consumption, the difference between them was still distinguishable. Specifically, bitterness seemed a main dominate sensation for subjects with the CP from the middle stage of consumption to the end (Fig. 5.1 A), while cocoa flavour was the main dominate sensation for subjects with the MP and the SP from the middle stage of consumption to the end (Fig. 5.1 C and E). In addition, subjects with the SP

displayed the most frequent change of dominant flavour at 40% of the consumption time (Fig. 5.1 E), while subjects with the CP presented more stable dominant flavour at 40% of the consumption time (Fig. 5.1 A) and the change in dominant flavour for subjects with the MP sits in the middle of these two (Fig. 5.1 C).

Similar results were found in the low off-flavour concentration (LG) sample. As Fig. 5.1 B D and F shows, the off-flavour of ginger in chocolate was the dominant sensation at all times when subjects with the CP were consuming the sample (LG). However, the TDS curve for subjects with the MP and SP presented multiple dominant sensations (Fig. 5.1 D and F), while the dominant sensation for subjects with the SP still shows the highest change frequency (Fig. 5.1 F).



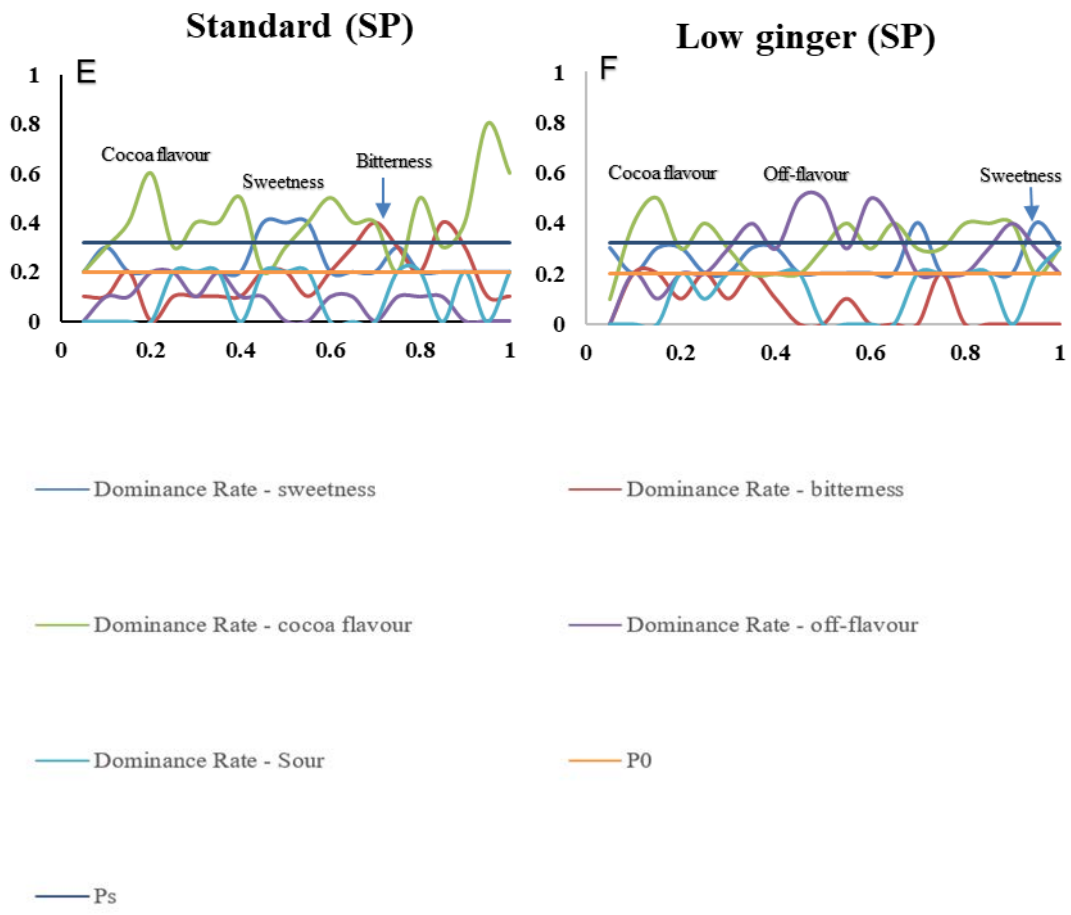


Fig 5.1 Dominance rate of each flavour attributes by TDS curve (Standard and LG)

In terms of samples with standard and LG (Fig. 5.1), the TDS assessment was significantly influenced by OP. These could be strongly correlated with the eating behaviour of each OP group. As Slotnick et al. (1988) stated, taste perception (Non-volatile flavour) is affected by the surface contact area between the tongue's surface and the sample. The aroma perception (volatile aroma) is influenced by air flow in the mouth which is dependent on chewing behaviour (chewing rate and duration) (Tarrega et al., 2007). The chewing preference with the highest chewing rate results in the volatile aroma (cocoa flavour) being the dominant sensation at the beginning of sample consumption (Fig. 5.1 A) due to the highest frequency of jaw movements. However, the highest chewing rate also results in an increased surface

contact between the sample and the tongue by reduction of sample size into many pieces with a smaller size via mastication. This results in increased perception of a non-volatile flavour (bitterness) as Fig. 5.1 A shows. For subjects with the MP and SP, a lower chewing rate results in lower air flow in the mouth and less surface contact between the sample and the tongue. This may determine a gradual release of each flavour (tastants and odorants) during oral processing, resulting in the dominant sensation changing more frequently than for subjects with the CP (Fig. 5.1 C D E & F). In addition, some studies found that saliva and oral mucosa are also important factors in flavour release or perception during oral processing (Haahr et al., 2004; Blissett et al., 2006). According to Haahr et al. (2004), more aroma compounds in chewing gum were retained in the aqueous phase with a high volume of saliva. During food consumption, food is broken down and mixed with saliva during mastication to form the bolus. The saliva flow and content during mastication influences the viscosity of the bolus, and this also influences the aroma release and perception (Guichard, et al., 2017). As Gonçalves and Lannes (2010) observed, the flavour persistence and the rate of flavour release during oral processing are associated with the rheological behaviour of the chocolate bolus. They found that increasing the viscosity of the bolus may lead to an increasing of persistence of aroma compounds during mastication. In addition, Buettner et al. (2005) observed that oral mucosa could interact with about 30% to 40% of aroma compounds when they were released from food during oral processing. In this research, subjects with the SP presented the longest consumption time. This may lead to more interaction with saliva and mucosa, as a result displaying a more complex TDS profile.

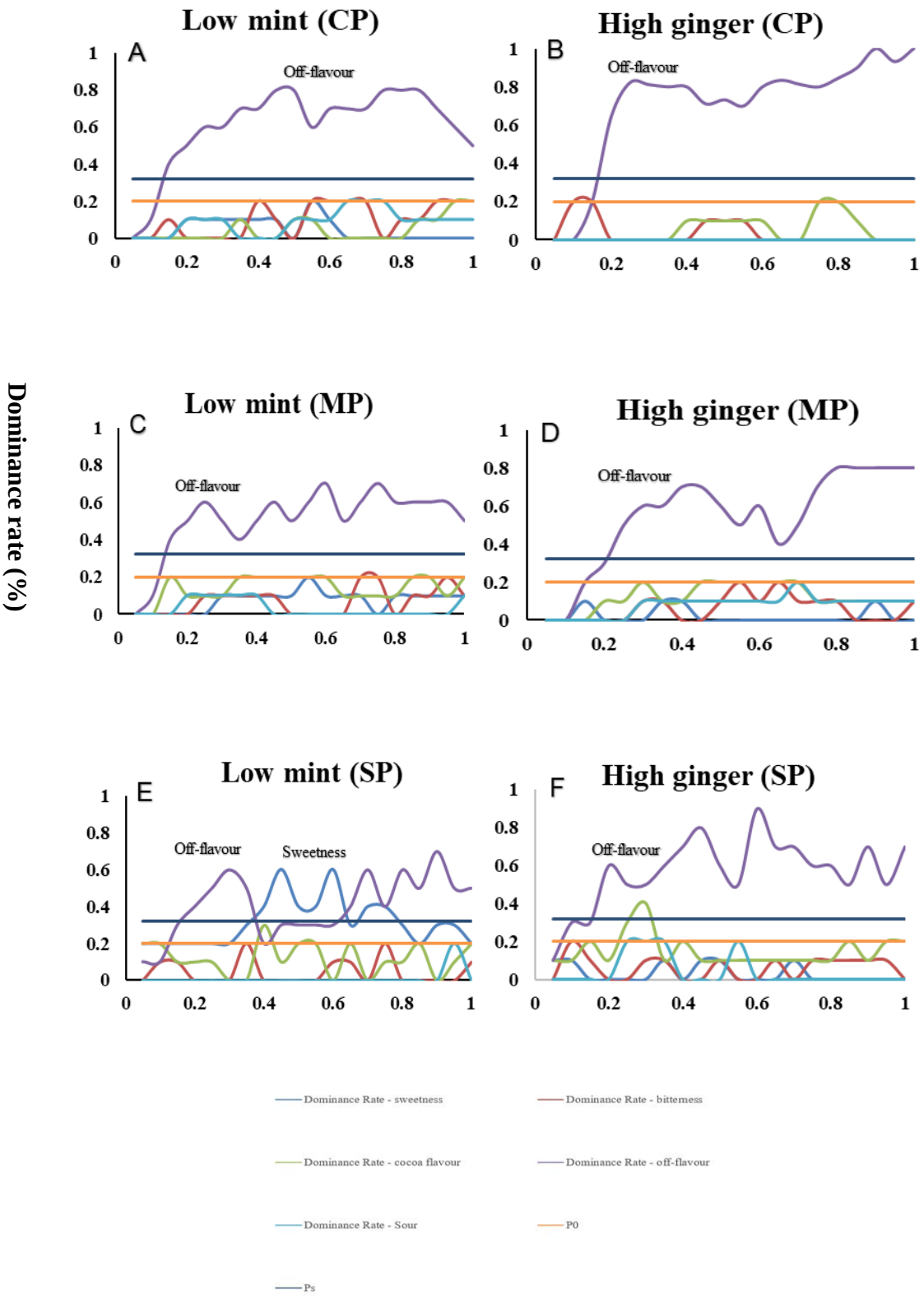


Figure 5.2 Dominance rate of each flavour attribute by TDS curve (LM and HG)

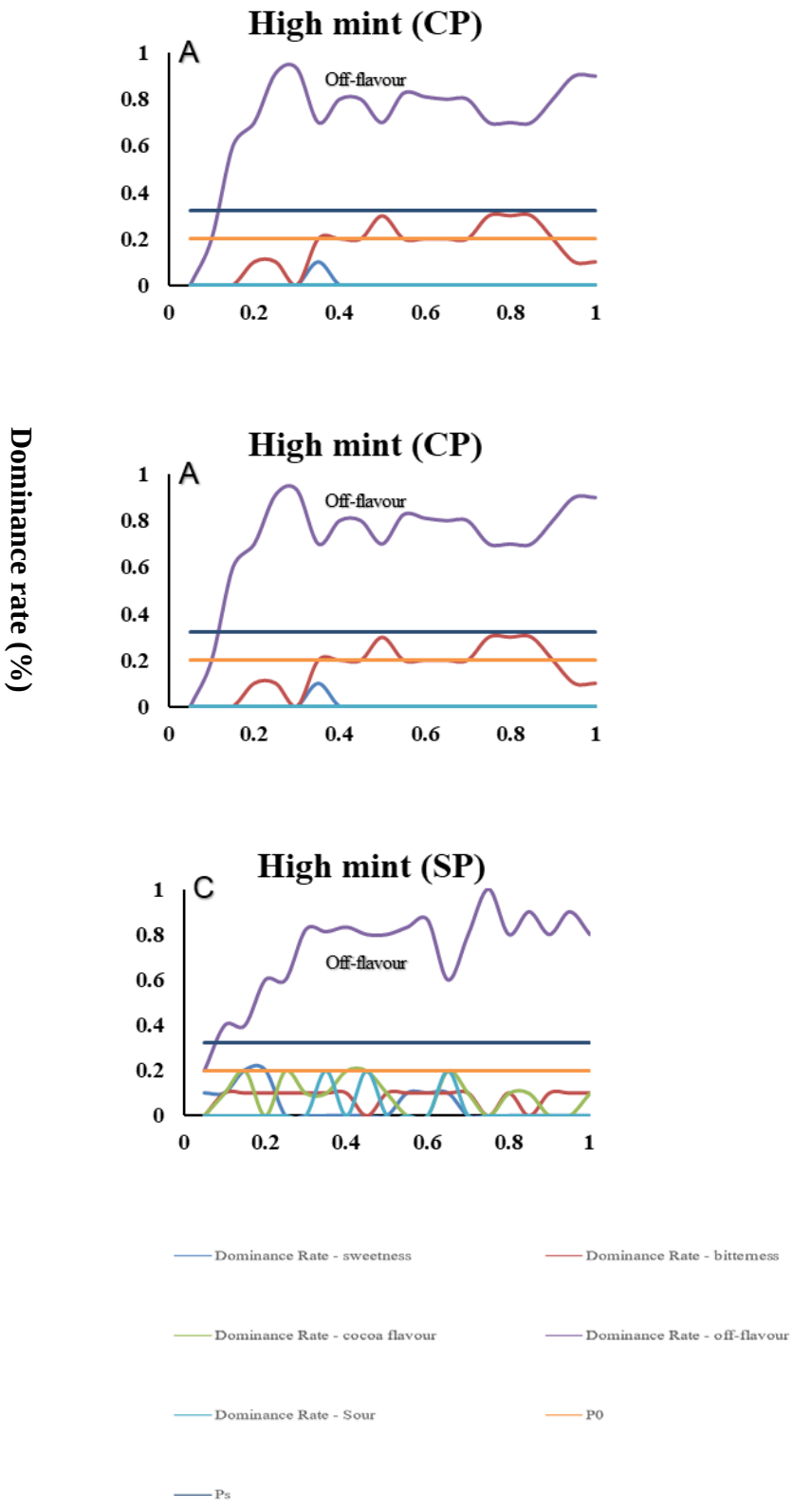


Figure 5.3 Rate of dominance for each flavour attribute by TDS curve (HM)

When subjects consumed samples with a medium “off-flavour” concentration (LM and HG), only the SP subjects presented multiple dominant sensations (off-flavour and sweetness) and a higher frequency of dominant sensation change during oral processing (Figure 5.2 E). The off-flavour (menthol or ginger) was only a significant dominant sensation for subjects with the CP and MP for the whole time (Fig. 5.2 A B C & D). When consuming samples with a high off-flavour concentration (HM), the off-flavour (menthol) is the dominant sensation for all OPs from the beginning of oral processing to the end (Fig. 5.3). This phenomenon reflects the fact that the flavour intensity of the sample may be the main contributor to the dominant sensation during oral processing rather than OP. A similar finding was reported by Rodrigues et al. (2016). They found that the TDS curve presented multiple dominant sensations and higher frequency of dominant sensation change for chocolate with low cocoa concentrations (28%-34%) during oral processing. With the cocoa content increasing, the dominant sensation tends to increase and there is a lower frequency of dominant sensation change, until only one dominant sensation is experienced throughout sample consumption (70-85% cocoa content). In addition, except for different flavour intensity, the different TDS profiles between LM and LG samples can be explained by the effect of the trigeminal system on flavour perception. According to the research of Viana (2011), the sensation of the trigeminal system is very sensitive to many chemical stimulations, such as burning, itching and cooling. Compared with the ginger sample , menthol chocolate provides an extra cold sensation, and this may enhance the flavour perception. This could explain the difference between LG and LM on TDS profile.

5.4 Conclusion

In this study, the features of three OPs have been identified through testing eating behaviour. During chocolate oral processing, subjects with a chewing preference presented the shortest

consumption time and the highest chewing rate, while subjects with a sucking preference presented a longest consumption time and, naturally, the lowest chewing rate.

An interesting phenomenon was discovered in this study. The flavour intensity of the samples influenced the eating behaviour of each preference group, which may result in a prolonging of consumption time and a decrease in chewing rate. This could also reflect subjects adapting to samples with different flavour intensity by changing their oral behaviour. In addition, the TDS testing indicated that a difference in oral processing would affect perception of dominant flavour during different samples consumption. However, when consuming samples with the strongest flavour (such as HM sample), the intensity of the samples was the main contributor to the perception of the dominant flavour, rather than the oral preference of each subject.

6 Research of difference in flavour perception and microstructure of bolus by different oral preferences

In this chapter, the aim of this study is to use alternative sensory methods to prove or supplement the previous studies (Chapter 4 & 5). An analysis of the bolus structure was also employed, and it was related to the Time-intensity (TI) test to discover the relationship between flavour perception and the restructuring of food through oral processing. The results of TI clearly describe the effect of different OP on flavour perception during samples consumption, and through the research of bolus microstructure, we found a strong connection between bolus structure and different OP in terms of different flavour perception.

6.1 Introduction

Research on the sensory evaluation of chocolate has long since recognised that, in addition to the perception of texture, the food matrix is also influenced by oral processing and the eating behaviours of the consumer. The oral processing of food includes the comminution of food from large particle sizes to smaller particle sizes and mixing them with saliva to form a bolus, and then swallowing (Selway & Stokes, 2014). During this complex processing, the mechanical properties of food are changed, and the sensory perception of texture and flavour are affected accordingly. A study by Tournier et al. (2014) found that the release of flavour from food is largely relative to a subject's mastication style and the time taken for oral processing. According to their research, a higher flavour (salt) release was induced by a

higher number of chewing cycles, and the longer duration of chewing led to maximum flavour (sodium) concentration. Some studies found that, compared with saliva flow, swallowing behaviour and the food's properties, chewing rate and frequency may play a more important role in flavour release (Mestres et al., 2006; van Ruth et al., 2003). As van Ruth et al. (2003) state, the flavour release from food during the chewing process is increased by the subject's increased chewing rate. More specifically, in research on the relationship between oral behaviour and flavour release, Hansson et al. (2003) and Haahr et al. (2004) found that the frequency of masseter muscle activity (MMA) directly and significantly affected aroma intensity in the air phase, and higher frequency of MMA in the chewing stage led to higher intensity of aroma release and slower aroma decrease. Moreover, Salles et al. (2011) claim that sensory perception is affected by the eating strategies of each individual, such as different chewing patterns during oral processing and different saliva characteristics (saliva composition and flow rate) between individuals. As Kohyama and Mioche (2004) found, people of different ages or gender present significant differences in chewing patterns, which result in variations in flavour perception or release during oral processing.

The bolus structure is an important parameter used to analyse food comminution through oral processing (Woda & Peyron, 2006; Eberhard, et al., 2012; Engelen, et al., 2005). Most recent studies about the bolus mainly focus on the relationship between food texture and bolus properties at different stages of oral processing (Chen et al. 2013; Shiozawa & Kohyama, 2011; Rodrigues et al. 2014; Young et al., 2016). The perception of food texture during oral processing is a dynamic process. Devezeaux et al. (2015) divided dynamic texture perception (dominant sensations) during emulsion-filled gel consumption into three parts namely "firm" at the beginning of mastication, "elastic", "sticky", "refreshing" and "moist" in the middle, and "grainy", "creamy" and "melting" at the end of oral processing. They also found the

specific texture sensations are strongly related to the bolus properties. Similar results have been found by Jourdren et al. (2016). In their research, they found that the change of bolus properties during oral processing significantly influenced the perception of certain attributes, such as “dry”, “soft” and “doughy”. In a later study, Jourdren et al. (2017) observed that bolus properties not only influenced texture proception during oral processing, but also influenced volatile release and perception due to saliva addition. In terms of the effect of oral processing on texture perception, De Lavergne et al. (2015) found that subjects with long eating duration displayed different bolus structure than subjects with short eating duration, and this influenced the dynamic texture perception during consumption of sausages. In current research, there is still a gap in knowledge in relation to the connection between flavour release or perception, and bolus structure. As previous studies have posited (Chapter 4 & 5), there are multiple eating patterns in consumer’s oral preference (OP), such as the chewing preference (CP), the sucking preference (SP) and the mixed preference (MP) during chocolate consumption. The objective of this study is to continue the investigation into the features of different oral preferences on flavour perception, as they are connected to changes in the microstructure of the chocolate bolus.

6.2 Methods and materials

6.2.1 Time intensity (TI) test

60 subjects were recruited and grouped by OP into three categories (Chewing preference: CP, sucking preference: SP and mixed preference: MP) in line with previous research (3.3.1). SensoMaker software (Version 1.91, Nunes and Pinheiro, 2012) was applied to conduct TI test and data collection. In this test, two flavour parameters (ginger and menthol) with different concentration (See 3.1.1) were selected for this study. Before TI test, a training

session was carried out with subjects in order to ensure they understood the operation of the TI software (SensoMaker) and the session's procedure. Subjects were allowed to ask any questions in relation to the test. Subjects fasted for at least two hours prior to the TI session, and it took approximately 30-40 minutes to complete each session. A 2–3 min break was given between samples and a glass (250 ml) of water was provided to rinse the subjects' mouths before the next sample. The testing session time was between 10:00–11:30 a.m., after breakfast.

6.2.2 Bolus microstructure

6.2.2.1 Bolus collection

Thirty subjects were recruited from 100 untrained subjects to attend a vivo test on the microstructure of the chocolate bolus. These subjects were grouped by OP into three categories (Chewing preference: CP, sucking preference: SP, and mixed preference: MP) in line with previous research (3.3.1). Bolus collection was designed to determine the microstructure of the chocolate bolus of subjects with different OPs. The subjects were asked to eat samples using their own OP for three different consumption times which are:

• time of first swallow,

• time of last swallow

• time between these two.

At these different times the subjects were asked to expectorate the sample in their mouth into a 50 ml SterilinTM container, and these samples were sealed and frozen at -18 °C for subsequent testing (microstructure of bolus). In this session, five samples (SC, GC: LG and

HG; MC: LM and HM, as referred 3.1.1) were served to subjects, and two samples were given for each type of sample.

6.2.2.2 Slide preparation

The chocolate bolus produced by subjects from various consumption times and OPs were analysed using a confocal laser scanning microscope (CLSM - Olympus FV1000, USA). The procedure of slide preparation for this study was adapted from Auty et al. (2001) with modifications. Nile Red (Sigma-Aldrich, New Zealand) was used to stain the cocoa butter in the bolus due to its ability to locate the hydrophobic phase (such as cocoa butter, cocoa butter alternatives and vegetable oil). 0.5 mg of Nile Red per g of bolus was added. After that, the containers were sealed and wrapped in aluminium foil and transferred to a dark, cold place. All samples were stained for at least 4 hours, but no more than 12 hours before carrying out the image test. After staining, samples were heated to 40 °C with a water bath for 15 minutes until they were completely melted. For analysis of bolus structure, the sample treatment (heating and cooling) did not present significant difference when compared to samples without treatment (see Appendix C). Melted samples were transferred onto a microscope slide with single, concave surface with a metal spatula, and a coverslip was quickly placed on top of it.

6.2.2.3 CLSM imaging and image analysis

The slides of the samples were imaged by a 20-x lens in order to gain a clear and global microstructure of the bolus. During imaging, three concurrent image channels with different excitation wavelengths (405, 473 and 559 nm, Table 3.2) were applied and layered to generate the final image. In addition, in order to collect an individual image from each channel, the collection wavelength was set at 425–460 (blue), 499–561(green), and 655–755

nm (red). ImageJ Fiji software (National Institutes of Health, Bethesda, MD, USA) was used in the analysis of particle size in this process. Particle count and total area of particle was the output of the software, and the data were exported to Excel (Office 365, Microsoft, USA) for data analysis.

6.2.3 Data analysis

The results from the parameters obtained from the time-intensity curves (I_{max}, Ti_{max}, T_{tot} and Area) and the results from ImageJ were compared and interaction between OP groups (oral preference x sample) were by using analysis of variance (ANOVA) with a Tukey's multiple comparison test with a fixed effect model at a level of significance of $p < 0.05$ (SPSS version 20, IBM Corporation, USA).

6.3 Results and discussion

6.3.1 Time-intensity analysis of the added flavour: ginger and menthol)

As this test mainly focussed on flavour perception during oral processing, an aftertaste was not considered. The added flavour in this test would be present a long time after the last swallow and therefore, the TI curves (Fig. 6.1) do not display the entire flavour perception trend. Table 6.1 shows the mean values of the parameters for the added flavours (ginger and menthol) of each chocolate sample by subjects with different OPs. In terms of low ginger (LG) chocolate, significant differences ($P < 0.05$) were found between the three OPs in relation to I_{max}, Ti_{max}, T_{tot} and Area under the curve (AUC). Subjects with a SP presented the longest time of maximum intensity, duration of the stimulus and lowest maximum intensity during sample oral processing. Subjects with a CP presented the shortest time of maximum

intensity, duration of the stimulus and highest maximum intensity during sample oral processing. As Hansson et al. (2003) and Haahr et al. (2004) found the frequency of masseter muscle activity (MMA) directly and significantly influenced aroma intensity in the air phase. In addition, Phan et al. (2008) also suggested that rapid flavour release was related to high bite force, and high chewing rate presented a quick flavour release during oral processing, and slow flavour release was caused by a long chewing cycle during oral processing. This finding is consistent with previous research of features of each OP; subjects with the CP had the fastest sample consumption time due to highest chewing rate; subjects with the SP had the slowest sample consumption time due to having the lowest chewing rate. An interesting phenomenon was observed in that subjects with the MP presented the highest AUC compared to the other subject groups (Table 6.1). This could indicate that in this study, subjects with MP and medium chewing rate can perceive more added flavour than subjects with SP or CP when they consume samples with low ginger concentration .

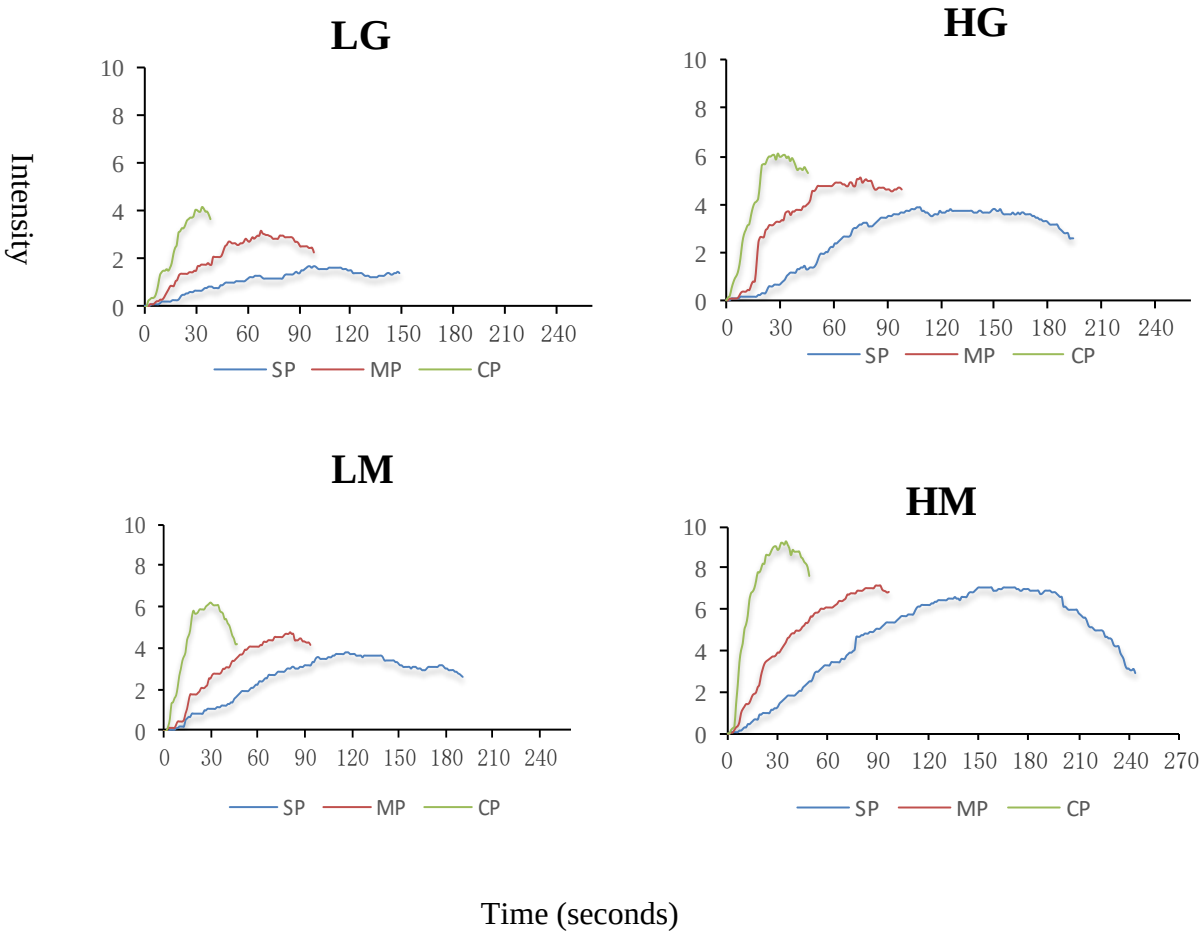


Figure 6.1 Time-intensity curves for added flavour (ginger and menthol) of chocolate samples for subjects with different OPs

For the TI test for LM and HG samples (with medium intensity of flavour), significant differences ($P < 0.05$) were found between the three OPs in relation to I_{max}, Ti_{max}, T_{tot} and Area. Subjects with a CP exhibited the shortest maximum intensity time, duration time of the stimulus, smallest area and highest maximum intensity during sample oral processing. Subjects with a SP exhibited the longest maximum intensity time, duration time of the stimulus, largest area and lowest maximum intensity during oral processing. Subjects with a MP sat between the other two OP groups.

With respect to HM samples (with highest intensity of added flavour), significant differences ($P < 0.05$) were found between the three OPs in relation to Ti_{max}, T_{tot} and Area. An interesting phenomenon was observed in maximum intensity between MP and SP. According to the TI curve, the subjects with an MP and an SP presented similar maximum intensity when they consumed samples with the highest amount of added flavour (HM). In addition, subjects with a CP still presented the highest perception of maximum added flavour intensity during sample oral processing.

Table 6.1 Mean value of TI parameters from curve (Area, Ti_{max}, I_{max} and T_{tot})

Area	LG	HG	LM	HM
CP	96.22 ± 30.98^{cC}	202.72 ± 52.53^{cB}	206.75 ± 57.81^{cB}	341.28 ± 64.09^{cA}
MP	219.91 ± 58.75^{aC}	354.6 ± 82.76^{bB}	318.12 ± 88.11^{bB}	468.78 ± 103.2^{bA}
SP	158.28 ± 46.23^{bC}	509.37 ± 115.62^{aB}	481.16 ± 108.21^{aB}	1142.72 ± 303.43^{aA}

Ti_{max}	LG	HG	LM	HM
CP	34.46 ± 8.19^{cA}	29.4 ± 7.77^{cA}	30.66 ± 10.28^{cA}	35.72 ± 12.38^{cA}
MP	68.06 ± 15.18^{bC}	75.93 ± 16.02^{bBC}	81.58 ± 22.93^{bB}	89.87 ± 24.64^{bA}
SP	96.24 ± 24.74^{aC}	107.31 ± 30.71^{aBC}	117.26 ± 29.58^{aB}	150.73 ± 39.48^{aA}

I_{max}	LG	HG	LM	HM

CP	4.13±0.35^{aC}	6.12±0.42^{aB}	6.20±0.63^{aB}	9.25±1.21^{aA}
MP	3.14±0.29^{bC}	5.14±0.39^{bB}	4.77±0.43^{bB}	7.19±1.21^{bA}
SP	1.71±0.22^{cC}	3.86±0.38^{cB}	3.76±0.37^{cB}	7.08±1.33^{bA}

T_{tot}	LG	HG	LM	HM
CP	39.37±6.12^{cB}	46.62±7.43^{cAB}	47.94±6.79^{cAB}	49.35±5.58^{cA}
MP	99.22±10.45^{bA}	98.36±12.53^{bA}	94.25±16.76^{bA}	97.39±14.89^{bA}
SP	148.75±22.36^{aC}	195.9±28.85^{aB}	191.64±27.45^{aB}	244.31±35.97^{aA}

Note: Superscript letters (lower case) in each column denote significant differences (oral preference x sample) at $P < 0.05$; Superscript letters (upper case) in each row denote significant differences (between samples) at $P < 0.05$.

Comparing the four different flavour added chocolate samples, the results of the parameters from the TI curve changed with the increase of added flavour intensity in the samples. For subjects with SP, significant differences ($P < 0.05$) were found in I_{max}, Ti_{max}, T_{tot} and Area between the three types of intensity in the samples (low, medium and high added flavour intensity). Specifically, the sample with the highest added flavour intensity showed the highest Area, I_{max} and longest Ti_{max} and T_{tot} while the sample with the lowest added flavour intensity presented the lowest Area, I_{max} and shortest Ti_{max} and T_{tot}. In terms of subjects with CP, a significant difference ($P < 0.05$) was found in I_{max}, T_{tot} and Area between the two types of sample intensities (low and high added flavour intensity).

According to Table 6.1, with an increase in added flavour intensity, HM samples presented the highest I_{max} and Area. However, the results for subjects with an MP with regard to Ti_{max} and T_{tot} show no significant difference between medium and high intensity flavour added chocolate. For subjects with an MP, only Area and I_{max} presented an increase trend with an increase of added flavour intensity in the samples. There was no significant difference between the sample with medium intensity of added flavour and the sample with high intensity added flavour. In addition, compared with the three samples with different flavour intensities, subjects with the MP presented similar T_{tot} value during oral processing. Previous studies (Chapter 5) on the features of the OP suggested that subjects with an MP are easily influenced by the flavour intensity of samples, and may switch to a different processing style, either chewing or sucking. The results of the TI curve for the MP are consistent with previous research (Chapter 5).

6.3.2 Bolus structure analysis (CLSM)

Cocoa butter was stained with Nile Red dye which shows as a yellow colour in images, facilitating the analysis of the transformation of chocolate's microstructure during oral processing. Figure 6.2 shows the progressive change in bolus structure (standard chocolate as an example) by subjects at various consumption stages (10%, 50% and 80%， more consumption stages on Appendix B). During oral processing, the chocolate structure is transformed gradually from a predominantly composite solid (< 10% of consumption time) to an oil/water emulsion. At 10% of total consumption time, there are large amounts of cocoa butter (yellow) showing in an accumulated state at the beginning of oral processing for subjects of all OPs. At the middle stage of oral processing (50%), the cocoa butter in the accumulated state is separated by particles of irregular shape and size through oral behaviour

(chewing or sucking during oral processing). In the later stage of oral processing (before swallowing), the larger drops of cocoa butter have been broken down into smaller and more regular particles (droplet) and suspended in and mixed with saliva. Selway and Stokes (2014) hold that oral processing of food contains three main steps; breaking down food into small particles; mixing with saliva to form a bolus and swallowing the bolus. The food structure is progressively reorganized during oral processing, and continuous oral behaviour changes the solid food into a cohesive and soft bolus to meet the requirements of swallowing (Hutchings & Lillford, 1998; Olthoff et al., 1984). Therefore, the changes in distribution and form of the cocoa butter in the image (Figure 3.3) clearly display the changes in microstructure of the chocolate samples. In addition, during sample oral processing, the chocolate structure is transformed gradually from a composite solid to an oil/water emulsion between 10% and 80% of total consumption time for all OPs. This links to the phase inversion of chocolate during oral processing. During oral processing, continuous saliva secretion and mixing with the melted chocolate causes a phase inversion from a water-in-oil-phase to an oil-in-water phase. The decrease in cocoa butter surface area with an increase in saliva surface area (Figure 6.2) demonstrates the process of chocolate phase inversion during oral processing.

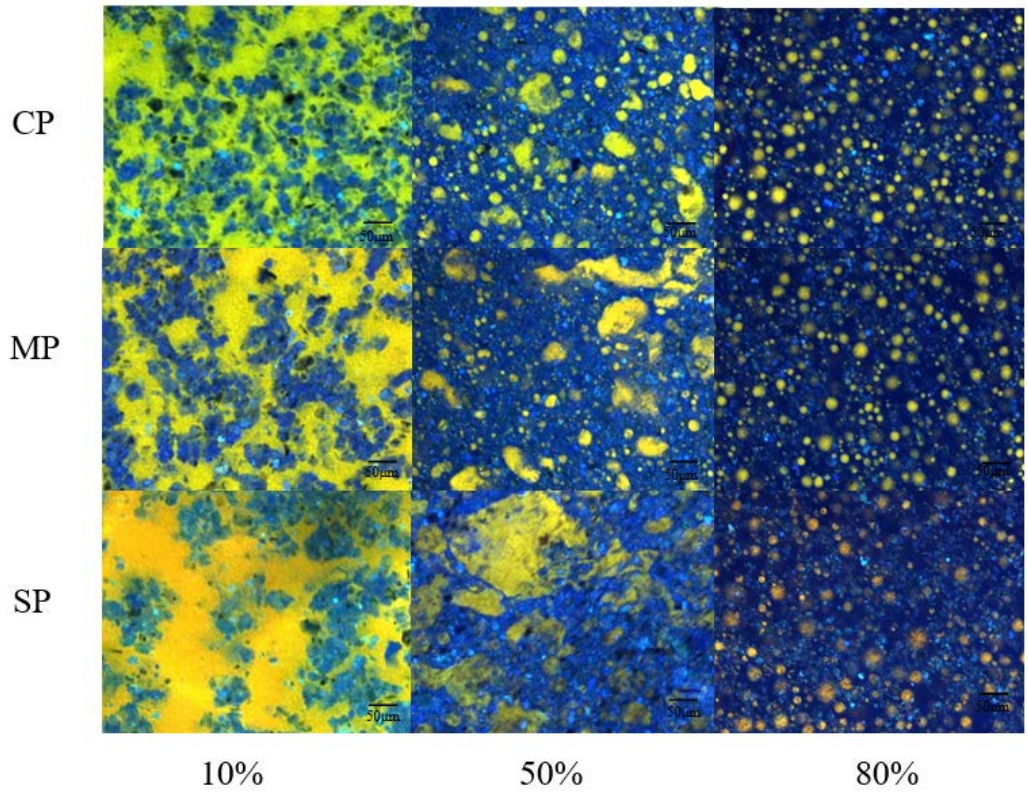


Figure 6.2 CLSM image of standard chocolate bolus at 10%, 50% and 80% of total consumption time by three oral preferences; Yellow area: Cocoa butter, Dark blue area: saliva, Light blue/aqua: cocoa solids, Black surround: air bubble.

A notable observation was discovered in relation to the distribution of cocoa butter when comparing the three OPs at the same stage of consumption. At 10% of total consumption time, the distribution of cocoa butter for all subjects presented large size, accumulated and connected particles (Figure 6.2); however, subjects with the SP demonstrate the slowest phase inversion compared with subjects with the MP and CP due to slowest mixing of saliva. In addition, at 50% of total consumption time, the water-in-oil phase has been transformed into an oil-in-water phase for all OPs; however, fat particles in the boluses for subjects with an SP are larger than for subjects with the CP and the MP. As Carvalho (2013) posited that phase inversion depends on the mixture of saliva and the mechanical forces of the tongue and teeth during oral processing. According to previous studies, subjects with a CP or an MP

exhibit higher chewing rate than subjects with SP. Therefore, the rate of phase inversion and particle breakdown has a strong connection to a subject's OP

The difference in bolus microstructure for different OPs may well be due to the difference in chewing times and frequency for each OP. In order to prove the difference in transformation of the bolus microstructure during oral processing, ImageJ was used to quantify the state of the cocoa butter (count and particle size) and describe the distribution of cocoa butter in the bolus. Table 6.2 shows particle size (area) and cocoa butter particle count for the four types of samples by the different OPs at different consumption times (time of first swallow and time of maximum intensity perception) which were obtained from the TI curve and previous study. The consumption time of first swallow and maximum added flavour intensity perception differ due to the subject's OP, as was investigated in the previous experiment (Figure 6.1). In terms of the LG sample which has low added flavour intensity, subjects with the CP presented the largest number of cocoa butter particles and the smallest average particle size ($P < 0.05$). Subjects with the SP exhibited smallest number of cocoa butter particles and the largest average particle size ($P < 0.05$). This phenomenon can clearly prove the difference in different OPs. Specifically, the largest number of cocoa butter particles and the smallest average size of particle reflect the feature of subjects with a CP who have the highest chewing times and highest chewing rate during oral processing. On the other hand, the smallest number of cocoa butter particles and the largest average size of particle reflect the features of subjects with the SP who have the lowest chewing action and the lowest chewing rate during oral processing. At the time of maximum intensity perception (Table 6.1 and Table 6.2), subjects with the CP also presented the largest number of cocoa butter particles ($P < 0.05$), while subjects with the SP had the smallest number of cocoa butter particles ($P < 0.05$) due to the difference in the OP. However, the average size of cocoa butter

particle between the different OPs is similar ($P > 0.05$). This phenomenon reflected that saliva content during each oral preference was different. Compared to subjects with CP, subjects with MP and SP displayed longer consumption time (Table 5.1) due to their special features of oral behaviour - this resulted in a higher amount of saliva addition during oral processing, which would dilute the observed fat content.

Table 6.2 Count and particle area of cocoa butter of four types of samples with different oral preference at either time of first swallowing or time of maximum flavour intensity by ImageJ

LG	**Count**	**Average Size(μm²/pixels)**
Time of first swallow		
CP	244.33±30.52[a]	196.61±31.22[c]
MP	143.57±31.98[b]	385.25±122.17[b]
SP	90.94±22.03[c]	580.51±116.42[a]
Ti_{max}		
CP	785.55±89.03[a]	39.77±3.40[a]
MP	591.28±79.36[b]	41.565±7.92[a]
SP	424.875±82.76[c]	39.18±9.12[a]
HG	**Count**	**Average Size(μm²/pixels)**
Time of first swallow		
CP	213.61±54.87[a]	236.41±68.89[b]
MP	122.8±45.05[b]	517.34±139.16[a]
SP	116.45±25.07[b]	536.76±129.10[a]

Ti_{max}		
CP	826.16±87.60[a]	38.70±10.73[a]
MP	556.64±89.92[b]	36.92±8.79[a]
SP	426.90±95.34[c]	35.85±7.49[a]
LM	**Count**	**Average Size(μm²/pixels)**
Time of first swallow		
CP	240.27±59.39[a]	207.38±62.60[a]
MP	123.51±44.21[b]	539.38±83.88[b]
SP	118.55±34.08[b]	523.11±103.87[b]
Ti_{max}		
CP	821.51±56.49[a]	36.31±4.22[a]
MP	523.75±119.51[b]	36.77±5.49[a]
SP	419.09±86.76[c]	38.25±3.45[a]
HM	**Count**	**Average Size(μm²/pixels)**
Time of first swallow		
CP	234.11±55.41[a]	214.38±67.58[b]
MP	119.33±40.58[b]	561.28±141.42[a]
SP	117.36±24.62[b]	516.95±129.71[a]

Ti_{max}		
CP	858.41±101.81[a]	42.27±8.42[a]
MP	535.37±124.52[b]	41.83±8.08[a]
SP	382.53±67.84[c]	41.92±8.20[a]

Note: Different letters in each column denote significant differences at $P < 0.05$

In terms of the HG and LM samples with medium added flavour intensity, subjects with a CP presented the shortest first swallow time ($P<0.05$), the largest number of cocoa butter particles and the smallest average particle size ($P<0.05$). However, subjects with the SP and the MP presented a similar number of cocoa butter particles and an average particle size ($P>0.05$). As a previous study has suggested (Chapter 5), subjects with the MP easily change their oral behaviour when consuming samples with a stronger flavour (such as HG, LM and HM samples), gradually reducing their chewing time and decreasing chewing rate to reduce flavour release from the food. In this way the results for subjects with an MP tend to be similar so those of subjects with an SP. At the time of maximum intensity perception, subjects with the CP also presented the largest number of cocoa butter particles ($P<0.05$), while subjects with an SP showed smallest number of cocoa butter particle ($P<0.05$) due to the difference in OP. However, the average size of cocoa butter particle between different OP is still similar ($P>0.05$).

6.3.3 Relationship of bolus microstructure and flavour perception

In terms of a comparison of the same OP with different samples, subjects with the CP presented stability ($P>0.05$) in the number of cocoa butter particles and particle size at the time of first swallowing and Ti_{max} when they consumed samples with different added flavour intensity. A similar trend was also observed in subjects with the SP and the MP. Combining

these results with the results from the TI curve, added flavour intensity perception differs depending on the flavour concentration in each sample. The similarity ($P>0.05$) in the state of the cocoa butter (number of cocoa butter particles and average size) at the time of maximum intensity perception when subjects consumed each sample displayed a strong connection between flavour perception and transformation of food structure by oral processing. This means that the maximum intensity may correspond to a similar ($P>0.05$) microstructure of the bolus (state of cocoa butter: number of cocoa butter particles and average size). In addition, there are no significant differences ($P>0.05$) in the average size of the cocoa butter particle for subjects from different OP groups or all samples with different added flavour intensity at the time of maximum intensity perception. This result may reflect the fact that maximum flavour release from food occurs when cocoa butter area is about 35.85 to 42.27 μm^2/pixels.

It is also worth noting that there is no significant difference ($P>0.05$) on I_{max} for subjects with a CP consuming any sample of added flavour intensity. However, the I_{max} for subjects with an SP or an MP showed significant differences ($P<0.05$) between low added flavour samples (LG) and high added flavour samples (HG). According to previous studies (Chapter 5) on the effect of flavour intensity on OP; high added flavour intensity in samples influences the eating behaviour of subjects, with subjects slowing down their jaw movements (sucking and chewing rate). The performance of subjects with the CP may indicate that the change in oral behaviour may happen after $Ti_{max.}$ On the other hand, the performance of subjects with an MP or a CP indicated the change in oral behaviour may have started before $Ti_{max.}$

6.4 Conclusion

This study has demonstrated the features of each oral preference style through the TI test. During chocolate consumption, subjects with the CP perceived the highest intensity of the added flavour in the shortest time for all samples with added flavour levels, while subjects with the SP perceived lowest added flavour intensity over the longest time when they consumed samples with low and medium levels of added flavour. In addition, subjects with the MP and the SP appear to change their oral processing with an increase in added flavour concentration in the chocolate samples.

In regard to bolus structure analysis, the distribution of cocoa butter clearly represented the differences in oral processing when subjects consumed the chocolate samples. The cocoa butter particle count and average particle area could be used to easily differentiate the differences in the three OPs. In addition, the bolus structure as revealed by CLSM may have a connection with flavour perception during food oral processing. It is notable that flavour intensity can influence a subject's oral behaviour, and, in turn, the oral behaviour can be an important factor in flavour release as was seen through the analysis of TI and the bolus structure. These findings are consistent with the results of previous studies (TI, TDS and test of oral behaviour).

7 General discussion

This chapter will summarize the findings of the previous chapters and provide possible future directions for further research. First the chapter will discuss the effect of flavouring ingredients on the sensory perception of chocolate. The applicability of different methods for determining sensory perceptions will be discussed next. Finally, there will be a discussion of research that could be pursued in the future.

7.1 Effect of flavouring ingredients on the sensory evaluation of chocolate

Texture perception of chocolate mainly depends on the transformation of its microstructure during oral processing (Silva et al., 2013). Different composition (such as ratios of cocoa butter, sugar, cocoa solids or others) can significantly impact the texture of the final chocolate product. In terms of the chocolate sample used in this test, standard chocolate had different concentrations of flavouring ingredients (ginger and menthol) added to it. This modified the formulation of the standard chocolate and changed its composition.

Chocolate samples with both low and high concentrations of ginger and menthol ingredients in this study presented no sigificant difference ($P>0.05$) to standard chocolate in regard to melting point. The instrumental tests (Sections 4.3.1 & 4.3.2), showed that low concentrations of ginger and menthol in the chocolate (LG and LM) brought no significant

change to viscosity and hardness. However, samples with higher concentrations of ginger and menthol (HG and HM) presented significant differences ($P<0.05$) in these parameters compared to the standard sample. In terms of sensory perception (MQDA), no subjects could perceive a sigificant difference in the texture of any chocolate variety in terms of either smoothness, thickness or snapping (Section 4.4). In terms of the perception of *flavour* of each sample, subjects with different OPs could clearly distinguish the difference in low or high flavour intensity in the chocolate samples. It was notable that subjects with a chewing preference presented a sigificant difference to the subjects with a sucking preference or a mixed preference in perceiving the cocoa flavour intensity of LG, HG and LM (Section 4.3.4). As Afoakwa et al. (2008) have put forward, as both volatile and non-volatile flavours are released into the saliva, a profile of texture and taste is then progressively developed in the mouth during oral processing. The difference in perception of cocoa flavour intensity found in this study was due to differences in oral processing as subjects consumed the chocolate samples.

7.2 Applicability and limiations of testing methods

7.2.1 Test of oral behaviour

The subjects in this study exhibited different oral preferences when they consumed chocolate. Before the MQDA test, subjects were classified into three groups according to oral preference (Section 3.3.1). The test for oral behaviour looked at the subjectss' different oral preferences. In this test, four parameters: number of chews before first swallow, total eating time, total number of chews, and time of first swallow were recorded on video, and the data were collected by visual analysis. The results show that subjects with a chewing preference had the shortest consumption time and highest chewing rate, and subjects with the SP presented the

longest consumption time and lowest chewing rate. The interesting phenomenon found in this study was that flavour intensity in the chocolate samples affected the oral behaviour of each subject, which resulted in an extension of consumption time and a decrease in the chewing rate as Table 5.1 shows (Section 5.3.3). These results were clear across subjects with different oral preferences. However, in terms of the chewing rate before first swallow, the results showed that samples having different flavour and flavour concentration (LM, HM, LG and HG) had no significant ($P>0.05$) influence on the oral behaviour of subjects with a mixed preference in terms of their chewing rate before first swallow. This result conflicted with the results of the bolus microstructure analysis. Through analysis of bolus microstructure, MP subjects presented a significant change in their oral behaviour, which tended toward those of subjects with SP before the time of first swallow.

In terms of the bolus microstructure test (CLSM, Section 6.3.2), subjects with the MP presented a significant difference ($P<0.05$) in average size of cocoa butter particles at the time of first swallow for the LG sample compared with the other samples. The larger average size of cocoa butter particles when subjects with the MP consumed the other samples (HG, LM and HM) indicated that their oral behaviour had changed. The differences in the results between the bolus microstructure test and the oral behaviour test could reflect a limitation in the use of visual analysis of oral processing. During visual analysis, subjects' jaw movement when consuming the samples was the only parameter to determine whether they were performing a chewing action. However, movement of the sample by the tongue during oral processing often also leads to jaw movement and this can also be observed visually. This could be a limitation of the oral behaviour test.

7.2.2 Temporal dominance of sensations (TDS) test

Temporal dominance of sensations (TDS) can reveal the dominant sensations over time during food consumption (Pineau et al., 2009). In this test, the perception of five flavour factors (sweetness, bitterness, sourness, cocoa flavour, off-flavour: ginger or menthol) were used to analyse the difference in perception of each OP when subjects consumed the samples (Section 5.3.2).

According to the results, the TDS curve clearly displayed the difference between the subjects in each OP category in regard to dominant sensations during sample consumption. When consuming the standard sample (SC), subjects with the SP and MP perceived more dominant flavours ("cocoa flavour", "bitterness" and "sweetness") or a high frequency alternation of dominant flavours compared to subjects with the CP (only "cocoa flavour" and "bitterness"), as Figure 5.1 (C and E) shows (Section 5.3.2). Similar results were found when subjects consumed the LG sample. As Figure 5.1 (B,D and F) shows, the off-flavour of the LG chocolate sample was the dominant sensation for the entire consumption time for subjects with the CP. However, subjects with the MP and SP presented multiple dominant sensations (Fig 5.1 D and F) when consuming the LG sample. In addition, the dominant sensation (flavour) for subjects with the SP presented the most frequent change.

The TDS test could clearly identify the differences in dominant flavour perception for subjects with different OPs when they consumed samples with low added flavour concentration (ginger) or samples without flavouring. However, when subjects consumed samples with medium or high "off-flavour" concentrations (LM, HG and HM), the differences between OPs tended to be inconspicuous, as Figure 5.2 (B,D,F) shows. Altough, Rodrigues et al. (2016) also found that an increase in flavour ingredients (cocoa) made the dominant sensation concentrate on the flavour ingredient and presented a lower frequency in

dominant sensation change, Thereby, high flavour intensity in samples could be a limitation in the investigation of features of each oral preference.

7.2.3 Time-intensity (TI) test

The TDS test (Section 5.3.2), showed that subjects with different OPs displayed sigificant differences in dominant flavour perception over time when they consumed samples with low and medium concentrations of flavouring ingredients. This finding could be attributed to the difference in flavour peception or flavour release for the different OPs. In order to more deeply research the effect of oral preference on flavour perception, the time-intensity method was used to obtain a dynamic picture of flavor perception and its continuous change during oral processing.

As Haahr et al. (2004) and Hansson et al. (2003) reported, the frequency of masseter muscle activity (MMA) can sigificantly affect aroma intensity in the air phase during food consumption. Moreover, Phan et al. (2008) also suggested that the speed of flavour release from food was strongly relative to chewing rate, with a high chewing rate resulting in a rapid flavour release from food and a low chewing rate (long chewing cycle) resulting in slow flavour release from food during oral processing.

In this research into TI (Section 6.3.1), similar results were found. When consuming the LG sample, subjects with the CP (shortest chewing cycle) presented the shortest time to maximum intensity, shortest duration of the stimulus and highest maximum intensity during sample oral processing. Subjects with the SP (longest chewing cycle) presented the longest time to reach maximum intensity, longest duration of the stimulus and the lowest maximum intensity during sample oral processing (Table 6.1). Similar results also occurred for medium

and high flavour intensity samples. These findings are consistent with previous studies (Phan et al., 2008; Haahr et al., 2004; Hansson et al., 2003). However, it was also found that subjects with the SP and the MP appear to change their oral processing style with an increase in flavour concentratrion of added flavours in the chocolate samples. This was consistent with the results of the oral behaviour test (Section 5.3.1), where the flavour release from food influenced the eating behavior for each preference group with a decrease in chewing rate and an extension in consumption time.

The TI test clearly shows flavour perception or flavour release dynamics for subjects with different OPs. Through the TI curve, the features of each OP can be described in detail, even when subjects consumed samples with medium and high flavour intensity (HG, LM and HM).

7.2.4 Confocal Laser Scanning Microscopy (CLSM)

The processing of food in the mouth involves the breakdown of the food's initial structure by mastication and provides sufficient lubrication from saliva to allow swallowing. As seen in the literature review (Section 2.5.3) along with airflow in the mouth and food surface area, the breakdown of the food matrix has an impact on flavour release.Therefore the relationship between food structure breakdown and flavour perception by subjects should be considered.

In this test, the overall state of the bolus by subjects from the three OPs were determined by distribution of cocoa butter. Through the CLSM test, the distribution of cocoa butter clearly indicated the result of oral processing by subjects of different OPs and consumption time (Figure 6.2). Through ImagJ Fiji analysis, cocoa butter particle count and average particle area could be used to easily distinguish the differences in the three OPs. At the time of first swallow, subjects with the SP presented the smallest number of cocoa butter particles and

largest average particle size ($P < 0.05$) when consuming the sample with the low flavouring concentration ingredient (LG), however, there was no sigificant difference between subjects with the SP and subjects with the MP in the number of cocoa butter particles and average particle size when they consumed medium and high flavour intensity samples (HG, LM and HM). In addition, subjects with the CP displayed the largest number of cocoa butter particles and the smallest average particle size ($P < 0.05$). Subjects with the CP always presented the largest number of cocoa butter particles and the smallest average particle size ($P < 0.05$).

These findings are strongly consistent with the test of oral preference (Chapter 4) on oral behaviour, those subject with the CP having the highest chewing rate and largest number of cocoa butter particles and a smaller average particle size ($P<0.05$), while the subjects with the SP and MP had a lower (or no) chewinng rate and presented a smaller number of cocoa butter particles and a larger average particle size ($P<0.05$). The most interesting finding is the similarity ($P>0.05$) in average size of cocoa butter particles at Ti_{max} (Table 6.2) when consuming each type of chocolate sample suggesting a strong connection between flavour perception and food structure transformation through oral processing. This could imply that the maximum intensity subjects perceive corresponds to a similar ($P>0.05$) bolus microstructure.

Compared with other sensory tests, analysis of bolus microstructure can clearly display the state of sample transformation during oral processing. The difference in cocoa butter distribution reflected the features of different OPs. Subjects with a high chewing rate (CP) presented a large number of cocoa butter particles, with a small average size, and subjects with a low chewing rate (MP and SP) displayed a small number of cocoa butter particles with a large average size. Combining the results of the TI and bolus structure tests, a bolus with a large number of cocoa butter particles and a small average size corresponded with high

flavour intensity perception. However, similar numbers of cocoa butter particles and average size also presented a difference in flavour intensity perception (subjects with SP and MP consuming HG, LM and HM) at the time of first swallow. This also suggests that the difference in OPs is not only influencing the transformation of the food matrix during oral processing, but also influencing flavour perception due to a difference in oral behaviour.

The technology used in this test did present some limitations however. In terms of bolus collection, subjects could not easily spit out the chocolate bolus, especially subjects with the CP. As subjects with the CP have a high frequency chewing action, some of the bolus stuck to their teeth. Therefore, bolus quantity could be irregular. In addition, subjects with the SP and MP had a longer oral processing time (without swallowing) to simulate the state of the end of sample oral processing. Excessive saliva in their mouth may have changed the nature of their oral processing.

7.3 Future research

7.3.1 The effect of saliva fat on flavour perception

In this study, subjects with different OPs perceived different flavour intensities (maximum intensity, see Table 6.2) in the samples. The off-flavour (ginger or menthol) intensity perception by subjects may be influenced by different chewing rates, with subjects with high frequency chews (CP) presenting higher off-flavour intensity perception, and subjects with low frequency chews (MP and SP) presenting lower off-flavour intensity perception. However, many studies have stated that saliva plays an important role in aroma release and thus perception. As mentioned in Chapter 2 (Section 2.5.1) the functions of saliva during oral processing include; cooling hot food, cleaning food, construction of the food matrix and

providing lubrication for swallowing (Bornhorst & Singh, 2012). In addition, Taylor & Linforth (1996) stated that for fat-based foods, the change of emulsion phases during oral processing (from water-in-oil to oil-in-water) can lead to a significant change in aroma release. De Roos (2003) explained that flavour compounds can be entrapped by a lipid phase in partly solid, or fully solid, food products. Improved flavour release occurred during oral processing (when food matrix is destroyed by the chewing action and mixed with saliva). They also observed that flavour release from food is strongly connected to chewing efficiency and volume of saliva added during oral processing. Pedersen et al. (2002) also put forward that saliva can affect flavour release and perception through interaction and dilution of flavour compounds during food consumption. Haahr et al. (2004) also reported an increase in the amount of saliva in the mouth reduced the transportation of flavour compounds in chewing gum, as more flavour compounds would be retained in the aqueous phase. Perhaps more saliva could dilute the flavour compounds in chocolate, and in this way influence the perception of flavour. Guichard et al. (2017) reported that saliva properties also influence the release of volatile flavours. They found that the lipolytic activity and sodium content in saliva had a significant effect on the perception of aroma, with subjects with high lipolytic activity and low sodium content in their saliva perceiving more intense aromas. As Dawes (1981) mentioned, food smells, climatological environment and previous stimulation can affect saliva flow rate and function. Subjects with MP and SP could have more saliva produced in the consumption of chocolate samples than subjects with CP due to their longer consumption time. This may result in more interaction between aroma compounds and saliva. In addition, differences in chewing rate of the three oral preferences lead to a different rate or degree of food destruction (or transformation from water-in-oil to oil-in-water). These may be the other important factors that explain the different profiles of TI and TDS test (Chapter 5 & 6).

Therefore, saliva (amount and chemistry) and its relationship to flavour release by different OPs would be the next step in this research.

Different flavour perception may relate to the different flavour intensity in the chocolate samples or interaction between flavours during oral processing. It is possible that the different fat content in each sample influenced not only the texture perception but also flavour perception. Carrapiso (2007) stated that increase of fat content led to a decrease of aroma concentration in nosespace and headspace. Goncalves and Lannes (2010) reported that fat content significantly influenced rheological behaviour of chocolate, and flavour release and persistence of flavour in the mouth are related to the rheological behaviour of chocolate. They found that the persistence of flavour compounds in the mouth may be extended by an increase in the viscosity of the chocolate bolus. Samples (LM, HM, LG and HG) with lower fat content could have enhanced the perception of aroma flavour, which in turn limited the perception of other flavours. In addition, some studies also pay more attention to research on interactions between fat and aroma compounds, since most of these compounds can be more easily solubilized in fat rather than water due to their hydrophobic properties (Guichard et al. 2008; Roudnitzky et al. 2003; Guichard et al. 2018). The concentration of aroma compounds in fat influences their release into the air phase during oral processing, and this will influence perception. In addition, aroma release is also influenced by the nature of fat and its physico-chemical properties. Guichard et al. (2008) observed that compared with milk fat, ethyl hexanoate presented better release in palm kernel oil. However, diacetyl with less hydrophobic compound displayed the opposite behaviour. Moreover, Roudnitzky et al. (2003) stated that the melting point of fat also influenced aroma release. In this study, all the samples contain the same fat (cocoa butter), while the content of fat in samples are slightly different due to different concentration of flavouring ingredients added to the samples (extra ingredients diluted the concentration of original ingredients in chocolate). In addition, the two

flavouring ingredients (menthol and ginger) have different physico-chemical properties (fat soluble and water soluble respectively). This may have led to the difference in flavour perception during oral processing. Thereby, the interaction between fat and flavour ingredients is also an important part of future research.

7.3.2 Improvement of sensory perception methods

7.3.2.1 Retronasal aroma trapping device (RATD)

Temporal dominance of sensations (TDS) and time-intensity (TI) sensory methodology in this research provided a dynamic picture of dominant flavour perception and overall flavour perception over time during oral processing. However, the results of both these sensory methodologies were based on the subjects' own judgement. This could be subject to errors in the results when looking at the intensity of flavour in chocolate samples, especially with untrained subjects. In addition, subjects needed to undertake some additional actions (not just oral processing) when they are carrying out the TDS and TI tests. These extra operations could also influence subjects' oral processing.

In recent years, studies in vivo experiments have been designed to trap the volatile organic compounds (VOCs) in order to gain insight into the effect of oral processing, oral physiology and the food matrix on the aroma compounds released from food during consumption (Munoz-Gonzalez et al., 2014; Bonneau et al., 2018). The simplest device for laboratory testing and the most widely used method to trap the VOCs is the retronasal aroma trapping device (RATD), as shown in Figure 7.1.

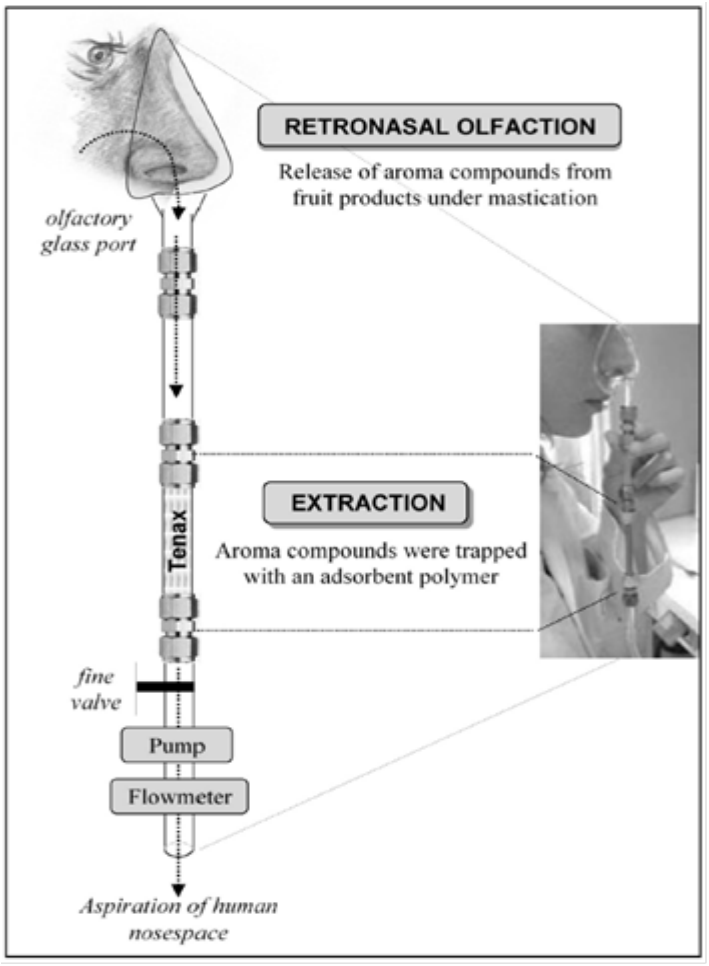


Figure .7.1：Retronasal aroma trapping device (Source: Bonneau et al., 20018)

The RATD consists of three basic components, the olfactory glass port, trap and pumping devices. Subjects first place their nose in the glass port and exhale through the nose during sample consumption. The air flow of the breath from the nasal cavity is transported into a removable trap connected to the glass port. The trap is usually filled with an adsorbent polymer, and the aroma compounds in the air flow are adsorbed. In order to ensure a steady air flow during aroma trapping, a flowmeter with a rotameter are connected to the end of the device. After the test, the adsorbent polymer is removed from the trap component and transferred to a gas chromatography-mass spectrometry (GC-MS) instrument for analysis of the aroma compounds.

Compared with TDS and TI, the operation of a RATD is simpler for subjects when they are conducting the test, though more intrusive. Subjects only need to place their nose into the olfactory glass port when they are consuming samples and no other action is required of them. In addition, this methodology can help to build a connection between flavour perception and flavour release during oral processing as it can provide more objective results, from subjects with different subjective emotions, than TI and TDS. In terms of research on flavour release during oral processing, proton transfer reaction mass spectrometry (PTR-MS) is also a widely used and effective method for monitoring of VOCs (Farneti et al., 2012). The main mechanism of PTR-MS is using H_3O^+ to detect VOCs by transferring H^+ (on H_3O^+) onto the VOC, and then the $VOC{\cdot}H^+$ is detected by mass analysis (Boamfa et al., 2004; Granitto er al., 2007; Frank et al., 2011). In general, PTR-MS mainly consists of four components:

1. Sample inlet: draw the detected gas into the collision dissociation chamber.

2. Ion source: produce H_3O^+ ions.

3. Collision dissociation chamber: for reaction between VOCs and H_3O^+ ions

4. Mass analyser: identify trace compounds and determine their concentration.

Compared with the RATD method, PTR-MS has a lot of advantages. Firstly, this method can detect the VOCs in real-time (Granitto er al., 2007). During oral processing, the flavour release is a dynamic process due to the dynamic change in the environment of the oral cavity and food structure. Thereby, real-time detection can display a better profile of aroma release from food, and it can easily be linked to results from TI and TDS testing (which display a dynamic flavour perception). In addition, PTR-MS can directly trap the VOCs without any complex operation for sample preparation, as compared with RATD (Farneti et al., 2012).

7.3.2.2 Other sensory technologies

During the oral behaviour test, the chews and swallowing action as subjects consumed the samples were recorded by researcher observation. This may result in some errors in judgement of jaw movement as was mentioned in Section 7.2.1. In order to obtain more accurate results in regard to oral behaviour, other techniques to monitor chewing action during oral processing should be considered. Electromyography (EMG) is a tool that records muscle movement, and it has found wide application in the investigation of oral behaviour (Farfan et al., 2010; Iguchi et al., 2015). It can monitor the activities of muscles during food consumption by attaching electrodes onto a subject's face (Reaz et al., 2006). Through analysis of the EMG signal, subjects' chewing action can be identified. Although this technique is more accurate than outside visual observation, there are issues of signal-to-noise ratio and distortion of the signal and these need to be considered.

8 Conclusion

This chapter summarises and integrates the findings presented in this thesis.

In recent years, chocolate producers have been influenced by increased demands for healthy or functional products. The main contribution of this thesis focuses on the effect of different oral processing preference on flavour perception during oral processing. In addition, it may provide a potential guide to development of functional chocolate for manufacturers.

One of the main findings of this study is that a subject's oral preference (OP) be an important factor impacting on flavour perception. For the design of the study of chocolate with new formulations, extra flavouring ingredients were added to chocolate samples and resulted in a change in rheological behaviour and texture of the final products. Even where added ingredients were found to change the rheological and mechanical properties of the chocolate samples in this study, consumers were not able to distinguish any textural change. This indicates that the flavour of ingredients could be more notable for overall sensory perception of a final product and this could be a challenge for functional chocolate development for manufacturers. In addition, flavour perception was influenced by subjects' OPs, with subjects with a chewing preference perceiving higher intensity of cocoa flavour than the other groups. This is related to the difference of each OP in the release of flavour.

The three OPs presented different characteristics during oral processing, with the CP presenting, the highest chewing rate and the shortest consumption time, while the SP, displaying the lowest chewing rate and the longest consumption time. This difference between the OP groups influenced the most dominant perception during consumption, with

subjects with a higher chewing rate and shorter consumption time perceiving less alternation in dominant flavour, and subjects with a lower chewing rate and longer consumption time displaying more alternation in dominant flavours during oral processing. In addition, subjects with the highest chewing rate perceived the highest intensity of flavour over the shortest time, while subjects with lower chewing rates perceived maximum flavour intensity over the longest time. These results indicated that difference in OP significantly influenced individual flavour perception, and this explains the difference in flavour perception of consumers of the same product. In addition, it is interesting to note that a food's flavour intensity can influence a subject's oral behaviour, leading to a decrease in chewing rate and prolonged consumption time.

In terms of bolus microstructure research, the distribution of cocoa butter clearly displayed differences between the OP groups when subjects consumed chocolate samples. In addition, the structure of the bolus (cocoa butter particle count and average particle area) showed a strong correlation with subjects' flavour perception. More specifically, boluses with a large number of cocoa butter particle and a small average size (produced by subjects with high chewing rate) corresponded with high flavour intensity perception. A bolus with a smaller number of cocoa butter particles with a larger average size (produced by subjects with a low chewing rate) correspond to low flavour intensity perception. However, similar bolus structures, produced by subjects with different OPs, also presented differences in flavour intensity perception. This reflects the fact that flavour perception is contributed to by the combination of the transformation of the food matrix and oral behaviour during oral processing. On the other hand, flavour intensity (especially strong flavour intensity) in food can also influence a subject's oral behaviour, which results in a change in chewing rate and bolus structure.

Overall, people's oral preference (OP) presented a significant influenced on flavour perception of chocolate products. The difference OPs presented different features in oral behaviour, and it resulted in different oral environments (different airflow in the mouth) and bolus formation time (food degradation in the mouth). The addition of flavour ingredients (or functional ingredients with a strong flavour) into food products should also be considered in the context of consumer oral preference in order to obtain higher acceptability of overall sensory perception.

Appendices

Appendix A

Questionnaire of MQDA test

Session 1

Date: ……

Gender: ……. Age: ……

BMI: …… (Formula: BMI= Weight in Kg/ Height in Metres squared)

INSTRUCTIONS 1

1) Eat the sample

2) Wash your mouth out with water provided

3) You may re-taste the sample as many times as you would like until you are satisfied you have identified as many descriptors as you can

4) Write descriptors on line scales as shown in INSTRUCTIONS – PART 2

INSTRUCTIONS 2

Date: ……. Session Number: …….

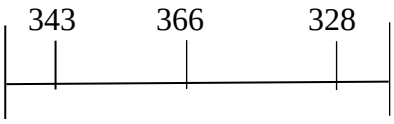
INSTRUCTIONS - PART 2

1) Draw a line on the scale that represents where you rate each sample.

2) Above each line you draw, write the number that corresponds to that sample

Sweetness

Example



SENSORY EVALUATION STARTS HERE

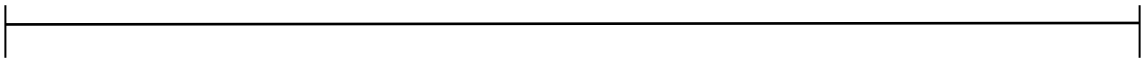
Section1: Select your chocolate eating behaviour

1. Chewing 2. Suck 3. Chewing and suck

Section 2

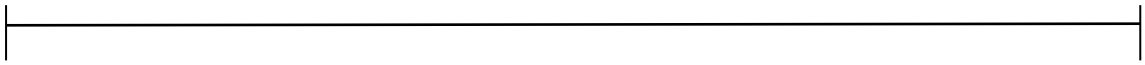
AROMA and FLAVOUR

1) Earthiness (cocoa intensity)



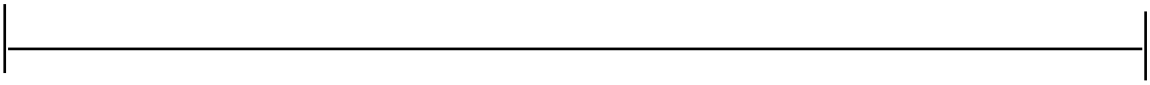
Low High

2) Persistence of flavours on the palate



Short long

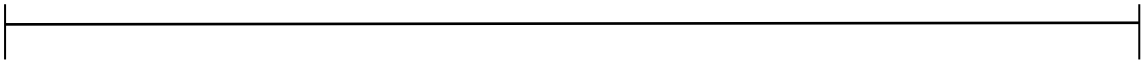
3)……. Off-flavour intensity (depend on which flavours or ingredients will be added into chocolate)



Low High

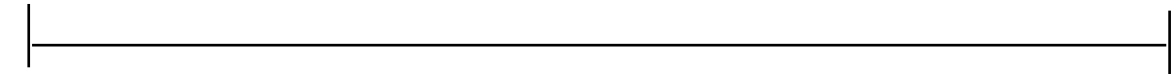
PALATE (Texture)

4) Smoothness



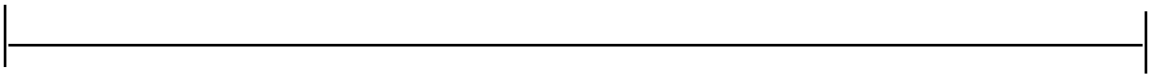
Low High

5)Thickness



Low High

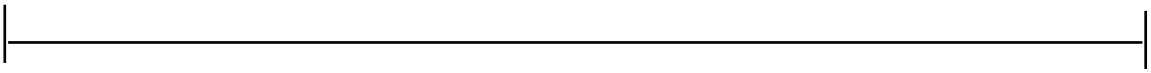
6) Snapping



Low High

Preference

7) Preference for aroma and flavour (flavour intensity acceptability)



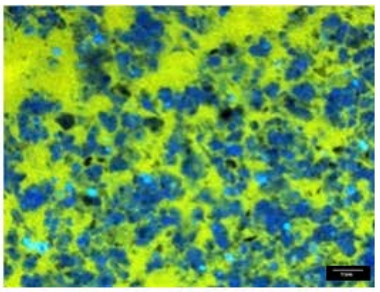
Dislike Like very much

Approved by the University of Auckland Human Participants Ethics Committee on 11-Oct-2017 for three years.

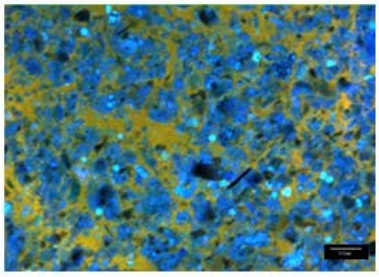
Reference Number: UAHPEC 021267

Appendix B

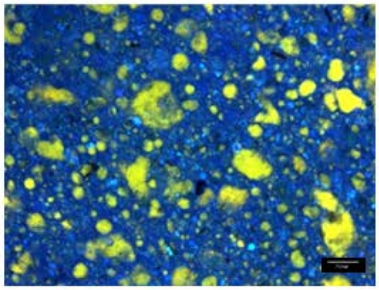
Example of transformation of microstructure of bolus (CP)



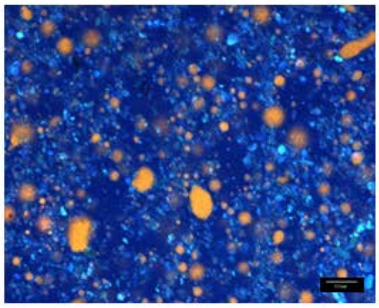
10% consumption time



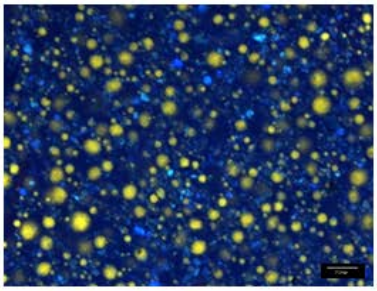
25% consumption time



50% consumption time

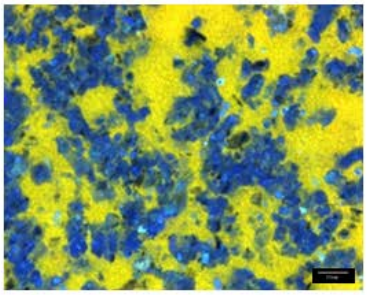


65% consumption time

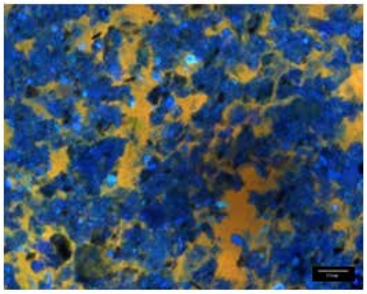


80% consumption time

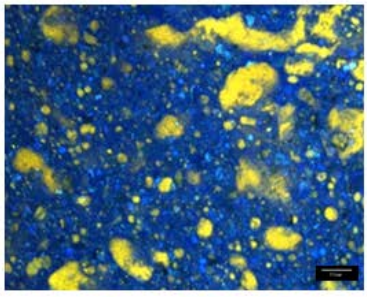
Example of transformation of microstructure of bolus (MP)



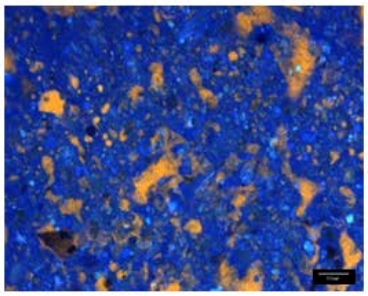
10% consumption time



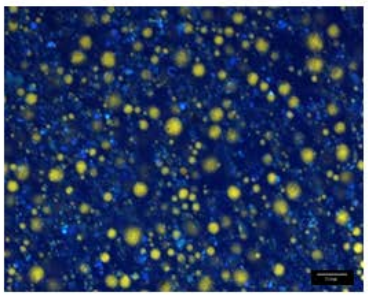
25% consumption time



50% consumption time

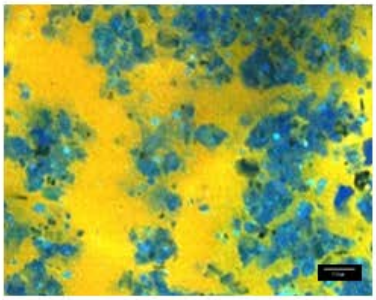


65% consumption time

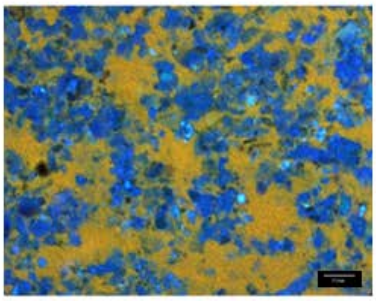


80% consumption time

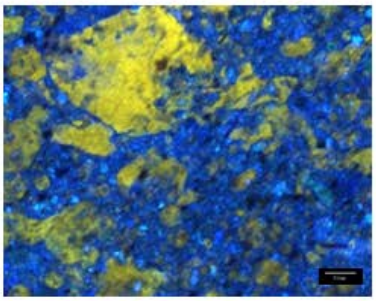
Example of transformation of microstructure of bolus (SP)



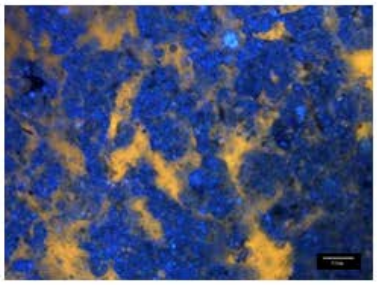
10% consumption time



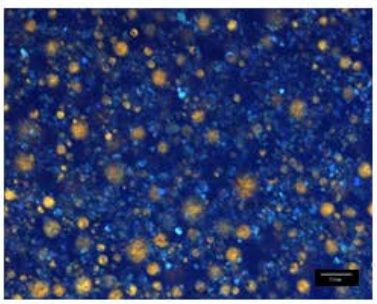
25% consumption time



50% consumption time

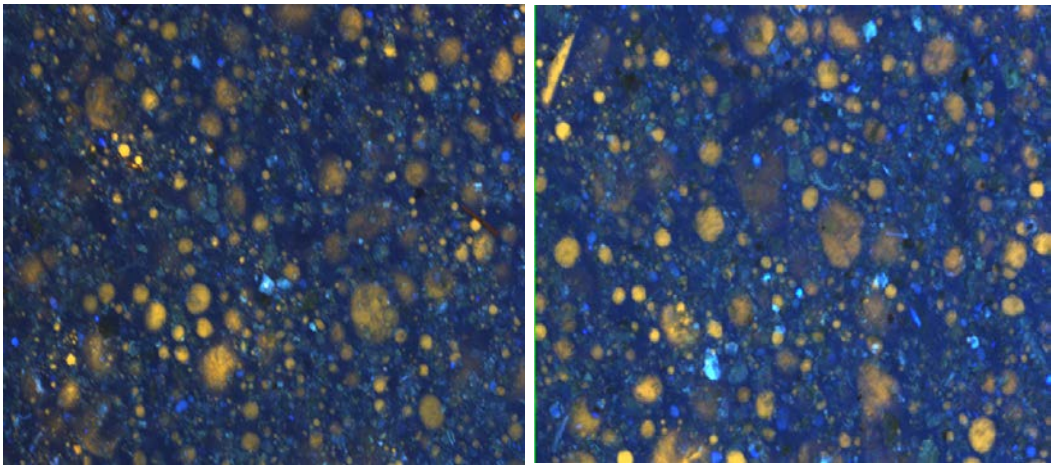


65% consumption time



80% consumption time

Appendix C



Example for CLSM image of standard chocolate bolus at the time before swallowing by chewing preferences; Yellow area: Cocoa butter, Dark blue area: saliva, Light blue/aqua: cocoa solids, Black surround: air bubble. Sample with treatment (heating and cooling) on left and the sample without treatment on right.

Table: Average count and size of standard chocolate (treatment and non-treatment) by chewing preference at the time before swallowing.

Standard (Chewing)	Count	Average Size(μm²/pixels)
Time before swallowing		
Treatment	901.16±103.44[a]	34.39±9.26[a]
Without treatment	866.90±92.13[a]	38.25±7.53[a]

Note: different roman letters in each column donate significant differences at $P < 0.05$

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