# RELATING SENSORY PERCEPTION TO CHEWING DYNAMICS

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## ABSTRACT

Understanding the mechanism behind the dynamic changes of food structure during oral processing is the key area for food texture studies. Food texture is a sensory perception derived from the structure of food, and oral processing plays an important role in this perception. This research aimed to establish a method to explore the relationships between oral processing and sensory perception, using biscuits of varying compositional and structural properties as a model food system.

The initial study verified the capability of the Temporal Dominance of Sensations (TDS) technique to describe the textural aspects of a model food system whose structural properties change throughout oral processing. By standardizing the time-axis of the TDS curves from first bite to swallow, the technique was able to discriminate the textural properties of the samples of different sugar to fat ratios over the consumption period. The key differences of this type of standardization method were the attribute dominance rates and range in times to select the first dominant attribute. Moderate training of panellists on the definitions of the attributes showed performance improvement; clearer TDS curves (higher dominance rates) and reduced times to make the first dominant attribute selection (at least 10% faster). It was observed that subjects used a greater number of chewing cycles to process a food sample when they were also performing a sensory task such as TDS. The observation holds regardless of the level of training.

When the TDS task was performed initially (the first sample), subjects need time to explore and learn the task hence slower chew frequency. This effect can be eliminated by introducing warm up samples before each session to ensure familiarity to samples and task. Overall, the samples were discriminated in their textural properties throughout chewing. The TDS technique appeared to be relevant to relate to the changing food properties in the mouth. The hardness levels of the sample marked an influence at the early stage of a chewing sequence, influencing the first dominant attributed selected and the oral processing. As food evolves during oral processing, other associated attributes become dominant in response to changing structural properties in the mouth. All samples undergo various structural changes in the mouth before reaching a definite state

before swallowing. Two types of masticatory adaptations were present; adaptation to the task performed and adaptation to the altered textural properties in the mouth.

The reproducibility of the TDS curves was also performed. This study demonstrated that for a food sample with five attributes to be evaluated in triplicate, at least 10 subjects were needed (i.e. 30 observations). This is true as the TDS technique is based on food evolution in the mouth. Further exploration of the TDS technique was performed using Discrete Point TDS. The technique offered new information that the typical TDS technique could not. The method was capable of differentiating the dominant attribute at each specific stage during mastication. This is not measurable with the conventional TDS technique and is less time consuming. In addition, intensity scores were found to complement the standard TDS data.

The present study also showed the need to combine the TDS technique with masticatory recordings to investigate the dynamic mechanisms of the food behaviours throughout food oral processing. The simultaneous recording of the TDS technique, electromyography (EMG), and electromagnetic articulograph (EMA) confirmed that human masticatory apparatus adapted (chew frequency) to the altered textural properties caused by changing food sample composition and structure which also continued to evolve in the mouth. Evaluation of dynamic changes in sensory perception at various mastication stages helped in explaining the food evolution in the mouth and its responding oral processing strategies. The early chewing stage was dedicated to the fracture mechanism of the food where attributes such as *hard* and *crunchy/crispy* were most dominant. Mid chewing was dedicated to effort used to masticate food into a bolus which was suitable for swallowing; this included effort to reduce food particle sizes and incorporate saliva and the attributes *crumbly* and *dry* were most dominant. The end of chewing was dedicated to removing food materials from around the mouth for swallow, where *sticky* was most dominant. These associations supported the hypothesis that masticatory parameters are controlled by the sensory input and are linked to food properties, where a range of different food structure is responsible for the changes in the chewing strategies.

Findings from this research demonstrated the strong correlation between the TDS profiles and chewing dynamics provided a new and improved technique for the food industry, in particular for designing foods with desired sensory properties. Moreover, the study confirmed that a complete understanding of texture can only be obtained through collaboration among different disciplines.

# ACKNOWLEDGEMENTS

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## TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

# LIST OF PUBLICATIONS AND PRESENTATIONS

## Peer-Reviewed Publications

1. Ashley K. Young, Jean Ne Cheong, Duncan I. Hedderley, Marco P. Morgenstern and Bryony J. James. (2013). Understanding the Link between Bolus Properties and Perceived Texture. *Journal of Texture Studies. 44*, 376-386.

2. Foster KD, Grigor JM, Cheong JN, Yoo MJ, Bronlund JE and Morgenstern MP. (2011) The Role of Oral Processing in Dynamic Sensory Perception. *Journal of Food Science. 76*(2), R49-R69.

## Conference Presentations

1. Jean Ne Cheong, Kylie D. Foster, John M.V. Grigor, John. E. Bronlund and Marco P. Morgenstern. *Development of a Dynamic Sensory Technique for Relating Texture Perception to Oral Processing Behaviour*. 2nd International Conference on Food Oral Processing - Physics, Physiology, and Psychology of Eating. Beaune, France. 1st - 5th July 2012 (*Oral Presentation*).
2. Jean Ne Cheong, Kylie D. Foster, John M.V. Grigor, John. E. Bronlund and Marco P. Morgenstern. *The Effect of Sensory Training on Dynamic Sensory Perception and Chewing Behaviour*. Biomouth 2011. Palmerston North, New Zealand. 28th - 29th November 2011 (*Oral Presentation*).
3. Jean Ne Cheong, Kylie D. Foster, John M.V. Grigor, John. E. Bronlund and Marco P. Morgenstern. *Dynamic Texture Perception of Solid Foods*. The NZIFST Conference - Science to Reality: New Zealand and Beyond. Rotorua, New Zealand. 27th June - 1st July 2011 (*Oral Presentation*).
4. Jean Ne Cheong, Kylie D. Foster, John M.V. Grigor, John. E. Bronlund and Marco P. Morgenstern. *The Use of Temporal Dominance of Sensation (TDS) for Dynamically Evaluating Biscuits with Small Structural Variations*. 5th Annual New Zealand and Australia Sensory Symposium, The New Zealand Institute of Food Science and Technology. Christchurch, New Zealand. 8th - 9th February 2011 (*Oral Presentation*).
5. Jean Ne Cheong, Kylie D. Foster, John M.V. Grigor, John. E. Bronlund and Marco P. Morgenstern. *A Multidisciplinary Approach to Investigate Dynamic Sensory Perception*. 10th Annual Functional Foods Symposium - Brains, Bounce & Baby Boomers. University Of Auckland, Auckland, New Zealand. 16th November 2010. P13 (*Poster Presentation*).
6. Jean Ne Cheong, Kylie D. Foster, John M.V. Grigor, John. E. Bronlund and Marco P. Morgenstern. *An Improved Approach to Investigate Human Masticatory Behavior and Sensory Perception*. International Conference On Food Oral Processing - Physics, Physiology And Psychology Of Eating 2010. University Of Leeds, Leeds, United Kingdom. 5th -7th July 2010. P74 (*Poster Presentation*).

# CHAPTER 1

# INTRODUCTION

Food textural properties are perceived during the complex process of eating. Texture assessments are made throughout the chewing process and, alongside flavour assessments are highly influential in the overall sensory experience (compared to tactile or visual assessments). From acquisition to swallowing, food is manipulated continuously in the mouth and modulated by the changing food bolus properties, thus influencing what sensations are perceived. These sensations change in intensity and dominate at different times as bolus properties change throughout oral processing (Pineau et al., 2009).

Only by understanding how food behaves in the mouth and what perception are perceived can the link between food properties, oral processing strategies, and perceived sensations be fully understood. Techniques exist for exploring each area in isolation. However, exploring both simultaneously requires more consideration. Hence, a multidisciplinary approach is useful in understanding the relationship between eating physiology, food structure, and sensory responses.

The search for a better understanding of how food behaves in the mouth revealed the importance of research into oral processing (Chen, 2009). Taking into account all factors affecting texture perceptions, the knowledge from different disciplines needs to be combined. The properties of food (structure), the structural breakdown properties (rheological and mechanical properties), the process of food breakdown in the mouth (oral physiology), and the sensations perceived (sensory science) are the main factors defining texture perception (van Vliet, van Aken, de Jongh, & Hamer, 2009).

A relatively new dynamic method, Temporal Dominance of Sensations (TDS) makes it possible to evaluate the changing responses of several attributes simultaneously during food chewing (Pineau et al., 2009). Information obtained from the TDS technique, when combined with masticatory behaviour measurements may provide useful links between food sensory perception and structure. This thesis hypothesized that a range of different food structures may be responsible for changes in the chewing strategies used at different points during oral processing. Hence, it is critical to understand how food structure is broken down in the mouth, where food texture is sensed, and how food texture is perceived.

The main aim of this research was to further understand relationships between sensory perception and oral processing. Specific objectives were to:

1. Investigate the capability of using the TDS technique to describe the structural aspects of model foods for food that has one or more sensory properties,
2. Investigate whether the process of dynamic sensory judgment affects normal human oral processing behaviours,
3. Investigate whether moderate training has an effect on both the dynamic sensory judgment and oral processing behaviours,
4. Investigate the use of the TDS technique to understand chewing dynamics, and
5. Investigate masticatory parameters most appropriate for relating food structure to textural measures.

This PhD thesis has been organized in the following ways: Chapter 2 reviewed the literature with a focus on the relationship between the mechanical properties of foods and texture properties during the dynamic chewing process in humans. The review concluded that the relationship has not been investigated adequately and there has been little attempt to understand the dynamic changes at a fundamental level. The remainder of this thesis is reported as a series of experiments exploring the need for multidisciplinary techniques to understand the link between sensory perceptions and chewing dynamics. The first experiment examined the effect of performing the dynamic sensory evaluation (Temporal Dominance of Sensations, TDS) on human oral processing behaviours (Chapter 4). The second experiment studied the development of the sensory technique (Chapter 5). Further work explored the potential of providing sensory attribute training to subjects for better subject performance (Chapter 6). An alternative way of collecting the sensory perceptions was explored using paper format (Chapter 7). The experimental work (Chapter 8) was then extended to measuring the masseter muscle activities by surface electromyography (EMG) and recording of the mandibular movement by tracing the trajectory of the lower incisor (using the electromagnetic articulograph, EMA). Finally, the simultaneous recordings verified the relationship between chewing strategies and food texture.

# CHAPTER 2

# LITERATURE REVIEW

The objective of this literature review is to provide background on the importance of investigating the effect of sensory sensations related to solid food systems on human mastication behaviours. van Vliet et al. (2009) have suggested the importance of having a thorough background in fracture mechanics, the interaction of saliva with products, oral physiology and sensory sciences in order to have a thorough understanding of the texture perception of solid foods. This review of oral processing is written with the main focus on food texture, with special reference to solid foods.

## 2.1 The Importance of Texture

Today, texture is no longer looked at as the absence of defects, but as a positive quality attribute. Texture is one of the main contributors to food quality, acceptability, identity, and human's enjoyment of eating foods besides other main sensory factors; appearance, taste, and aroma (Bourne, 2002; Engelen & van der Bilt, 2008). Szczesniak (1971) published results from a word-association-test given to 151 consumers, showed that the frequency of responses related to texture was higher than flavour, and was more important for solid foods than for liquid foods. Not only is texture important for food appreciation, but also for recognition of food and often used as an indicator of food quality. The acceptability of the textural attributes depends entirely on the food of interest. Soft and dry apples (Ioannides et al., 2009), soggy cornflakes, thin chocolate mousse, tough steak or wilted spinach are associated to bad texture whereas soft and air hollandaise sauce is associated with very good texture. In addition, texture is also used to determine if a food is safe for transportation to the digestive system without accident (Nishinari, 2004). A safe swallow food bolus needs to have suitable rheological and surface properties, particle size, and water content (Lucas & Luke, 1986; Prinz & Lucas, 1995; Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Chen & Lolivret, 2010; Loret et al., 2011). The ease to swallow has been associated with the length of oral residence time in the mouth (time from ingestion till swallowing) in particular foods that may require extensive mastication to reduce food sizes (Chen & Lolivret, 2010).

Food texture basically refers to food structure (Hutchings & Lillford, 1988). The structure of a food appears to be the main factor determining textural perception (van Vliet et al., 2009). Structure means “the nature and relationship between component parts of a body or material” (Jowitt, 1974). Since texture is one of the most important determinants for consumer acceptance, the knowledge of how food structure influences sensory texture assessment is of great interest.

### 2.1.1 Definition of Food Texture

Texture is mostly perceived when food is transformed in the mouth, which includes particles being reduced and saliva added to form a bolus of many sizes, shapes, and consistency. Therefore, texture can be defined in many different aspects. Among definitions available from literature, texture can be defined as “the attribute of a substance resulting from a combination of physical properties as perceived by the senses of touch (including mouthfeel and kinaesthesis which includes muscle and tendon), sight, and hearing” (Jowitt, 1974). The International Standards Organization (5492, 1992) defined texture as “all the mechanical, geometrical, and surface attributes of a product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory”. The definition from Szczesniak (2002) stated that “texture is the sensory and functional manifestation of the structural, mechanical, and surface properties of foods detected through the sense of vision, hearing, touch, and kinesthetics”. Nevertheless, these definitions point towards texture as a sensory property that can be described with multi-parameter attributes, detected by few human senses, which is derived from food structure with regards to the way the material is handled and felt (in the mouth or responses by muscles and tendons). All these definitions also pointed out that the geometry (the physical structure of the material) and the mechanical and surface properties (the way the material handles and feels in the mouth) were key elements of texture (Szczesniak, 1963). As a result, there is a need to standardize or control interrelated properties if only one of the textural elements is studied in isolation.

### 2.1.2 Food Texture Perception

The texture of a product is perceived by the sense of sight, touch, and sound. In some products, only one sense is used to perceive food texture, but in other cases, texture is perceived by a combination of a number of senses (Lawless & Heymann, 1998). Since texture is made up of many attributes, different types of foods may be described with different vocabularies, during different eating events (from its journey from the plate to the stomach). Although some textural properties can also be performed visually or by manual manipulation, the main evaluation occurs in the mouth, which is the focus of this thesis. Prior to food ingestion, food texture information can be gathered through visual, tactile, and auditory stimuli. Visual inspection of appearance includes color, size, shape or surface features. Tactile inspection includes manual manipulation with hands or tools (i.e. knife, fork or spoon).

Liquid Foods

In the mouth, liquid foods do not require fragmentation by the teeth and are deformed primarily using the tongue by compressing the food material towards the palate and then to the back of the mouth. Under this condition, textural characteristic such as thickness, viscosity, sliminess, creaminess, fattiness, roughness, and stickiness are perceived (van Vliet, 2002). These characteristics are often found in milk, soup, beverages, and honey.

Semi-Solid Foods

Semi-solid foods are processed depending on initial properties. More solid like products are bitten off by the incisors before being further processed between the tongue and palate; soft solids are processed directly on the tongue-palate (Foegeding, Cakir, & Koc, 2010). Important sensory attributes for these products are firm, soft, creamy, sticky, spread-ability, slippery, thin, and thick (Prinz & de Wijk, 2007; van Vliet et al., 2009). These characteristics are often present in yoghurt, scrambled egg, puree, and pudding.

Solid Foods

Solid foods require more chewing actions such as first bite, chewing, transportation, bolus formation, and swallowing. During the first bite of a food, textural characteristics related to mechanical properties of food are detected (i.e. hardness, crispness, brittleness, plasticity) (Brown, Langley, & Braxton, 1998b; van Vliet et al., 2009). As the food is manipulated and food sizes are reduced, saliva is added to facilitate bolus formation. During this condition, textural characteristics related to bolus particle size and adhesions to the oral surfaces are detected (Peyron et al., 2011). After swallowing, the remaining residue adhered to the tongue, teeth or palate in the mouth creates a mouth-coating sensation (Foegeding et al., 2010). These characteristics are often found in biscuit, potato, cheese, and sweet.

### 2.1.3 Texture Perception Measurement

Texture is the most important sensory characteristic in solid foods and can be measured by sensory evaluation or instrumental methods (Bourne, 2002). Human subjects evaluate the sensory and physical properties of the food, and the method is often compared with instrumental analysis describing the physicochemical properties of food (Ross, 2009).

In solid foods, texture (mechanical parameters) is generally measured using compression or cutting tests on an Instron Universal Testing Machine (Wang & Stohler, 1990; Foster, Woda, & Peyron, 2006; Kim et al., 2009) or a Texture Analyzer (Hutchings et al., 2009) which measures force and deformation over time. Texture Profile Analysis (TPA) is most commonly used to predict texture sensations including hardness, firmness, chewiness, gumminess, adhesiveness, crumbliness, and elasticity (Szczesniak, 2002; Kim et al., 2009). Although these instrumental methods can generate parameters which can correlate to sensory terms in some cases (Bourne, 2002; Peyron et al., 2009), the method is often poorly correlated with sensory evaluation as instruments generally use a single measurement (with maximum two compressions representing two initial bites) and fails to mimic the complexity of the dynamic nature of food processing in the mouth (e.g. changes in temperature and moisture) (Brown, 1994; Rosenthal, 1999). The initial bite is an important aspect of texture perception and provides information on the mechanical parameters of the food. However, it represents only 2 - 10% of the total mastication time (Bourne, 1975), whereas with subsequent chews, other textural sensations can become more apparent making the above mentioned instrumental analysis redundant. It is worth noting that Rosenthal (1999) pointed out that many sensory sensations cannot be related to a single physical property of the food in a simple way. To make the analysis more complicated, these sensory sensations change from chew to chew throughout oral processing.

Hutchings and Lillford (1988) explained the dynamic aspects of texture assessment in the mouth with a three-dimensional model. The model consists of three axes; degree of structure, degree of lubrication, and time. Each food example follows its own breakdown pathway within the three dimensions during oral processing. This approach to characterize the perception of food texture has turned texture appreciation from a static process to a dynamic one. This model has been proposed since 1988, but there has been no follow-on research and lack of exploration using the model. The textural complexity of solid foods, the lack of knowledge of food oral processing breakdown, and the lack of techniques to characterize and quantify the three dimensions could be the reason for the lack of usage of the model (Chen, 2009).

Most sensations associated with food texture occur only when food is manipulated, deformed or moved across oral receptors, and this makes the mouth a very challenging system to mimic. In an effort to take this into consideration, many researchers have tried making more physiologically adapted measurements.

### 2.1.4 Physiological Measurement of Eating Behaviours

The most common method to record masticatory function has been the combination of kinesiography and electromyography (EMG) recordings. The former gives information about jaw movements and the latter follows the bioelectrical activities of masticatory muscles which are known to be closely related to the forces developed during mastication (Boyar & Kilcast, 1986a; Brown, 1994). These simultaneous recordings make it possible to study the dynamics of chewing of various cycles and stages during mastication.

Human mastication behaviour can be studied by following the mandibular movements during a masticatory sequence. Each sequence is made of a succession of chewing cycles and each cycle is formed by one jaw-opening followed by one-jaw closing movement (downward followed by an upward movement). Because of the regular/repetitive succession of cycles, rhythm is a major characteristic of mastication (Woda, Foster, Mishellany, & Peyron, 2006a). The rhythmical jaw movements of repeating chewing cycles are known to be of many rates and directions (Hiiemae, 2004) dependent on individuals, mastication stages, and the type and state of food in the mouth (Mioche, Bourdiol, Martin, & Noel, 1999; Lucas, Prinz, Agrawal, & Bruce, 2004; Mishellany, Woda, Labas, & Peyron, 2006). In humans, opening movements during chewing cycles are generally vertical while closing movements contain a strong horizontal component (Agrawal, Lucas, & Bruce, 2000).

Mandibular movements can be studied by following the trajectory movement of the teeth with a sensor or marker attached to the lower incisor. A typical chewing cycle in a repetitive jaw movement sequence starts with slow opening followed by fast opening, and ends with fast closing followed by slow closing (Figure 2.1). In between these phases, teeth come in contact (occlusion phase) with food which slows the closing phase. The slow closing phase allows feedback from mechanoreceptors sensing the food structure via the central pattern generator (CPG) to decide and/or alter the subsequent chewing strategy (Agrawal, Lucas, Bruce, & Prinz, 1998) whilst assessing texture. During the occlusion phase, early closing period, the jaw moves laterally to one side of the mouth to fracture food into small particles (Lucas, 2004) and the pathway is more centered during the later closing stage. As time passes for a chewing sequence, the amplitudes of the tooth trajectory decrease (Hiiemae, 2004). At the end of a closing phase is the occlusion phase, which is followed by the beginning of an opening phase. During the occlusion phase, the tongue plays an important role by catching falling food, mixing the food with saliva, and pressing it against the hard palate for sensory analysis.

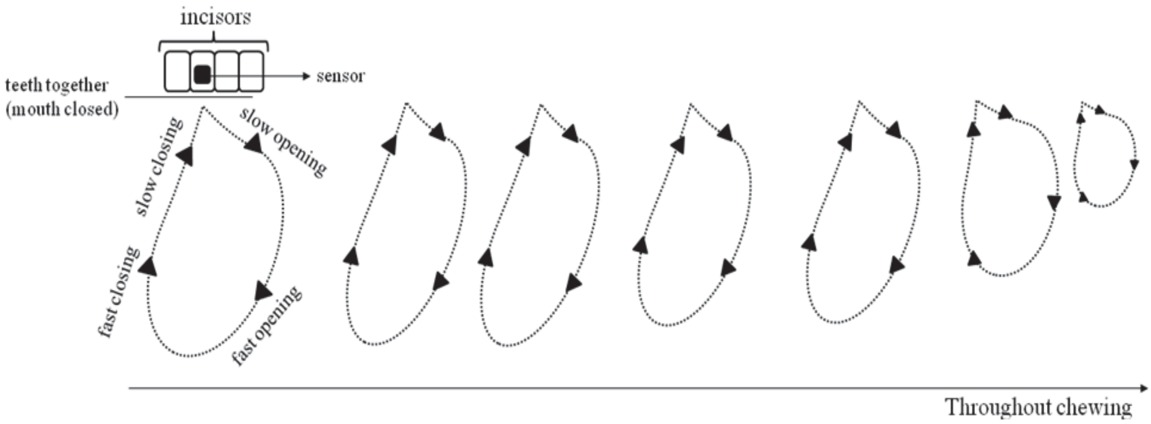



**Figure 2.1: Typical tooth trajectory of a point on the mandible (such as the incisor) throughout a chewing sequence**

Recording of Jaw Movements

The Electromagnetic Articulograph (EMA) has become one of the major techniques in tracking jaw movement during oral processing (Peyron, Mioche, Renon, & Abouelkaram, 1996; Lassauzay, Peyron, Albuisson, Dransfield, & Woda, 2000; Karkazis, 2002; Kohyama & Mioche, 2004; Foster et al., 2006). Despite high variability among individuals, these studies highlighted the importance of studying jaw movements in the oral processing of food. It has been shown that the masticatory process is influenced by food properties, including the size of food sample (Thexton, Hiiemae, & Crompton, 1980), its hardness (Peyron, Maskawi, Woda, Tanguay, & Lund, 1997; Peyron, Lassauzay, & Woda, 2002), and other physical properties (Foster et al., 2006) that cause texture sensations.

Figure 2.2 displays common EMA parameters derived from jaw tracking. The amplitudes (mm/s) are reported in terms of opening and closing velocities (OV and CV), opening and closing amplitude (OA and CA), opening and closing durations (OD and CD), and right and left lateral amplitudes (RLA and LLA). Data generated has been used to relate food structure and mandibular movements (vertical and lateral movements as the two dimensional units are commonly used).

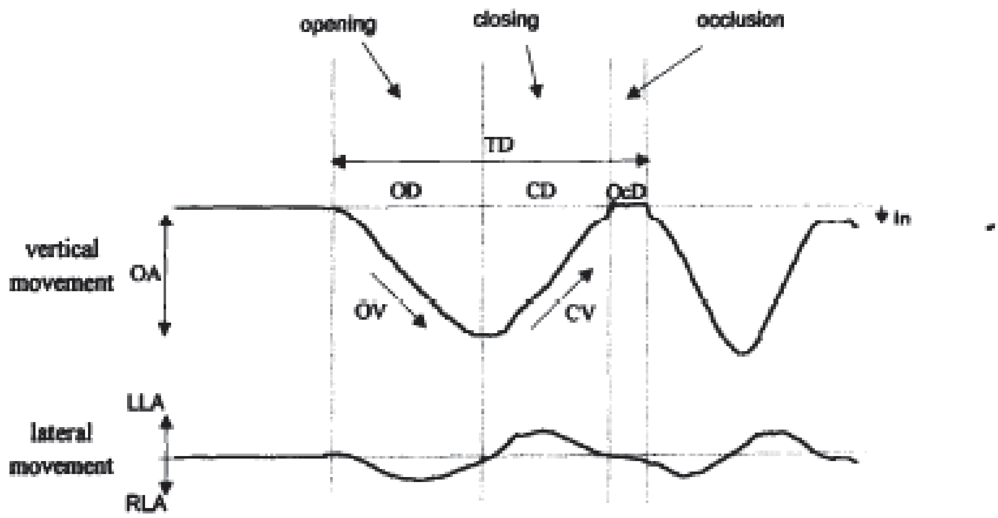



**Figure 2.2: Common chewing parameters derived from articulograph data (Source: Peyron et al., 1996)**

Currently, the AG 500 (Figure 2.3) developed by Carstens Medizinelectronik (Lenglern, Germany) is the most developed and reliable three-dimensional (3D)-EMA recording system that permits real-time display of articulators (usually placed at tip and body of tongue, lips, lower jaw, and soft palates) (Yunusova, Green, & Mefferd, 2009; Carsten Medizinelektronik GmbH, 2012). A typical EMA set up has six transmitter coils fixed in the EMA cube, a carrier (holder for all AG500 components), a receiver unit, and multiples sensors. Raw data are collected as voltages by the sensors and are based on distance and angle between the axes from each transmitter. The data collected are digitized and corrected (removal of head movements by means of reference sensors) to correspond with the three coordinate planes of motion; x (anterior-posterior), y (superior-inferior), and z (lateral-medial) and two angles; φ (tilt) and υ (yaw). The corrected data can be then processed following instructions provided by the manufacturer (Yunusova et al., 2009).


**Figure 2.3: Parts of the Electromagnetic Articulograph (EMA): (a) EMA cube (b) carrier (c) transmitter**

During mastication, large muscles contribute to the mandible movements (Figure 2.4). Various phases of a chewing cycle are attributed to different muscle contractions. It is well known that among the masticatory muscles, the masseter muscle is the main chewing muscle (Boyar & Kilcast, 1986b) that does most of the work during food crushing, shearing, and clenching (Naeije, McCarroll, & Weijs, 1989), and the temporalis muscle is a displacement muscle which is mostly responsible for the mandible movement (Plesh, Bishop, & McCall, 1986).

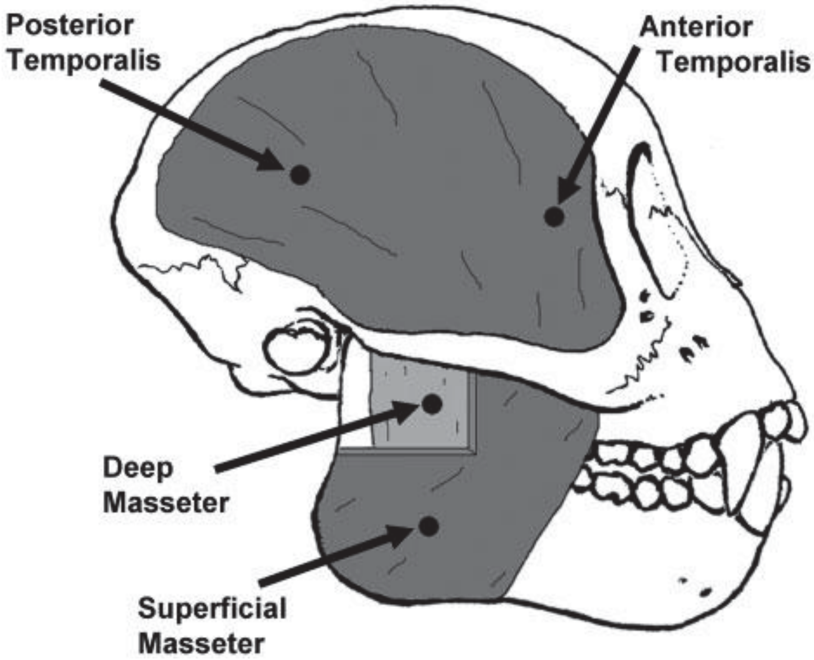



**Figure 2.4: The large muscles of mastication (temporalis and masseter) that contribute to the mandibular movements (Source: Vinyard, Wall, Williams, & Hylander, 2008)**

During an opening phase of a cycle, the elevator muscles (i.e. temporalis and masseter) are silent (no activity). During a closing phase of a cycle, these muscle are activated and electrical bursts are recorded, starting from the initiation of the closing movements, and gradually increase towards the end of the movement. Greatest muscle activities are recorded after the silent period. Boyar and Kilcast (1986b) showed approximately 75% of the total EMG activities were recorded during the closing phase while the rest were recorded during the occlusal phase. It must be noted that although EMG activity can be related to specific muscles, the collected recordings are not necessarily attributed to one particular muscle. Activities from adjacent muscles including the suprahyoid musculature and anterior digastic may be picked up (Takada, Yashiro, Sorihashi, Morimotol, & Sakuda, 1996). Forces produced by these muscles may be studied individually or sometimes pooled together to study masticatory EMG patterns. The choice of using either one, combination of any two or all muscles greatly depends on the aim of the study (Gonzalez, Montoya, & Carcel, 2001b).

Recording of Muscle Activities

Electromyography (EMG) is a common technique used for recording muscle contractions (Boyar & Kilcast, 1986b). Measurements of muscle activities using EMG during food chewing offer the possibility of relating natural chewing processes to food texture without hindering the chewing movements. It is a non-invasive technique and does not significantly interfere with normal chewing behaviour (Gonzalez, Benedito, Carcel, & Mulet, 2001a). A typical EMG set up would consist of bipolar surface electrodes placed on the main masticatory muscles (right-left of massetar and right-left of temporalis) and a ground electrode attached to a piece of bony area of the body (e.g. shoulder or ear lobe) to prevent noise in the data. These muscles are commonly used in mastication studies as they are easily accessible from the skin surface (Brown, 1994; Agrawal, Lucas, Prinz, & Bruce, 1997). This technique allows real-time information on the muscle activity generated during chewing. In this technique, the electrode activity/bursts created throughout a chewing sequence are collected. The EMG signals collected need to be filtered, amplified, and conditioned (Gonzalez, Montoya, Benedito, & Rey, 2004) (Figure 2.5). These recordings can also be used to provide information about intermediately and final swallowing (Okada, Honma, Nomura, & Yamada, 2007).

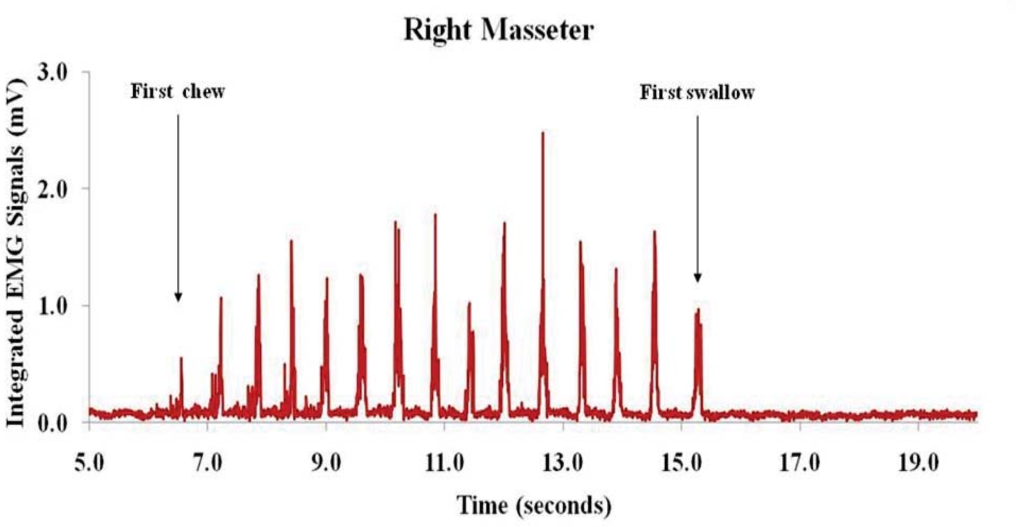



**Figure 2.5: A typical EMG records (rectified) during mastication for the right masseter from first chew to first swallow**

Simultaneous recording of jaw movements and muscle activities make it possible to mimic the dynamic nature of food oral processing (Figure 2.6).

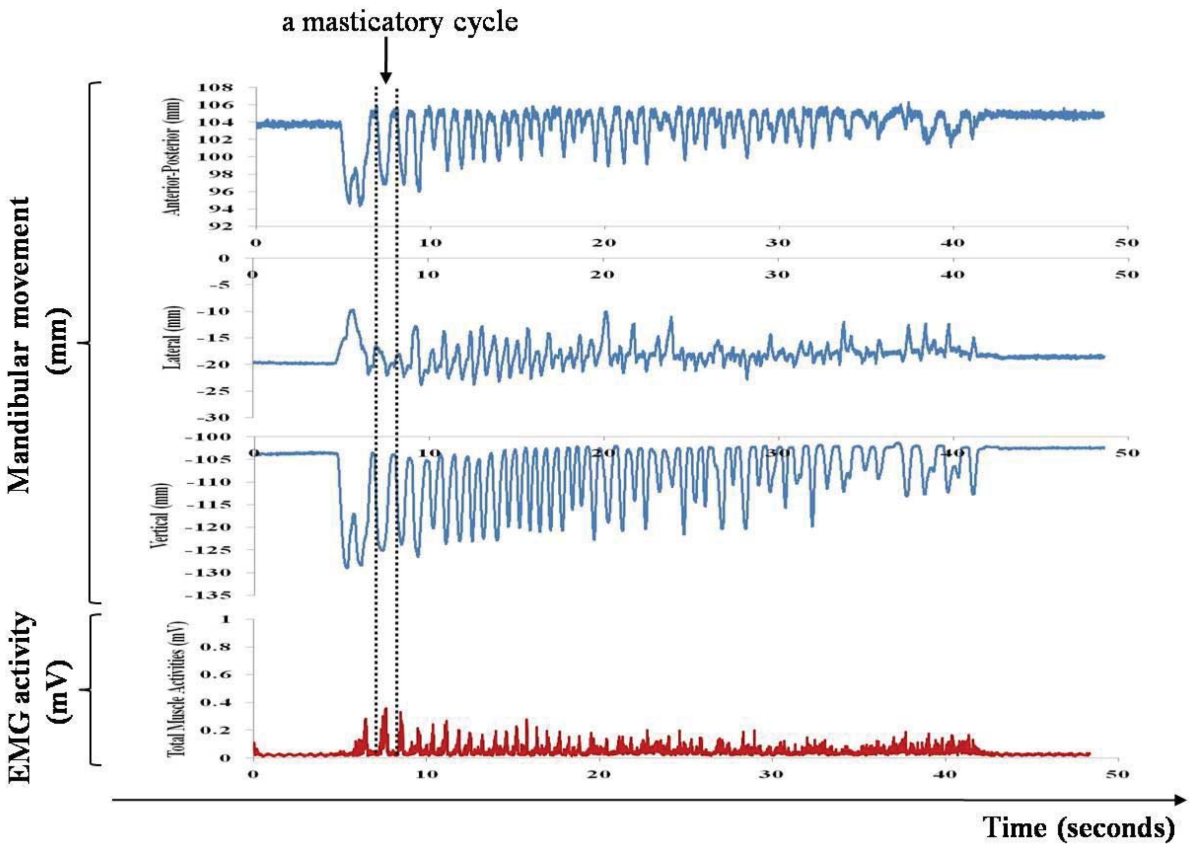



**Figure 2.6: Example of a simultaneous recording of electromyographic activities of chewing muscles and jaw movements obtained during chewing of a food sample. The top three traces are anterior-posterior, lateral, and vertical mandibular movements and the bottom traces are the total electromyographic (EMG) activities of right-left masseters muscles**

Brown, Eves, Ellison, and Braxton (1998a) have also highlighted the importance of having an efficient data recording system to monitor progressive changes in masticatory parameters during food breakdown in the mouth. A system combining both electromyography from muscle and kinesthesiology from jaw movement provided great flexibility and simplicity. The report demonstrated the differences in mechanism of oral breakdown used for different type of foods.

Mastication parameters extracted from these techniques (Table 2.1) can be used to investigate the relationship between chewing patterns and mechanical structure of a food. A collection of information generated from the simultaneous recordings such as the chewing rhythm, chewing rate, chewing work, and muscle effort could be used to describe the events happening (how food behave) in the oral cavity during the whole of the masticatory sequence. Data generated could be used to predict the variation in textural properties in food (Ioannides et al., 2009). For example, forces (work) during the first chew cycle may correlated to hardness, fracturability, and gumminess; chewing work and time can be used to relate tenderness, adhesiveness, and chewiness; post-maximum gradient may be linked with stickiness; cumulative intensity of forces may be used to describe crunchiness (Gonzalez et al., 2004).

**Table 2.1: Examples of oral processing data extracted from EMA and EMG for the purpose of studying food properties**

| Parameters | Definition |
|---|---|
| **From combinations of recordings** | |
| Chewing duration (s) | Total duration of the chewing sequence |
| Number of cycles | Number of chews used to complete a sequence starting from food ingestion to swallowing |
| Chewing frequency (1/s) | Number of cycles/chewing duration |
| Cycle duration (s) | Time requires to complete a single cycle (includes opening, closing, and occlusal phase) |
| Number of swallows (NS) | Number of swallows which include intermediately and final swallows |
| **From jaw movements recordings** | |
| Opening duration (ms) | Time required for the opening phase of a cycle |
| Closing duration (ms) | Time required for the closing phase of a cycle |
| Occlusal duration (ms) | Time required for the occlusal phase of a cycle |
| Vertical amplitude (mm) | The total up-down displacement of the mandible for a chewing cycle |
| Lateral amplitude (mm) | The total side-side displacement of mandible during a chewing cycle |
| Anterior/posterior amplitude (mm) | The total front-back displacement of the mandible during a chewing cycle |
| Opening and closing velocities | Average speed of jaw opening and jaw closing |
| **From muscle activities recordings** | |
| Total muscle activities (masseter and temporalis) | Sum of areas of the EMG curves for entire chewing sequence |
| Average muscle activity of each muscle | The total muscle activity of each muscle divided by the number of cycles in the chewing sequence |

## 2.2 Influence of Food Texture on Oral Processing

Food structure has been shown to influence various stages of a chewing sequence, from individual cycles to the whole sequence of the chewing process. Food oral processing can be divided into four stages for solid foods. *Acquisition* is probably the most important phase for the assessment of texture. Food can be accepted or rejected during this stage; if it is rejected, food may be spat out and if it is accepted (Lucas, 2004), food will be *transported from the front of the mouth to molar teeth* for further processing and this process involves simple jaw movements. Once food is placed between the molars, the jaw closes and the main chewing sequence is initiated. During this process, *particle sizes are reduced* by repetitive chewing cycles and saliva is incorporated. The last stage of mastication is the *clearance and swallowing phase* where the food bolus is transported to the back of the tongue before being pushed into the pharynx by tongue-palate interactions. The clearance phase uses the tongue to remove remaining particles that are not incorporated in the bolus. Swallowing can also occurs intermittently in a chewing sequence.

The following sections will discuss the influence of food properties on oral processing during the four main mastication stages. During these stages, food evolves into many mechanical and geometrical characteristics. Due to large amount of literature available, this section focuses on the most commonly studied textural attributes related to biscuits; *hard, crunchy/crispy*, *crumbly*, *dry*, and *sticky*. The attributes are discussed from both a combination of structural and sensory perspective.

### 2.2.1 Prior to Ingestion

Texture is also perceived extra orally (outside the mouth) or intra orally (inside the mouth). Prior to ingestion, visual observation on the color, shine, and surface texture provide textural information of the food. Additional information can be gathered with manual handling by stirring, spooning, and cutting or from the odor of the product. Inside the oral cavity, a different scenario can happen. The structural changes in food during manipulation provide textural information, which can be converted into sensory responses by human. These texture responses occur systematically and the order of appearance follows a definite pattern with regards to the order in which the characteristics are perceived. For example, Brandt, Skinner, and Coleman (1963) divided the textural order of appearance into first bite, masticatory, and residual phases. For example, the first bite is often associated with mechanical characteristics like hardness and brittleness, masticatory sequence encompasses of the mechanical characteristics such as gumminess, chewiness, and adhesiveness, and the last phase includes changes induced in mechanical and geometrical characteristics such as moisture and mouth coating.

### 2.2.2 The First Bite

The first bite may include acquisition of a food product or may be the first chewing cycle when subjects are given a constant sized sample in an experimental condition. It is usually a one-bite process and normally seen as the initiation of an oral process. Sensory information received from the first bite covers a wide range of textural features such as hardness and fracturability. Dan, Okamoto, Wada, Dan and Kohyama (2007) showed how the first bite was targeted for hardness recognition for cheese products based on the elongated first peak seen from the bite time-force curves created. The first bite was seen as an exploratory procedure for recognizing a specified attribute or to decide the subsequent chew trend. In most cases the masticatory parameters from first to the third bite were dependable on one another and usually increase with food properties (Heath, 2002). This may be related to learned behaviour that if a sample is hard, large bite force are used on the second bite.

The first bite is often made by the incisors (or the molars if standardized samples are given to subjects) begins the masticatory process. The force applied during the first bite is related to the mechanical and geometrical properties of food. Mechanical properties of food are probably the main determinant of the way food is handled and behaved in the mouth. It is clearly indicated that the mechanical properties of a food have a huge influence on both the biting pattern and biting length.

With reference to solid foods, the first bite is conducted by forcible occlusion of the opposing edges of the upper and lower incisors (Okada et al., 2007). The way an incisor penetrates into a food product influences the biting speed and force. The speed of biting, in particular during the late phase of tooth contact with the food, depends on food toughness (Ang, Lucas, & Tan, 2006). The bite force applied is likely to provide an initial evaluation of the work required for subsequent mastication. Mioche and Peyron (1995) demonstrated the influence of mechanical properties of foods on the biting force as applied by the incisor. Ten volunteers were asked to bite as they wish to the point of fracture on various standardized samples of food (elastic, plastic, brittle like). The first maximum bite forces of the initial bite(s) was recorded and used to plot bite force-time curves. Figure 2.7a shows several bites were used to fracture elastic product generating a symmetrical biting force (as deformations of elastomers tend to recover). In contrast, only a single bite could be made with plastic or brittle products (Figure 2.7 c & d); the former required the longest time to fracture (produced a bite force that increased till a plateau); the latter was fractured in less than 1second (creating a sharp increase and an abrupt decrease).

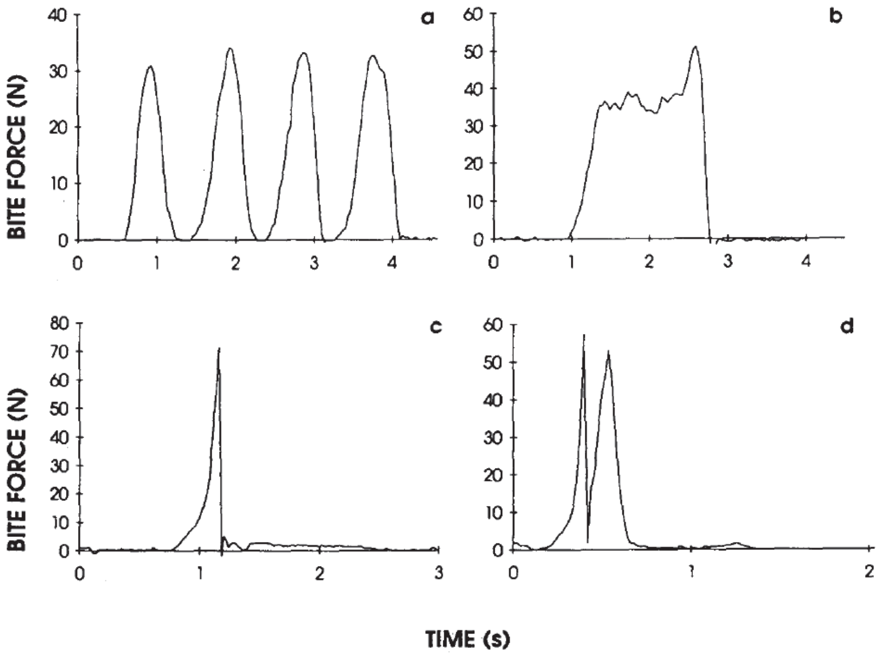



**Figure 2.7: Recoded pattern of bite-force on (a) elastic products, (b) plastic products, (c & d) brittle products (Source: Mioche & Peyron, 1995)**

Geometrical characteristics mainly refer to the food shape and size, and mainly reflect the appearance of a food (mostly sensed visually). Szczesniak (1963) grouped geometrical characteristic into 1) those related to the size and shape of the particle and 2) those related to shape and orientation. Although it has not been proven, but parameters for first bite could also probably be determined by visual, olfactory, and tactile inputs to the brain (Lund & Kolta, 2005). Peyron et al. (1997) showed food thickness significantly affects some parameters of jaw movements during biting, in particular the slow closing phase. Using the incisor teeth, subjects were asked to cut through a food then expectorate the piece (one bite), and to bite and chew a food while estimating the sample hardness on a 10 cm long scale. The authors found that although the type of bite had no significant effect on the hardness scores, the thickest samples were perceived as being harder. Woda et al. (2006a) also indicated that food thickness contributed to the variation in the first bite. Another contributing factor influencing the first bite is satiety; hunger led to larger bite sizes (de Wijk, Zijlstra, Mars, de Graaf, & Prinz, 2008).

### 2.2.3 Masticatory Sequence

Chewing is the next oral procedure after the first bite for both semi-solid and solid foods. The major functions of this stage of processing are to reduce food particle sizes (from macro to micro) and to produce swallowable food (Bourne, 2004). Particle sizes have to be reduced below a certain level and a certain degree of lubrication must be exceeded before swallowing can begin (Hutchings & Lillford, 1988). In a study with ten natural foods (peanuts, carrots, gherkins, stoned green olives, mushrooms, egg white, ham, chicken breast, emmental cheese, and coconut), Jalabert-Malbos et al. (2007) stated that particles of less than 2 mm were required for safe swallowing, unless food particles were soft enough to be swallowed at a larger size. In another study with food mixture of brazil-nut and natural yoghurt, Prinz and Lucas (1995) also found that particle sizes of below 2 mm were small enough to be swallowed except for concentrations of 20% or above where they must be chewed further to wet the food surface.

The reduction series involve cycles of repetitive jaw movements (vertical, horizontal, and lateral directions) and masticatory muscular activities (masseter and temporalis) which constantly change throughout the chewing sequence in response to the changing physical and chemical properties of foods. Continuous particle size reduction increases the surface area, promoting saliva lubrication, and releases juices or oil from food to enhance the flavour release. Other functions during this stage include temperature adjustment, sensory assessment, and to increase surface area promoting rapid digestion in the stomach (Bourne, 2004).

During a masticatory sequence, it has often been observed that the amplitude of vertical and lateral jaw movements tend to decline gradually, as did the EMG activity in the jaw closing muscles (Peyron et al., 2002). This is associated with the food particles being reduced and the bolus being softened (Hiiemae et al., 1996). Horizontal jaw movements also reduced with an increase of chewing cycles (Foster et al., 2006). Jaw movements also vary with food type. In general, foods with a firm texture are chewed between molars with slower jaw-closing velocities (Plesh et al., 1986; Takada et al., 1996) and more lateral movements compared with foods with soft texture, which are often ruptured between the tongue and hard palate (Foegeding & Drake, 2007). There are also shown differences between elastic and plastic textures for lateral movement (Foster et al., 2006). These examples show the role of food textures in influencing the rhythmic oral processing behaviours.

Hardness

Hardness is often used to describe solid food characteristics and is defined as "the amount of force required to bite through the sample between the teeth" (ISO 5492, 1992). However, Woda, Mishellany, and Peyron (2006b) described hardness as a loose term that covers many food attributes such as elasticity, plasticity, brittleness, and toughness. This is understandable because these attributes are often correlated or often occur simultaneously (Szczesniak, 1963).

Hardness is not only important for the first bite, it also plays a role during the masticatory sequence. Often reported as a sensory attribute, hardness can also be quantified by measuring the force developed during the process of chewing (where food is crush). Since the masseter muscles are well known to be responsible for jaw closing (during food crushing), many studies have focused on the activities of the masseter muscles and demonstrated an influence of hardness properties on masticatory parameters.

Generally, an increase in food hardness is reflected by an increase of chew in EMG values (Anderson, Throckmorton, Buschang, & Hayasaki, 2002; Brown, 1994; Karkazis, 2002). Boyar and Kilcast (1986a) observed distinct differences in the chew forces obtained using the EMG when chewing on gelatin and carrageenan gels. Plesh et al. (1986) found that more EMG activities were observed for harder foods. Horio and Kawamura (1989) together with Ashida, Iwamori, Kawakami, Miyaoka and Murayama (2007) agreed that the masseters were found to be the largest and mastication time was the longest when chewing on the harder samples. Kohyama, Ohtsubo, Toyoshima, and Shiozawa (1998) also found the masseter activities increased as rice hardness increased (as measured by a texturometer). Mioche et al. (1999) combined both the masseters and temporalis activities to investigate chewing pattern during mastication of different food samples ranging from canned frankfurters without skin, fresh coconut, toffee, French Comte cheese to Swiss Sbrinz cheese. It was found that the total muscle work increased steadily with food hardness and that the total muscle work was a highly discriminating variable among the food samples.

When the studies of muscle activities are combined with mandible movements, each masticatory cycle can be divided into open, closed, and intercuspal phases also known as the occlusal phase where tooth to tooth contact occurs (Takada, Miyawaki, & Tatsuta, 1994). The duration and velocity of these movements are often found changing with hardness. Takada et al. (1994) found unchanged opening time but increased closing time with hardness. Plesh et al. (1986) also showed long occlusal phase but prolonged opening phases during chewing of hard gums. Nakajima, Hideshima, Takahashi, Taniguchi and Ohyama (2001) have suggested that the occlusal phase was food dependant. The resistance to food slows the jaw (Lucas, 2004) and requires more chew effort. Generally, when chewing on harder foods, more chew effort/chew efficiency is required and this results in an increase in lateral movements during the chewing cycle

(Yamashita, Hatch, & Rugh, 1999). The same finding was also observed during a study with cheese, carrot, and gum (Lundeen & Gibbs, 1982), gum (Plesh et al., 1986), jellies (Takada et al., 1994), and visco-elastic model foods (Peyron et al., 2002).

More recent study found that these chew effort produced greater acceleration (increased velocity) of mandible in all phases, except the occlusal phase of closing. Although it did not change the cycle shape but a greater pathway excursion can be seen in all axes of motions (Anderson et al., 2002). Piancino, Bracco, Vallelonga, Merlo and Farina (2008) observed similar chewing pattern in the frontal plane where it was significantly higher and wider, with smaller closure angle when chewing on hard bolus. On another note, Shiga, Kobayashi, Arakawa, and Shonai (2003) also pointed out that the stability path of a masticatory movement was influenced by the type of food and also the chewing strategy used (i.e. unilateral or bilateral).

The vertical amplitude and anterior-posterior jaw movements also change with hard foods; increase in food hardness has been said to induce an increase (Horio & Kawamura, 1989; Peyron et al., 1997), a decrease (Proschel & Hofmann, 1988) or no effect on vertical amplitude (Plesh et al., 1986; Bishop, Plesh, & McCall Jr, 1990; Takada et al., 1994). These results were observed from the usage of natural foods. Using a controlled visco-elastic model food, Peyron et al. (2002) showed vertical amplitude was higher with increasing of hardness although Takada et al. (1994) did not find changes in the anterior-posterior and vertical directions from soft to hard jellies. Mizumori, Tsubakimoto, Iwasaki, and Nakamura (2003) assessed lateral movements during mastication of seven different foods using an Asymmetry Index (AI); by subtracting the number of right side strokes by the number of left side strokes, and dividing by the total number of stokes. The hardest food had a significantly higher AI than softer foods.

Other masticatory parameters that have also been used to study food hardness were total sequence durations (Hiiemae & Palmer, 1999), number of cycles per sequence, total sequence duration (Takada et al., 1994; Peyron et al., 2002; Peyron, Blanc, Lund, & Woda, 2004a; Engelen, Fontijn-Tekamp, & van der Bilt, 2005; Woda et al., 2006a), and chew swallow ratio (the total number of chews/total number of swallow) (Hiiemae et al., 1996). These masticatory parameters were widely reported to increase with increasing food hardness.

Almost all EMG and jaw movements' parameters including the total duration of a masticatory sequence, the total number of chewing cycles, the total muscular effort, and the mandibular movements (anterior-posterior, vertical, and lateral trajectories) are affected by increasing of food hardness. Although these masticatory parameters have proven to relate to food texture, Peyron et al. (2002) recommended using the number of chewing cycles, EMG activity of any one of the two temporal or the two masseter muscles, and the opening mandibular amplitude as the masticatory parameters to characterize a chewing sequence, in particular to describe hardness.

When assessing the effect of hardness on masticatory behaviour, most researchers tended to compare food of different hardness levels and by dividing the food into soft and hard foods (for natural food). Some of the examples are such as apple, banana, and biscuits (Hiiemae & Palmer, 1999), bread, toast, melba toast, breakfast cake, peanuts, and cheese (Engelen et al., 2005), and jellies (Takada et al., 1994). Although these natural foods were chosen to have a range of hardness, variation in other textural parameters would have an effect on the masticatory response (as texture is a multi-attribute parameters).

Whatever the food chosen, it depends on the research purpose. For example, to study the masticatory adaptations to bolus hardness, Piancino et al. (2008) used chewing gum as the soft bolus and wine gum as the hard bolus. The study reported that the EMG peak amplitude and chewing pattern increased (higher velocity and wider chewing cycle) with hard bolus. This result was in agreement with studies by Wilding and Lewin (1991) and Anderson et al. (2002). The larger the chewing pattern, the higher the velocity and force for food breakdown, the higher the chewing efficiency with increased muscle force (reflected by higher EMG amplitude with a hard bolus).

Contradictory results have been found on the role of hardness on chew frequency. Some claimed that chewing frequency increases (Plesh et al., 1986) or have no effect (Horio & Kawamura, 1989; Yamada & Yamamura, 1996) with hardness of food. Again, it must be noted that these results are gathered from observations using natural foods. A review by Woda et al. (2006a) explained that chewing frequency remained constant with increasing of hardness if the rheological properties of the foods are the same. Work by Peyron et al. (2002) conducted with gelatin gels identified that almost every chewing parameter was different between gels of varying hardness except for masticatory frequency. Research by Foster et al. (2006) also concluded that hardness had a minimal impact on the frequency of chewing.

Crunchiness

Crunchiness has always been closely correlated with hardness. Guraya and Toledo (1996) described crunchiness as “the perceived cumulative intensity of force required for repeated incremental failures of the product by chewing up to five times with molars”. Although crunchiness appears to be an independent characteristic, samples such as biscuits have to be relatively *hard* to be perceived as *crunchy* (Brown et al., 1998b). These authors found a significant correlation between the attribute hardness and crunchiness, and that these two attributes depended on one another and often create an ambivalent relationship. Brown et al. (1998b) also indicated more chewing effort was required for mastication of harder and crunchier samples, with an increase of mastication muscle effort between the first five chews and the next five chews with a subsequent decline in effort to the end of the chewing sequence. During the first few chews on biscuits, the principle activity is towards reducing sample size, and when some fragments become softer (softened by saliva), only the remaining solid samples will continued to require high forces to fracture.

It must be noted that crunchiness differs from crispness. Vickers (1984) described crunchiness as “firm and brittle, snaps easily with typical sound that has a lower pitch, less loud, and last longer than for crisp”. Szczesniak (2002) defined crispness as “firm and brittle, snaps easily emitting a sound upon deformation’ and Brennan (1984) defined crispness as “the degree to which the rupture is heard during the first bite”. Irrespective of the definitions, there is general consensus that both *crunchy* and *crispy* sensations are related to the fracture properties (Vincent, 1998). Fillion and Kilcast (2002) claimed that consumers were able to describe the differences between the two by judging the sound a food makes; a *crisp* sound was short and a *crunchy* sound was long. The same approach using sound to distinguish between the two sensations was used by Vickers (1984). The author claimed the distinguishing property between these two sensations was the pitch of the sound; the pitch of *crispy* products was higher than for *crunchy* foods.

Crumbliness

Another sensation which is easily confused with crunchiness and crispness is crumbliness. It is defined as "the rate, ease, and degree to which the sample breaks down during chewing" (ISO 5492, 1992). Brown et al. (1998b) assessed three different textures attributes (hardness, crunchiness, and crumbliness) and reported a significant inverse correlation between hardness and crumbliness, and a weak but significant relationship between crunchiness and crumbliness (negative correlation).

Dryness

Dryness often relates to moisture content and consumers often relate it to foods such as toast, crackers, and cereal (Szczesniak & Skinner, 1973). It is an attribute studied during mid-chewing (Pereira, de Wijk, Gavião, & van der Bilt, 2006) or throughout chewing (Peyron et al., 2011). Pereira et al. (2006) studied the effect of added fluids on the perception of solid food. Subjects evaluated specific attributes that have been divided into at first bite and during chewing on foods with and without added fluid. One of the attributes evaluated during chewing was drying and the attribute was described as saliva absorbing. Engelen et al. (2005) have also shown that dry and hard foods required more chewing cycles before swallowing, which was in agreement with Prinz and Lucas (1995). More time was needed for food fragmentation and enough saliva addition to form a cohesive bolus suitable for swallowing. In another chewing study, Peyron et al. (2011) studied several texture attributes including dryness using the method of Temporal Dominance of Sensations (TDS), where panellists were asked to identify the dominant perception continuously during the eating period. Results showed that the dryness perception increased slightly at the end of the masticatory sequence. The progressive adsorption of saliva (exchange between the solids and aqueous phases) in the bolus may be linked to the increased perception.

### 2.2.4 Clearance and Swallowing

Food is continuously chewed while saliva is being added to form a food bolus before further transport to the back of the tongue for swallowing. This transportation is mainly performed by tongue pressing against the palate (Palmer, Rudin, Lara, & Crompton, 1992; Saitoh et al., 2007) and has no distinctive jaw movement associated. Often, the ready-to-swallow bolus needs to reach a “swallow threshold” before being considered as safe to swallow. Hutchings and Lillford (1988) described a “swallow threshold” as a combination of particle size and lubrication criterion. Researchers like Mioche (2004) and Mishellany et al. (2006) have also strongly suggest that a swallowing threshold probably exist as people tend to chew until an optimum particle size is obtained before swallowing, and the particle size distribution of the bolus before swallowing is often food-dependent (Peyron, Mishellany, & Woda, 2004b). An optimum particle size distribution has been recognized as critical in producing the stimulus of the start-point of swallowing, a fairly moist bolus often being considered as critical before swallowing.

From a sensory perspective, Loret et al. (2011) considered the tongue as the main organ in determining the textural properties in the mouth. The tongue senses the size and particle lubrication of the food particles (Jalabert-Malbos et al., 2007). Okada et al. (2007) have also emphasized that the tongue played an important role in recognizing and evaluating the food. The tongue senses if the food particles are small and moistened enough to be swallowed safely. Humans also sometimes swallow intermittently during chewing until all material is removed from the mouth (Okada et al., 2007).

Stickiness

The precise triggers for swallowing are still incompletely understood. While some scientists considered it depends on food particle size and particle lubrication threshold (van der Bilt, Engelen, Pereira, van der Glas, & Abbink, 2005), others believed that bolus rheology (flow-ability and stretch-ability) may be important (Chen & Lolivret, 2010). Stickiness is an important physical and sensory property of food. An excessive level of stickiness can be considered desirable or not desirable for a food product; excessive stickiness may be associated with inconvenience of handling and unpleasant oral experience. For example, in some countries, stickiness is used as a quality indication of a product, for example cooked rice must maintain a certain level of stickiness to indicate that it is properly cooked (Chen, Feng, Gonzalez, & Pugnaloni, 2008). Stickiness is often associated with cohesiveness and adhesiveness, although both have very different definitions. Cohesiveness can be defined as the resulting forces inducing particles to stick together and adhesiveness can be described as resulting external forces due to attraction between bolus and the mouth.

## 2.3 Influence of Food Properties and Oral Processing on Sensory Perception

Texture perception is a highly dynamic process as the physical properties of the foods continuously change when manipulated in the mouth. As a result, no single instrument has been able to accurately and comprehensively reproduce the events that take place in the mouth from first bite to swallowing. Several techniques have been used to measure textural characteristics of foods to establish the relationship between texture perception and food structure parameters, but the link to predict which food structures are responsible for types of texture sensations perceived is yet to be uncovered (Figure 2.8). Analyzing the modulation of chewing behaviours in response to food structure (which is very likely to be linked to food texture) offers a means of understanding the dynamic texture perception from first bite to swallow (Chen, 2009).

Many authors have studied the sensory assessments made during the first bite (Peyron et al., 1997; Heath, 2002; Agrawal & Lucas, 2003; Dan & Kohyama, 2007; Dan et al., 2007). The first chewing cycle is often an exploratory step and is generally different to subsequent chewing cycles/bites and very likely to provide an initial evaluation of the food and also work required during subsequent mastication steps. For example, the frequency of chewing is usually lower for the first cycle (Peyron et al., 2002; Foster et al., 2006). Duizer, Gullett, and Findlay (1996) measured tenderness in beef over an entire masticatory sequence by exploring instrumental texture profile analysis (ITPA) with time-intensity and EMG. The outcome showed that more than just the first bite was required to measure tenderness.

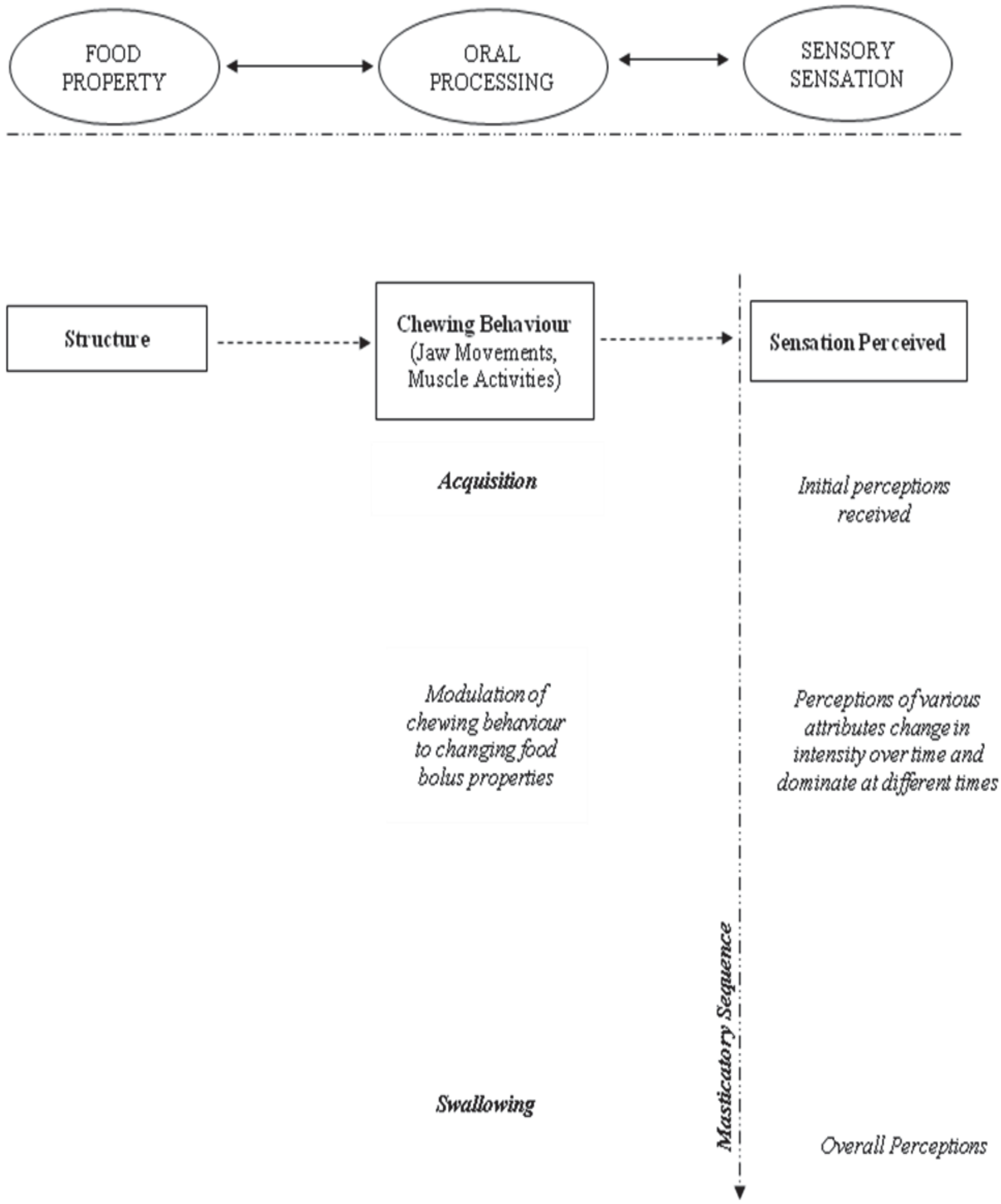



**Figure 2.8: Overview of interaction between food property, oral processing, and sensory sensation (modified from Source: Foster et al., 2011)**

Although the first bite is important, it represents only a small portion of the total mastication time. During subsequent chews, food is reduced in size and mixed with saliva and other textural attributes become more apparent. Despite this, only a few studies have looked into the sensory adaptations throughout a masticatory sequence with very different foods being used. Some used natural foods (Sakamoto, Harada, Matsukubo, Takaesu, & Tazaki, 1989; Agrawal et al., 1997; Agrawal et al., 1998; Veyrune & Mioche, 2000) and some used model food systems (Olthoff, van der Bilt, De Boer, & Bosman, 1986; van der Bilt, van der Glas, Olthoff, & Bosman, 1991; Slagter, van der Glas, Bosman, & Olthoff, 1992; Mioche & Peyron, 1995; Buschang, Throckmorton, Travers, & Johnson, 1997; Shiau, Peng, & Hsu, 1999; Fontijn-Tekamp, van der Bilt, Abbink, & Bosman, 2004; Kohyama et al., 2004) in an attempt to understand the total mastication process.

Kohyama and Mioche (2004) attempted to characterize chewing behaviour at five equivalent stages in mastication (based on chewing cycles) using six foods from a large range of textures (rice, beef, cheese, crispy bread, apple, and peanut). When data was analyzed stage by stage, it was found that samples differed most in the first stage of mastication and decreased gradually towards the end of mastication. The EMG voltage and vertical displacement were irregular and greater (for muscle activity) in the first stage of mastication. This could probably be due to the transportation of food with the tongue from the front of the mouth to the back of the teeth. In the middle of the mastication stage (second to third stages), the movements tended to become more stable and repetitive, with minimum chew-chew variations. During the last stage, cycle time lengthened, probably due to the increased inter-burst and occlusion duration. This suggests that the food effect continues until the final stage of the mastication sequence. The authors found that the influence of food properties was the strongest on jaw movements in the early stages of mastication. Food with high fracture properties displayed large vertical jaw opening during the first chewing stage but had the lowest during the last stage. Whereas complex structured foods like meat exhibited larger jaw displacements than other foods for any stage of mastication. The authors have also suggested that structural (peanuts and hard bread) and moisture changes (cheese and rice) during mastication had an effect on mastication behaviours.

In more recent studies, the recording of jaw movements and muscles activities were combined with sensory evaluation. These experiments were usually individually recorded (divided into several sessions). Pereira et al. (2006) aimed to investigate the influence of adding fluids to various solid foods on the physiological parameters of the chewing process, and on the perception of food. Two sessions were dedicated to collecting data from jaw movement and muscle activities, and the last session was dedicated to attributes evaluation on a 10 cm visual analogue scale (VAS) ranging from very little to very much. These researchers found that by adding fluid (water) to a food, it significantly lowered the muscle activity, number of cycles to prepare for swallowing, and various texture and sound perceptions; in particular for dry foods like melba toast and cake. Brown and Braxton (2000) also studied the process of food breakdown in the mouth by comparing measurements of chewing patterns to data from sensory assessment collected from two separate sessions. Results indicated that subjects can be classified according to their chewing efficiency and that these groups appeared to have different understanding of textural terms (hardness, crunchiness, crumbliness).

In other time, the sensory assessment was performed at a specified point of time. Peyron et al. (1997) investigated the relationship among jaw movements, physical characteristics of food, and sensory perception of hardness on carrot and cheese test foods. Subjects were asked to perform two types of bites with incisor teeth while jaw movements were recorded; in the first, subjects were asked to cut though a sample before spitting out the pieces and in the second, subjects were asked to bite and chew on the given samples. These procedures were repeated five times. At the first and fifth repetition, subjects were then asked to estimate the hardness of the samples on a 10 cm long visual analogue scale (VAS). The results suggested that the thickness of food has greater effect on hardness perceptions and there is no food type effect on chewing movement.

Alternatively, some researchers compared masticatory information to sensory assessment collected from different group of subjects and at different sessions. Peyron et al. (2002) compared information collected from different groups of subjects to compare chewing activity to sensory sensation perceived. The authors concluded that almost all EMG and jaw movements studied were affected the hardness of food (in this case a gelatin-based visco-elastic model food). However, these data were collected from two groups of subjects; seventeen female and male subjects aged 28.9 ± 0.7 and fifteen male subjects aged 22.6 ± 1.3). Later, Peyron et al. (2004a) adapted the masticatory recordings to study the influence of age on adaptability to food hardness. The experiment showed that age had little effect on the ability of older subjects adapting to changes in hardness of the test foods. The conclusion from this study were compared between masticatory information from the older subjects (29 males with mean age of 41.8 and 38 females with mean age of 42.0) to the hardness perceptions from the earlier study with young subjects.

Although these studies have significantly contributed to the food oral processing field, the jaw-muscle movements and sensory assessment need to be directly compared (using the same subjects) and simultaneously recorded. Examining textural changes during a masticatory sequence requires sensory techniques that can assess the changing attribute perception considering the additional dimension of time. Brown and Braxton (2000) have attempted to combined recordings of masticatory muscle activities, jaw movement patterns, and Time Intensity (TI) analysis. However, the method focuses on one single attribute (the wetness of sample). The dynamic characteristics of the food were not considered. Therefore, when multiple attributes need to be assessed during the same run, a suitable sensory method is crucial.

### 2.3.1 Selection of Appropriate Dynamic Sensory Method

Continuous monitoring of changing texture throughout mastication enables the understanding of sensory perceptual changes over time. Although there are other dynamic sensory methods available like Dual Attribute Time Intensity, the most commonly used are Time Intensity (TI) and Temporal Dominance of Sensations (TDS).

Time Intensity (TI)

The development and use of time-intensity methodology for sensory evaluation was reviewed comprehensively by Cliff and Heymann (1993) and Piggott (2000). Early use of this technique had subjects plotting perceived intensities on stationary graph paper before developments led to plots being recorded on moving paper. Later, time-intensity recordings were computerized (Guinard, Pangborn, & Shoemaker, 1985) and intensity was recorded with a computer mouse scale (Dijksterhuis & Piggott, 2000). The TI method continuously monitors the changes of a single sensory attribute over time when food is processed in the mouth (Cliff & Heymann, 1993; Peyvieux & Dijksterhuis, 2001; Sprunt, Raithatha, & Smith, 2002; McGowan & Lee, 2006; Ross, 2009). Common methodology of TI requires the panellist to rate their perceived intensity of a particular attribute over time on a line scale with anchors of (0) none and (10) extreme. Figure 2.9 shows an example of how data is registered through a computer mouse or sliding bar, which is controlled by the panellist.

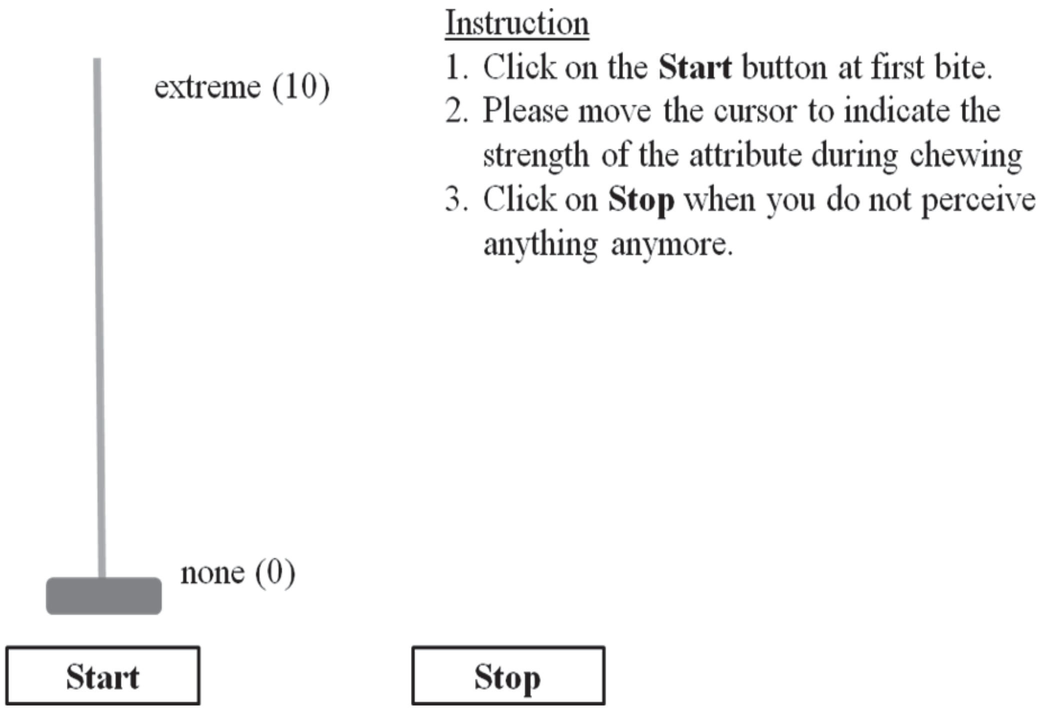



**Figure 2.9: Example of how data is registered through a sliding bar**

Although the TI method is widely used in dynamic sensory evaluation, the method has several limitations;

1. difficulties in generating agreement for sensory term definitions (Brown, Dauchel, & Wakeling, 1996),
2. extensive training is required (panellists need to be trained on the response device and should have good hand-eye coordination towards momentary sensation changes),
3. variation in response behaviour (panellists might translate responses differently, effect from different decision mechanism),
4. differences among product, individuals, and devices (Vanbuuren, 1992),
5. assessment of attributes continually induces a carryover effect from previous perceived sensation (Pineau et al., 2009).

Temporal Dominance of Sensations (TDS)

The Temporal Dominance of Sensations (TDS) method is a sequence representation of changing sensory perceptions over a period of food consumption. Using this method, a dynamic sensory description of a product can be established. This method was developed by the Centre European des Sciences du Guot in year 1999 (Pineau et al., 2009). TDS technique has received attention in the sensory community for its simple application, easy implementation, time and cost saving advantages, and the possibility to monitor up to 10 sensory responses at any one time (Le Reverend, Hidrio, Fernandes, & Aubry, 2008; Labbe, Schlich, Pineau, Gilbert, & Martin, 2009; Lenfant, Loret, Pineau, Hartmann, & Martin, 2009; Meillon, Urbano, & Schlich, 2009; Pineau et al., 2009; Foster et al., 2011;).

Since then, the TDS technique has been extensively compared to other conventional sensory methods. Le Reverend et al. (2008) described perceptions of six different attributes for hot beverages over time using TDS and compared it to TI. The author suggested that TDS was more suitable to be used to illustrate product perception patterns over time whereas TI was more suitable for determination of kinetic of one specific attribute. They also concluded that both methods brought similar information in terms of describing product's differences, attributes, and evolution over time. Pineau et al. (2009) used dairy products to describe the sequence of ten changing taste and texture attributes (sour, diacetyl, sticky, melting, fruity, smoky, fatty, nutty, pasty, and salty) to show how TDS curves are constructed and then compared the results to TI. These authors concluded TDS to be a better method when comparing several attributes over time and that TI is best suited when evolution of intensity in one attribute is of interest. Meillon et al. (2009) showed how TDS contributed to the sensory descriptions to differentiate two different types of partially dealcoholized red wines with three flavours (acid, bitter, sweet), three sensations (astringency, heat, pungent), and four aromas attributes (blackcurrant, red fruits, spices, woody). These researchers concluded TDS to be a reliable tool for providing product differences. Teillet, Schlich, Urbano, Cordelle and Guichard (2010) attempted to describe the taste of tap water by comparing results collected using sensory profiling, TDS, and free sorting task. They later developed a prototype which was best suited for their research. Labbe et al. (2009) compared TDS to conventional sensory profiling using gels containing different levels of odorants (peach and mint), citric acid, cooling agent, and xanthan gum. Results from this comparative study showed that TDS provides reliable information close to the standard sensory profiling method. In addition, the author claimed that TDS was able to provide information on the dynamic of perception after product consumption that was not available using a conventional sensory method. Sokolowsky and Fischer (2012) evaluated the bitterness in white wine using descriptive analysis, time-intensity analysis, and TDS analysis. They concluded that each technique provided additional information on the bitter perception in white wine and stated that TDS was better in discriminating all samples. Meanwhile, Ng et al. (2012) found the benefits of using a sequential approach of Quantitative Descriptive Analysis (QDA) and TDS as complementary methods for profiling blackcurrant squashes. TDS provided temporal information in differentiating products and how long the attributes were dominant for. Dinnella, Masi, Zoboli, and Monteleone (2012) compared results obtained by TDS and descriptive analysis to assess the impact of extra-virgin olive oils on the perceived sensory profiles of pureed beans and tomatoes. Sensory profile was more clearly described by the TDS. Paulsen, Næs, Ueland, Rukke and Hersleth (2013) also showed that TDS had a potential to provide complementary information compared to descriptive analysis in their study of salmon-sauce. Bruzzone, Ares, and Giménez (2013) stated that TDS was a useful technique to characterize yoghurt texture, providing information that was not gathered by Quantitative Descriptive Analysis (QDA). The methodology was also compared in solid food. Albert, Salvador, Schlich, and Fiszman (2012) compared the performance of TDS and key-attribute sensory profiling in assessing fish sticks and concluded that TDS had significant benefits in monitoring the appearance and evolution of different attributes over time (time saving and required practically no training). Saint-Eve et al. (2011) highlighted the additional information obtained using TDS to study the effect of texture on taste and aroma perception. The rapidity of the technique proven to be a better method than the profile method used.

TDS technique has also been used to enhance other consumer testing. Meillon et al. (2010) investigated the impact of wine on perceived complexity and temporality of sensation and attempted to link it to consumer preference. Results showed no relationship between individual liking and perceived complexity. Laguna, Varela, Salvador, and Fiszman (2013) linked TDS data to consumers' liking scores to study the eating quality of biscuits with different fat and fiber contents. The authors proposed TDS as a new tool for better understanding of biscuit eating quality.

Sensory scientists have also started using the TDS technique to gather temporal information for studying oral processing behaviours. Lenfant et al. (2009) applied TDS to texture evaluation. The researchers described a succession of perceptual events during mastication of wheat flakes. The wheat flakes had the same composition but different thickness and toasting levels. Panellists recorded their dominant texture sensations (hardness, crackliness, crispness, brittleness, lightness, stickiness, grittiness or dryness) for each product at different points of the masticatory sequence. Déléris et al. (2011b) used TDS to study the impact of swallowing on the dynamics of aroma release and perception during consumption of alcohol beverages. Between two swallowing protocols used, the swallowing of the product resulted in more complex perceptions. Déléris et al. (2011a) used TDS to establish the links between aroma release and texture during consumption of candies. Peyron et al. (2011) combined bolus mechanical analysis (using the texture profile analysis, TPA), bolus granulometric analysis (using dry manual sieving), and TDS to study the role of bolus properties in triggering swallowing. de Loubens et al. (2011) used TDS to quantify the profiles of salt release in model dairy products and discovered that fat had a major influence on food breakdown behaviour.

The TDS method has become one of the most exciting areas of dynamic sensory research, especially in flavour studies. The advantages of this technique have not been fully explored in texture studies of solid foods. At the point of this publication (Table 2.2), only few studies have applied TDS to solid foods (gels, cereals, candies, fish sticks, cheeses, and biscuits). The lack of TDS usage in studying textural studies in solid foods could probably be due to the complexity of the system. These publications used TDS to study flavour release (Labbe et al., 2009), taste and texture perceptions (de Loubens et al., 2011), combination of texture, aroma, and taste sensations (Saint-Eve et al., 2011), and texture attributes (Lenfant et al., 2009; Albert et al., 2012; Laguna et al., 2013).

**Table 2.2: Recent sensory researches using TDS technique (from 2008)**

| Researchers | Food | Rep | No of attributes | Number of panellists | Attributes trainings | TDS training | Evaluation Starts | Evaluation end |
|---|---|---|---|---|---|---|---|---|
| Le Reverend et al., 2008 | 6 hot beverages | 2 | 6 | 12 (trained in quantitative technique) | n/a | n/a | ^ | + |
| Labbe et al., 2009 | 9 gels with different levels of odorants | 1 | 5 | 43 (experienced in sensory profiling) | 3 sessions of 60 minutes | 1 session | Once swallowed | 5 minutes |
| Pineau et al., 2009 | 5 recipes of dairy products | 4 | 10 | 16 (untrained) | 12 sessions | 6 sessions | ^ | 120 s |
| Meillon et al., 2009 | 2 types of red wine with 4 different alcohol content | 3 | 10 | 16 (wine consumer) | 4 sessions of 1 hour | 3 sessions of 1 hour | ^ | + / 120 s |
| Lenfant et al., 2009 | 6 types of wheat flakes (different thickness and toasting levels) | 2 | 8 | 25 (untrained) | n/a | n/a | ^ | first major swallow |
| Teillet et al., 2010 | Tap water | n/a | 11 | 15 | n/a | n/a | ^ | n/a |
| Meillon et al., 2010 | 5 Syrah wine with different degree of dealcoholization | 3 | 7 | 8 (trained in TDS) | 5 sessions of 1 hour | | ^ | + / 120 s |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Deleris et al., 2011b | Alcoholic beverages | 3 | 4 | 10 (untrained) | 4 sessions | | n/a | + / 180s |
| Saint-Eve et al., 2011 | Model candies with varying textures | 2 | 6 | 12 (some experience in sensory evaluation) | 15 sessions of 45 minutes | 3 sessions of 45 minutes | n/a | + / 180s |
| Peyron et al., 2011 | Wheat flake cereals | 2 | 8 | 25 (untrained) | n/a | n/a | n/a | n/a |
| Sokolowsky and Fischer, 2012 | 13 commercial white wine | 3 | 8 | 18 (experienced in wine) | n/a | 3 sessions | ^ | n/a |
| de Loubens et al., 2011 | 4 model cheeses | 2 | 7 | 16 (trained) | n/a | n/a | ^ | n/a |
| Albert et al., 2012 | Fish sticks cooked differently | 3 | 7 | 9 (trained) | n/a | 2 sessions | ^ | + |
| Ng et al., 2012 | 11 commercial blackcurrant squashes | 3 | 9 | 11 (untrained) | n/a | 6 sessions of 2 hours | ^ | + / 60s |
| Dinnella et al., 2012 | Extra-virgin olive oil in vegetable foods | 4 | 5 / 9 | 13 | 3 sessions | 2 sessions | n/a | 90s |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Paulsen et al., 2013 | 6 combination of cooked salmon sauces | 3 | 8 | 9 (trained) | n/a | n/a | ^ | 50s |
| Laguna et al., 2013 | 6 biscuits with different fat and fibre contents | 3 | 7 | 13 (trained) | n/a | 4 sessions of 1 hour | ^ | At complete swallow |
| Bruzzone et al., 2013 | 8 yoghurts with varying fat content | 2 | 5 | 10 (trained) | n/a | n/a | ^ | + |

n/a = not available; ^ = when food is put in the mouth, + = no longer perceived sensations

TDS Procedures

Normal procedures in TDS require subjects to select the *Start* button on the computer screen when food is ingested (Figure 2.10). Then, as soon as panellists perceived a sensation, they choose the dominant sensation that triggers the most attention from the list provided (attributes appeared simultaneously on the computer screen). At the same time (not always included), panellists may be asked to score the corresponding attribute intensity on the scale given anchored at extremities with *not at all intense* to *very intense* (Le Reverend et al., 2008; Labbe et al., 2009) (Figure 2.11). At the final stage, when the first major swallow occurs, the run is stopped by clicking the *Stop* button.

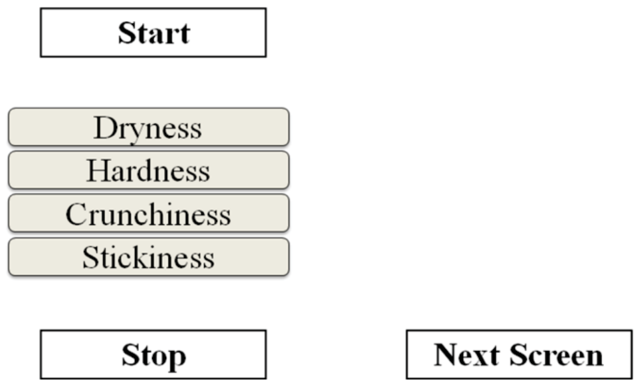



**Figure 2.10: An example of TDS procedure**

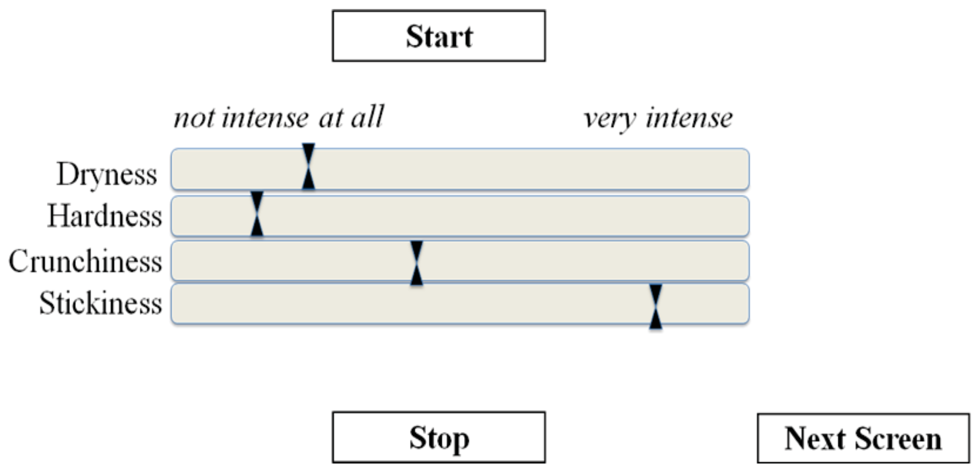



**Figure 2.11: An example of TDS procedure with intensity scale**

The word *dominant* has been described as “the most intense sensation” (Labbe et al., 2009; Albert et al., 2012), “a sensation that triggers the most attention at a point of time” (Lenfant et al., 2009), “the most striking perception at a given time” (Pineau et al., 2009), “the sensation that triggers the most attention during the eating process” (Saint-Eve et al., 2011), and “the sensations retaining the most attention” (Déléris et al., 2011a). The key element of these available dominant descriptors was “the attribute that capture the most attention”. Pineau et al. (2009) emphasizes that a dominant attribute is associated with the sensation that catches attention. The attribute is the sensation that pops up during chewing and should not be mistaken to the attribute with the highest intensity. For example, the strongest sensation can be hard in biscuits during initial chewing. At the same time, other less intense sensations such as crumbly and brittle can become dominant during chewing. Hence, subjects may choose hard at the beginning of the chewing, followed by crumbly or brittle as soon as the new sensation demands attention.

In constructing TDS curves (see Figure 2.12), the proportion of subjects who score each dominant attribute is computed at each point; in which a consensus of terms is determined. Hence, the time axis standardization is crucial. The axis is mostly standardized from first dominant attribute assessment (t=0) to the end of the evaluation when no more sensation are perceived, when swallowing occurs or when a fixed evaluation time is up (Table 2.2). Lenfant et al. (2009) plotted TDS curves with dominance rate against a standardized x-axis from first scoring (x=0) to major swallow (x=1). Albert et al. (2012) and Ng et al. (2012) took similar standardization approach for the TDS curves, the former standardized the x-axis from first scoring (x=0) to swallowing (x=100) and the latter standardized from when the *Start* button was selected (x=0) to when the *Stop* button was selected or after a fixed 60s of evaluation (x=100). The importance of x-axis standardization is unquestionable, most apparent in solid foods that are continuously processed in the mouth which changes the sensations perceived over time.

Table 2.3 summarizes the advantages and disadvantages of both TDS and TI methods. Although both methods are capable of producing reliable data, TDS makes it possible to illustrate product perception patterns over time and TI determines the kinetics of one specific attribute. Hence, the choice of method depends on the research interest.

**Table 2.3: Comparison between Temporal Dominance of Sensations (TDS) and Time Intensity (TI)**

| Temporal Dominance of Sensations (TDS) | Time Intensity (TI) |
|---|---|
| Monitor changing dominant attribute(s) continuously over the consumption period (attribute intensity may be also be recorded simultaneously) | Focuses on the intensity profile of a single attribute over time |
| Makes it possible to evaluate several temporal responses at one time | Potentially requires more time as TI focuses on a single attribute per single sensory run. Hence, difficulties in generating consistent data, which may lead to high variance between panellists and products |
| Provides data on sequence of perceived qualitative sensory changes | Provides data on profile of one attribute |
| Not much training needed | Extensive training is required |

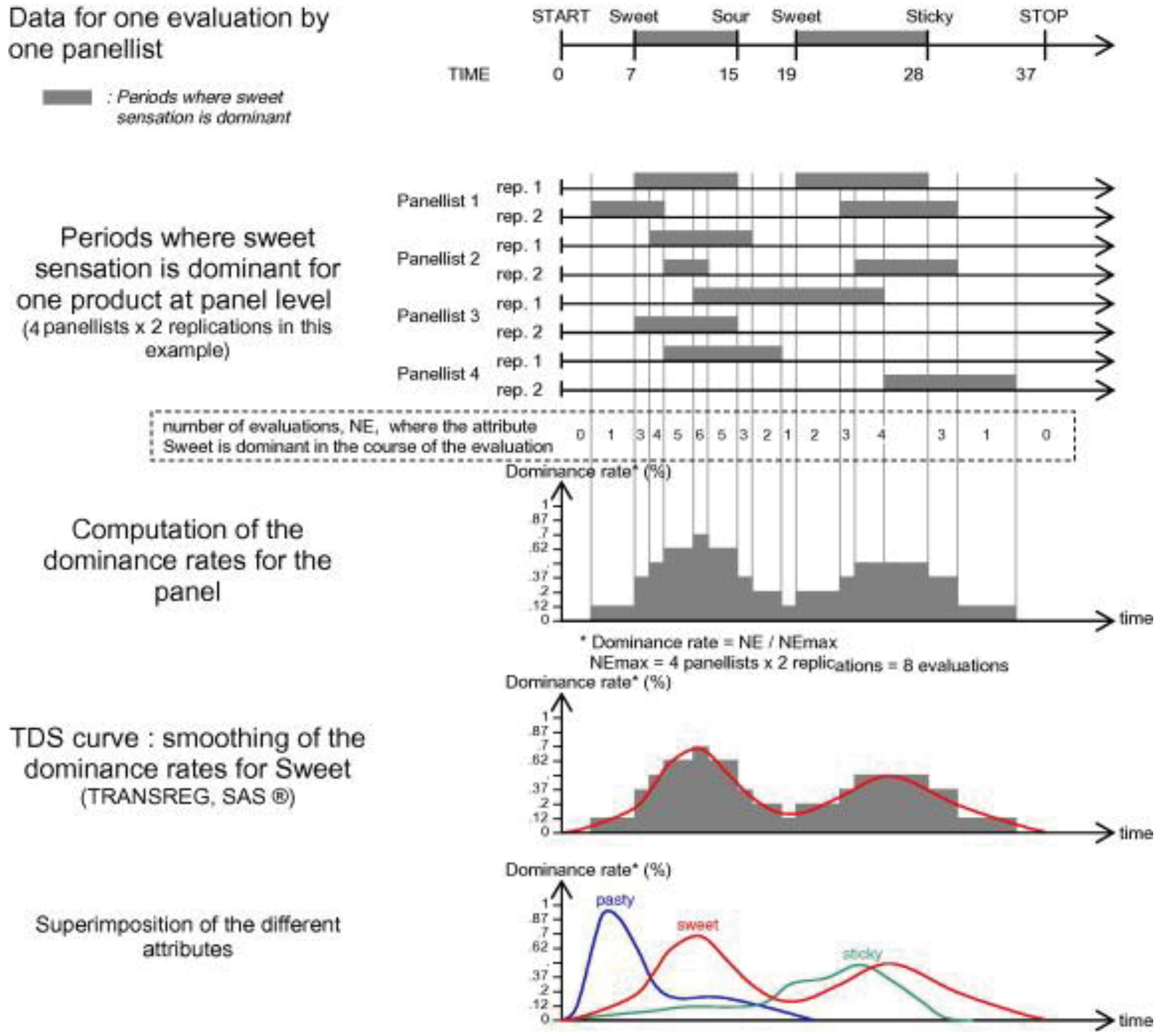



**Figure 2.12: Methods to compute TDS curves (Source: Pineau et al., 2009)**

## 2.4 Conclusion from Literature

From this literature review, we know that:

- The effect of investigating sensory sensations related to solid food system on human oral processing behaviours has been highlighted. However, the interaction of food structure, chewing behaviour, and textural perception are poorly understood.
- It has been discussed that several instruments can predict the link between mechanical properties of food, oral processing strategies to sensory attributes but these predictions often stop at first bite and often, these instruments often fail to mimic the dynamic activity in the mouth.
- In addition, examining textural changes during human chewing requires a sensory technique that can assess the changing sensory perceptions continuously.
- Hence, this study attempts to explore the suitability of using the TDS technique for understanding textural characteristics of solid food throughout out the oral processing.
- The sensory technique will be investigated in terms of its methodology capability and its feasibility to be combined simultaneously with other masticatory measurements to study the link between sensory perceptions and chewing dynamics.
- The impact of direct comparison of all instruments within similar subject group is also explored.

# CHAPTER 3

# MATERIALS AND GENERAL METHODOLOGY

## 3.1 Materials

### 3.1.1 Model Food System

A laboratory-made model food system that varies primarily in hardness level was designed to understand the food stimulus/masticatory response link. Hardness has been one of the most frequently studied attributes in mastication studies (Woda et al., 2006a) and it has been claimed that humans are more sensitive to the changes of hardness or elastic properties of solids than the changes of thickness or viscosity of liquids (Nishinari, 2004).

In food oral processing, there are few investigators that have designed food with specific properties (Foster et al., 2006; Ashida et al., 2007; Cakir et al., 2011) to study food oral processing. Some used everyday food (Kohyama & Mioche, 2004; Peyron et al., 2004b; Jalabert-Malbos et al., 2007), sometimes categorized on a scale of different hardness (Plesh et al., 1986; Piancino et al., 2008), and tenderness (Braxton, Dauchel, & Brown, 1996). These foods may differ in many other characteristics, which make it difficult to reduce to a single entity. Hence, a model food offers advantage of reproducibility whilst resembling commercial food products, and most importantly it permits swallowing allowing natural mastication processes to be observed.

### 3.1.2 Formulation of Samples

A series of biscuit recipes with a range of hardness levels, but with less variability in other textural attributes were designed by The Institute of Plant and Food Research Ltd., New Zealand for this research. The biscuits were produced using the procedures described in Kim et al. (2012). Biscuit doughs were formed by mixing the dry and wet ingredients (KM001 Series, Kenwood, UK) of different formulations. The doughs were then shaped into a rectangular cuboid, passed five times between sheeting rolls, cut lengthwise, and placed carefully over the other. The dough was re-rolled to 9 mm thickness, placed on a silicone gauze mat, rested for 15 minutes, baked in rotary oven (170 °C for 50 minutes), and cut out to the desired size. Three recipes were used in this study (Table 3.1). The recipes had similar fat to starch ratios, but with different levels of sugar; the least hard sample was soft crumbly and the hardest sample a relatively hard biscuit.

**Table 3.1: Ingredients used to formulate model food systems**

| **Ingredients** | **Sugar to Fat Ratios** | | |
|---|---|---|---|
| | **0.71 (the least hard)** | **1.47** | **2.24 (the most hard)** |
| Halo (soft) flour (g) | 665 | 610 | 572 |
| Castor sugar (g) | 165 | 280 | 355 |
| Margarine (g) | 250 | 203 | 167 |
| Salt (g) | 2 | 2 | 2 |
| Panodan Datem emulsifier (g) | 5 | 5 | 5 |
| Vanilla essence (g) | 2 | 2 | 2 |
| Water (mL) | 120 | 120 | 120 |
| Total weight (g) | 1207 | 1220 | 1221 |
| Fat to starch ratio | 0.46 | 0.41 | 0.36 |

Adapted from Kim et al. (2012)

Three batches of biscuit samples were produced for this study at different times using the same ingredients as listed above for five different sessions. Due to variations in temperature and humidity of the room or slight variations in manual or mechanical handling, there was always some variations in terms of hardness measures from bake to bake (Kim et al., 2012). During the first batch of production to obtain samples with three different levels of hardness (named as samples SP1, SP3, and SP5), samples SP3 and SP5 have similar mechanical hardness despite differences in their sugar to fat ratios. Nevertheless, taking into consideration that these biscuits were produced with different sugar to fat ratios, these samples were used in experiment for sessions 1 and 2. The second batch of biscuit samples of three different levels of hardness were produced for session 3. However, samples SP3 and SP5 were renamed as SP7 and SP9 due to the different hardness levels (as measured by fracture test). The third batch of biscuit samples were produced for the remaining of the sessions (named as Sample SP1, SP7, and SP9). These samples have similar mechanical hardness to those samples produced for session 3.

### 3.1.3 Instrumental Testing

Instrumental tests were performed on all samples produced. A fracture test (single-edge notched bend test, SENB) was carried out on each sample using an Instron Universal Testing Machine (Model 4444. Instron, High Wycombe, U.K.). This testing was chosen because it strongly correlated to the perception of hardness (Duizer et al., 2011). The critical stress intensity factor ($K_{IC}$, kPa $m^{1/2}$) was used as a measure of hardness. Biscuit samples were cut to approximately 45.0 ± 0.3mm (depth) x 14.5 ± 0.5mm (breadth) x 201.3 ± 0.8 mm (length). The SENB test involved a triple anvil apparatus. Force was applied using an anvil from the top to the centre of the test strip placed across the other two supporting anvils (150 mm apart) until fracture occurred. A force-displacement curve was plotted to determine the maximum force and area under the curve to this point. Values were then converted into measures of critical stress intensity factor in Mode I fracture ($K_{IC}$, kPa $m^{1/2}$). Measurements were performed in quadruplicate. Full procedures on the instrumental test are described in Kim et al. (2012).

### 3.1.4 Serving Method

Determination of suitable serving methods for this study involved collection of natural bite sizes from ten subjects. These subjects were potential subjects for the experiments and were selected based on the criteria as listed in Section 3.2.1. The length of the biscuits was used as a way of standardization in this study. The finalized averaged bite dimension was used was 22 x 15 x 15 mm (approximately 3.8 g) (Figure 3.1). Each biscuit was then coded and presented to subjects in triplicate. During each session, samples were presented in blocks of three; the first block of three included one of each level of hardness, as did the second and third blocks.

**Figure 3.1: Model food system used in this study**

## 3.2 General Methodology

### 3.2.1 Recruitment of Subjects

Subjects were recruited via advertising and word of mouth. The health and dental history of each subject was assessed using the administration of a screening questionnaire (Appendix A). Subjects also needed to be available, computer literate, willing to eat, and have no known allergies to any ingredients in the foods to be consumed. In general, subjects recruited were selected based on presenting good oral and general health. This included no known masticatory disorder, no dental treatment in the last 6 months, no medication that might influence salivation, and maximum of one missing tooth.

In addition to this, the subjects were all untrained/naive subjects in order to retain an element of normal consumer behaviour. This is also to study the feasibility of using naive subjects to perform selected sensory task.

This project was reviewed and approved by the Massey University Human Ethics Committee: Southern A (Application 10/13) (Appendix B).

### 3.2.2 Experimental Procedures

Experiments were carried out in 5 sessions while performing evaluation with and without TDS task whilst the number of chewing cycles and times used were recorded. The first session began with subjects chewing on samples whilst performing a TDS task, followed by the second session where subjects chewed on sample without any sensory assessments made. Half of the subjects completed these two sessions in reverse order with half performing only chewing during the first session and chewing and performing the TDS task during the second session. Prior to the third session, subjects were formally introduced to the definitions of sensory terms used with reference materials (training). Subjects also familiarized the use of the terms with commercial products and were briefed on the concepts of dominance and sequence before completing the TDS task. After the session, subjects were interviewed on how they made the dominant attribute selection via email. In the email, subjects were presented with their individual TDS curves and the mean TDS curves. Session four involved a TDS evaluation using paper format. Subjects were asked to indicate their perceived intensity for each dominant attribute presented (using a 15 cm unstructured line) on a paper questionnaire for each sample at four specific times of their mastication period at their own pace. Within each session, three samples of varying levels of hardness were presented in triplicate and the number of chewing cycles and times used were recorded. The last session (session 5) involved simultaneous recording of mandibular movements, muscles activities and evaluation with TDS task.

Temporal Dominance of Sensations (TDS)

Prior to the start of this study, the definition of texture was explained to the subjects as “sensory attributes that are perceived through the mouth using the teeth and tongue”. Definition of dominant and sequence were also explained to the subjects. Dominance was explained as “the sensation that triggers the most attention at a certain point of time” and sequence was being defined as “the sense transformation over time from first bite to swallowing”. Subjects were also asked to focus only on oro-sensory textural attributes (the focus of this thesis). The attributes used were reduced from a list generated by trained sensory experts from Plant and Food Research Institute Ltd., Palmerston North (none of which took part in this study) and were agreed and finalized by scientists involved. The descriptors used for all samples were *hard*, *crumbly*, *dry*, *crunchy/crispy*, and *sticky* which are defined in Table 3.2. These descriptors were based on commonly used force-related attributes describing fracture properties in the mouth. The number of descriptor was reduced to five in order to focus more on the development and understanding of the sensory method relative to chewing behaviours.

**Table 3.2: Definitions of attributes used for TDS task**

| Texture attributes | Definition |
|---|---|
| *Hard* | The amount of force required to bite through the sample between teeth (force required to compress, resistance to penetration). |
| *Crumbly* | The rate, ease and degree to which the sample breaks down during chewing. |
| *Dry* | Surface textural attribute which describes the perception of water absorbed by or released from a product. Sensation of dryness due to lack of saliva: absence of water. |
| *Crunchy/Crispy* | Sound associated with breakdown by chewing. |
| *Sticky* | Mechanical textural attribute relating to the force required to remove material that adheres to the teeth or to remove materials from around the mouth |

Adapted from ISO 5492: 1992 (E/F)

## 3.3 Data Acquisition

### 3.3.1 Recording the Sequence of Dominant Attributes with Temporal Dominance of Sensations (TDS)

TDS data were collected using an in-house written program where the sequence of mouse clicks was recorded and time stamped during evaluation (Labview 2009, National Instruments Corporation, Austin). The user interface consisted of buttons containing a list of relevant attributes which were pressed using a mouse. Figure 3.2 shows the TDS screen layout on the computer as seen by subjects.

Subjects were seated in front of a computer screen where a list of relevant attributes was presented. Subjects were told to select the *START* button to start evaluation when they began chewing after the sample was compressed between the molars. As soon as the first sensation was perceived, subjects selected the attribute considered as dominant from the list provided (all attributes appear on the screen). The attribute remained as the dominant attribute until the next attribute is selected. This process was repeated when the dominant attribute changed. Subjects were free to choose several times on the same attribute or never to select an attribute as dominant. Finally, subjects were also told they could select or ignore the *Swallow* button when intermediary swallows occurred (the *Swallow* button is more relevant to the last chapter to further study the differences in human chewing). Once the main bolus had been swallowed, subjects selected the *STOP* button.

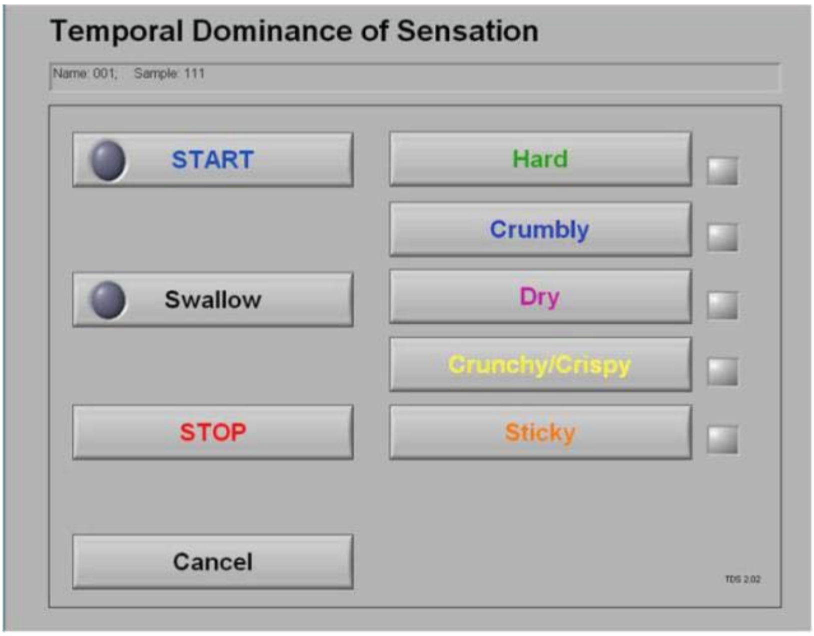



**Figure 3.2: TDS layout on computer screen**

### 3.3.2 Measurements of Oral Processing Behaviours

Jaw movements were recorded using an articulograph (Carstens AG500, Lenglern, Germany) which measured the movement of sensors fixed to the participant's teeth within a magnetic field. Muscle activities were measured using surface electromyography (EMG) (ADInstrument, Australia)

Recording of Jaw Movement: Electromagnetic Articulograph (EMA)
*AG 500, Carstens Medizinelectronik, Lenglern, Germany*

Sessions involved temporarily attaching five sensors to subject's teeth with adhesive glue (Cyano Veneer adhesive glue, Hager & Werken GmbH & Co. KG). Three sensors were glued to the left lateral incisor, right central incisor, and right lateral incisor and an additional two sensors were glued behind ears to create reference line. Subjects were asked to stand or sit in the centre of the articulograph cube and were told to refrain from moving and talking. During experiment, jaw movement data were collected with the mc5recorder programme from the AG500 data acquisition programme.

Measurement of Muscle Activities: Electromyography (EMG)
*Powerlab/4SP (ML750), ADInstruments Pty Ltd, Australia*

Session involved placing disposable surface electrodes (MLA1010B, ADInstruments) on the main masticatory muscles (the masseters). Before attaching the electrodes, the skin was cleaned with alcohol swabs (MLA1094, ADInstruments). Positive and negative electrodes were placed on the muscle, and a ground electrode was placed on a bony piece of the body, such as the elbow, shoulder blades or behinds the ear for reference. During the experiment, muscle activities were collected using Chart v5.5 for Windows, ADInstruments.

## 3.4 Data Analysis

### 3.4.1 Computing TDS Curves

A TDS session started when subjects clicked on the *START b*utton (food is taken simultaneously, t=0) and ended when the *STOP* button is clicked (t=*i*). During the TDS evaluation, attributes that triggered most attention were selected throughout a chewing sequence until the *STOP* button is clicked (when evaluation ended). These selected dominant attributes were marked using points, appointed to the corresponding attribute and time. Since only one attribute can be dominant at a point of time, one point is given to the active attributes; otherwise 0 point will be assigned. The attribute continued to be marked (as 1 or 0) at each time until another attribute was considered as dominant. Figure 3.3 shows an example of data generated by the TDS evaluation. These given points were summed up by subjects, samples, and attributes. The total points of an attribute received represent the number of times an attribute was selected as the dominant sensation. The dominance rates were then calculated from these points to plot TDS curve (dominance rate against individual mastication time).

Time →

| Subject | Sample | Attribute | t0 | t1 | t1 | t3 | t4 | t5 | t6 | t7 | t8 | t9 | t10 | t11 | t12 | t13 | t14 | t15 | t16 | t17 | t18 | t19 | t20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | SP1 | Hard | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP1 | Crumbly | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP1 | Dry | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP1 | Crunchy/Crispy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP1 | Sticky | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | SP3 | Hard | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP3 | Crumbly | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP3 | Dry | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP3 | Crunchy/Crispy | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | SP3 | Sticky | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP5 | Hard | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP5 | Crumbly | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 1 | SP5 | Dry | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP5 | Crunchy/Crispy | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | SP5 | Sticky | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

**Figure 3.3: Example of raw TDS data**

TDS curves were computed by considering each attribute separately. For each point of time, the dominance rate (proportion of subjects who selected each attribute) was obtained by dividing the number of citations of an attribute (all replications) by the number of panellists and number of replications. These dominance rates were then plotted against standardised time from first bite (t=0) and to swallow point (t=100) known as TDS curves. A high dominance rate indicated a high consensus among subject agreeing on a specific attribute as dominant.

In addition, the chance and significance levels were displayed to facilitate interpretation of the TDS curves (Lenfant et al., 2009; Pineau et al., 2009). The chance level $(Po)$ represents the proportion that an attribute could be selected by chance (Equation 1).

$$Po = \frac{1}{number\ of\ attributes} \qquad \text{Equation 1}$$

The significance level $(Ps)$ expresses the smallest value of the proportion that is significantly (p > 0.05) higher than the chance level (Equation 2).

$$Ps = Po + 1.645\sqrt{\frac{Po\ (1-Po)}{number\ of\ subjects\ x\ replications}} \qquad \text{Equation 2}$$

An attribute is considered to be dominant if the calculated dominance rate is higher than the significance level (Lenfant et al., 2009). In this study, the calculated chance level was 0.20 and calculated significance level was 0.28. When TDS curves from between change and significant levels to above the latter, the attributes are consistent at panel level (Meillon et al., 2009).

### 3.4.2 Oral Processing Behaviours

Chewing duration and number of cycles were manual counted by visual observation by experimenter. Chewing frequency was then calculated from these data. When physiology measurements were performed, chewing data was extracted from an in-house written programme (the MathWorks, Inc., US). Further details on data extraction will be discussed in data analysis section in relevant chapters.

Statistical analysis was performed with PASW Statistic (version 18, 2009). Further details on statistical analysis will be further addressed in data analysis section in each chapter.

# CHAPTER 4

# THE EFFECT OF PERFORMING A TEMPORAL DOMINANCE OF SENSATIONS SENSORY EVALUATION ON ORAL PROCESSING BEHAVIOURS

## 4.1 Introduction

Sensory evaluation can be performed using two approaches; static and dynamic. Common conventional sensory techniques are static methods where a single measurement is taken at a point in time (one point judgement). Unfortunately, this single measurement may be perceived differently at different points of time by different evaluators; during chewing or after taste if flavour is involved. As a consequence, consistent data can be difficult to generate and extensive training is required (Pineau et al., 2009). On the contrary, dynamic sensory methods involve sensory measurements over time therefore can provide information of sensory perceptions during oral processing.

In the attempt to monitor chewing behaviours in response to food structure, two masticatory recording devices were chosen to couple with a dynamic sensory technique (Temporal Dominance of Sensations, TDS). However, the effect of the evaluation procedure on the various strategies used to chew foods is unknown. For example, if the TDS technique is added to a chewing task, will the chewing results still be the same? Panel and panellists performance during for TDS has been assessed (Meyners & Pineau, 2010; Meyners, 2011), but none have investigated how subjects respond to the technique and the impact this has on chewing. In this chapter, TDS methodology and its effect on normal human oral processing behaviour was investigated by studying the texture perception of biscuit-like model foods throughout oral processing. In addition, methodological investigations of the TDS technique need to be completed in advance to ensure that this dynamic sensory method is suitable for dynamically investigating texture perception, particularly its suitability when combined with other masticatory recording devices planned for future experiments.

## 4.2 Materials and Methods

### 4.2.1 Model Food System

Biscuits were produced with the same procedures as described earlier in Section 3.1.2. The ratios of sugar to fat were modified to achieve samples with three increasing levels of hardness (SP1, SP3, and SP5). The measure of hardness was performed using a single-edge notched bend (SENB) test (Table 4.1). Each biscuit was cut to 22 x 15 x 15 mm in size (approximately 3.8 g in weight), coded, and presented to subjects.

**Table 4.1: Results from single-edge notched bend (SENB) of each recipe (mean ± SD)**

| Samples | *Critical stress intensity factor ($K_{IC}$, kPa $m^{1/2}$) |
|---|---|
| SP1 | 18.8 ± 4.4 |
| SP3 | 69.9 ± 8.3 |
| SP5 | 68.0 ±14.6 |

*procedure for instrumental test is described in Section 3.1.3

### 4.2.2 Subjects

Twenty-four untrained subjects participated in this study (83% females, 17% males: mean age 29.7 ± 5.1 years old) based on the criteria listed in Section 3.2.1.

### 4.2.3 Experimental Procedures

TDS evaluations took place during two sessions at the same time on two different days. In each of these sessions, nine (three samples x three replications) one-bite-size samples were presented according to a complete balanced experimental design. After each biscuit was chewed and swallowed, subjects rinsed their mouth thoroughly with water. Both sessions took place in a private and well lit room. A monitor was placed in front of the subjects while performing the TDS task, or else removed completely.

Sessions were carried out in a reverse order, where half of the subjects did the without TDS task first, the other half did the TDS task first. During these two sessions, oral processing times were recorded using a stop-watch and total number of cycles taken was counted visually by the experimenter. Chewing frequency was then calculated from these results.

without TDS task

During the session, subjects were asked to chew on samples in a normal manner, without attempting to make any sensory judgement on the samples served.

with TDS task

During the session, subjects were asked to chew and evaluate the samples using the TDS technique. Prior to this session, subjects were introduced to the notion of the temporality of sensations, the TDS technique, and the definitions of attributes used. Subjects were presented with a list of five attributes (*hard*, *crumbly*, *dry*, *crunchy/crispy*, and *sticky*) on the computer screen. Subjects put the sample into their mouth and simultaneously started the software by clicking on the *START* button. The complete procedures for TDS task has been discussed in Section 3.3.1.

### 4.2.4 Data Collection

The TDS data were collected using an in-house written program where the sequence of mouse clicks was recorded and the time stamped during evaluation (Labview 2009, National Instruments Corporation, Austin).

### 4.2.5 Data Analysis

Effect of Session Order and Sample Order

Subject's behaviour changes between and during the sessions, adjusting to the task and samples composition were investigated. Session order (half of the subjects did the normal task first, half did the TDS task first) and sample order factors (the first three samples, second three samples, third three samples) were added to the analysis along with the corresponding interactions with other factors.

Statistical Procedures

PASW Statistic (version 18, 2009) was used for statistical analysis. Normality tests were performed with Kolmogorov-Smirnov test. Oral processing behaviours (chew duration, cycle number, and chew frequency) were analysed using analysis of variance (ANOVA). General Linear Model procedure was used to study the effects of different factors on masticatory variables. When the F-test was significant, the Fisher's least significant difference (LSD) was used to compare differences of means. When data was not normally distributed, a Friedman's Analysis was performed.

### 4.3 Results

#### 4.3.1 Effect of Session Order and Sample Order during Evaluation of Samples with and without Performing the TDS Task on Oral Processing Behaviours

No session order effects were observed (Table 4.2) for all oral processing parameters (chewing time: F = 0.481, p = 0.495; number of cycle; F = 0.674, p = 0.420; chewing frequency: F = 0.099, p = 0.756). Sessions 1 and 2 pooled all data from these sessions regardless of whether the TDS task was performed or not. Insignificant results suggested that the subjects' chewing behaviours were consistent regardless of the task performed.

The order that the samples were presented was significant for the chewing time parameter within a session. The first three samples were chewed significantly longer than the second and third three samples when samples were chewed (F = 13.693, p = 0.000). Chewing frequency was also significantly affected by sample order (F = 8.307, p = 0.001). For both sessions, the samples in the first blocks of three were chewed slower compared to the second and third three samples.

**Table 4.2: Effect of session order and sample order on oral processing behaviours (mean ± SD) (n=24)**

| Sample Order | Chewing Duration (s) | | Number of Cycles | | Chewing Frequency ($s^{-1}$) | |
|---|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 1 | Session 2 | Session 1 | Session 2 |
| 1-3 | $27.2\pm9.3^{aA}$ | $26.9\pm8.7^{aA}$ | $34.0\pm11.7^{aA}$ | $34.0\pm10.1^{aA}$ | $1.27\pm0.21^{aA}$ | $1.30\pm0.24^{aA}$ |
| 4-6 | $25.1\pm8.1^{aB}$ | $26.0\pm8.9^{aA}$ | $33.2\pm11.3^{aA}$ | $34.2\pm10.7^{aA}$ | $1.34\pm0.22^{aB}$ | $1.36\pm0.25^{aB}$ |
| 7-9 | $24.8\pm8.9^{aB}$ | $27.4\pm10.1^{aA}$ | $32.6\pm11.5^{aA}$ | $35.4\pm11.2^{aA}$ | $1.34\pm0.23^{aB}$ | $1.34\pm0.26^{aAB}$ |

Different letters (a,b) indicate a significant difference between sessions ($p < 0.05$)
Different letters (A,B) indicate a significant difference between sample groups ($p < 0.05$)

### 4.3.2 Evaluation of Samples without Performing the TDS Task

No apparent differences were observed in chewing durations and number of cycles between samples SP1 to SP5 when these were chewed without performing the TDS task (Table 4.3). Sample SP1 had a significantly slower chewing frequency ($F = 7.662$, $p = 0.001$) than samples SP3 and SP5. These results indicated that sample compositions modified the chew frequency. The different compositions may have influenced the samples breakdown which includes interaction with saliva, and hence affecting the chewing strategies.

**Table 4.3: Effect of samples with different hardness levels on oral processing behaviours without performing the TDS task (mean ± SD) (n=24)**

| Samples | Chewing Duration (s) | Number of Cycles | Chewing Frequency ($s^{-1}$) |
|---|---|---|---|
| SP1 | $25.1 \pm 9.9^a$ | $30.4 \pm 11.4^a$ | $1.24 \pm 0.18^a$ |
| SP3 | $25.0 \pm 9.0^a$ | $32.6 \pm 11.5^a$ | $1.34 \pm 0.24^b$ |
| SP5 | $23.8 \pm 6.3^a$ | $31.2 \pm 9.2^a$ | $1.33 \pm 0.24^b$ |

Different letters (a,b) indicate a significant difference between samples ($p < 0.05$)

### 4.3.3 Evaluation of Samples whilst Performing the TDS Task

Table 4.4 shows the effect of evaluating samples while performing the TDS task on oral processing behaviours. Similar observations were seen when subjects evaluated the samples. Slightly longer chewing durations and numbers of chewing cycles were used when evaluations were made, but this difference was not significant. Again, sample SP1 ($F = 6.802$, $p = 0.003$) had a significantly different chewing frequency than samples SP3 and SP5.

**Table 4.4: Effect of samples with different levels of hardness on oral processing behaviours whilst performing the TDS task (mean ± SD) (n=24)**

| Sample | Chewing Duration (s) | Number of Cycles | Chewing Frequency ($s^{-1}$) |
|---|---|---|---|
| SP1 | $28.5 \pm 9.7^a$ | $35.2 \pm 10.0^a$ | $1.29 \pm 0.26^a$ |
| SP3 | $27.5 \pm 9.2^a$ | $36.6 \pm 11.6^a$ | $1.37 \pm 0.23^b$ |
| SP5 | $27.6 \pm 9.0^a$ | $37.3 \pm 10.8^a$ | $1.39 \pm 0.24^b$ |

Different letters (a,b) indicate a significant difference between samples ($p < 0.05$)

### 4.3.4 Effect of Evaluating Samples with and without Performing the TDS Task on Oral Processing Behaviours

Further ANOVA was used to compare the effect of tasks on oral processing behaviours when chewing samples with different hardness levels. Subjects mainly showed differences in the number of cycles used when performing different tasks (Figure 4.1). The number of chews used increased when TDS task was performed for all samples. Increment of mastication durations were also observed between the tasks, but only for sample SP5.

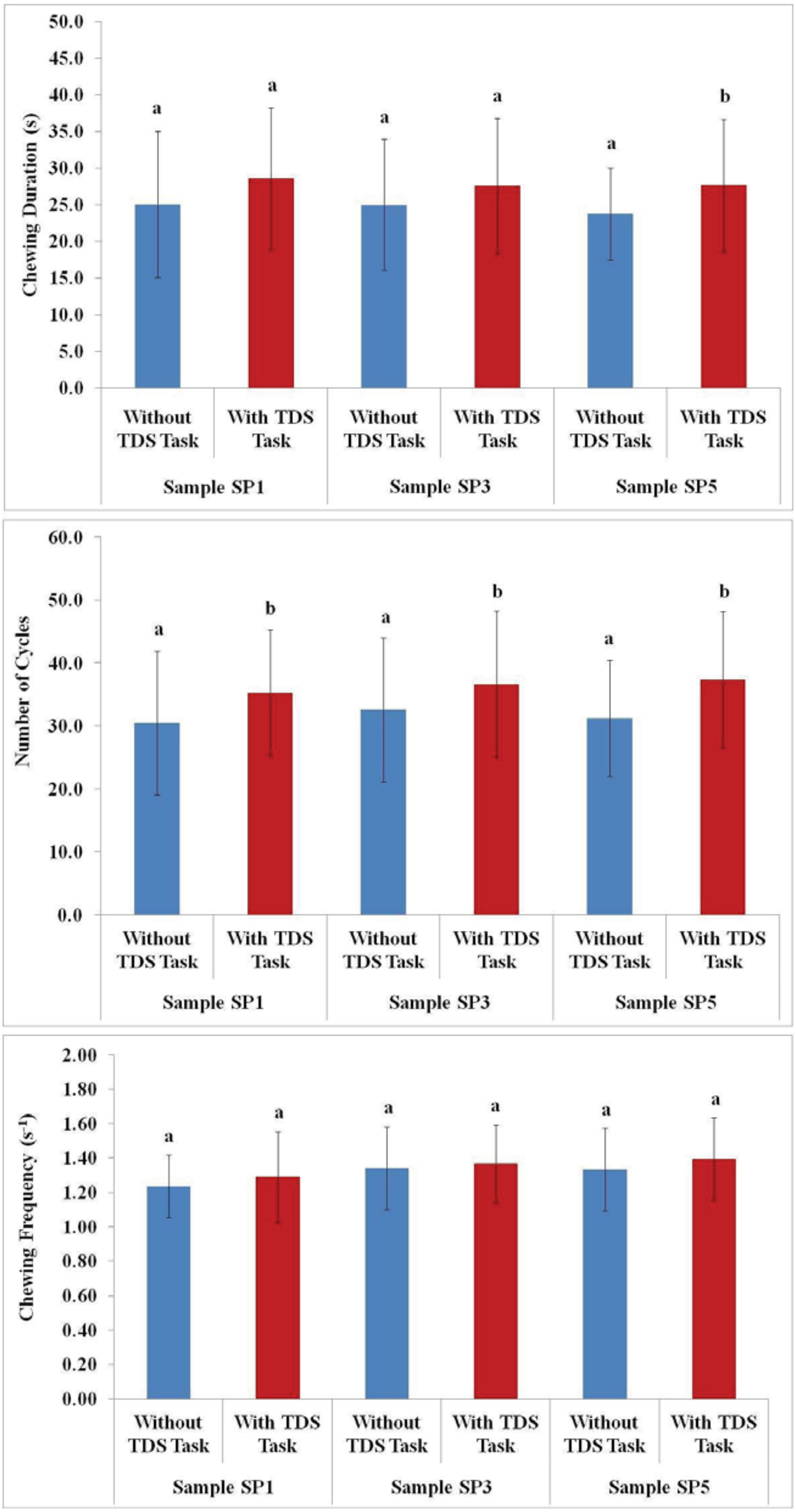



**Figure 4.1: Oral processing parameters (chewing durations, number of cycles, and chewing frequency) for different samples averaged across 24 subjects (different letters indicate significant difference between tasks)**

## 4.4 Discussion

### 4.4.1 Influence of Session Order and Sample Order on Oral Processing Behaviours

The order the tasks were performed did not influence the oral processing behaviours. Regardless of the order the tasks was performed, similar chewing strategies were used suggesting a consistency in human mastication. However, further into a session, subjects tended to chew faster. The difference in the mastication parameters for the first three samples could be explained by the need to experience the task for the first time or it may be a sign of fatigue. When the task was performed initially, subjects may have taken longer time to explore and familiarize themselves with the task, hence the slower chew frequency. When the task was performed for the second and third time, subjects may have adapted and been able to comfortably perform the task, hence faster chew frequencies were seen. In addition, fatigue is very unlikely as differences show up after as little as three samples. No complaints regarding fatigue were made. Published research using TDS for a range of 6-20 samples have not reported any issues regarding exhaustion (Le Reverend et al., 2008; Labbe et al., 2009; Lenfant et al., 2009; Meillon et al., 2009; Pineau et al., 2009; Teillet et al., 2010; Albert et al., 2012). This observation could be due to a psychological effect where subjects were eager to end the experiment. Lassauzay et al. (2000) observed incremental changes in chewing characteristics during chewing of elastic model foods from the first to the second sessions and small differences between the last two sessions. Chewing measurements varied in the repeated sessions and the variation may due to psychological variables such as emotional state and tiredness. These authors recommended to exclude results from the first session as valuable data could be expected from subsequent sessions. Chewing behaviours can also be influenced by factors like hunger, mood, and appetite. It would be interesting to determine if the changes in behaviours were a consequence of these factors in future (not in the scope of this thesis). It is therefore recommended in future experiment to 1) use warm up samples before each formal evaluation to ensure familiarity to samples, the TDS task, and the environment, and 2) ensure subjects had food two hours before evaluation to prevent hunger before data is collected.

### 4.4.2 Influence of Tasks on Oral Processing Behaviours

Between tasks, subjects tended to chew for longer (both in times and number of cycles) when performing the TDS task than when chewing in a normal manner. These results indicated that there was an effect of adding the TDS task on normal oral processing behaviours. The long chewing time was thought to link to the time needed to complete a sensory assessment and the effect of placing a computer in front of the subjects was also clearly shown. Human chewing behaviour may be sensitive to environmental conditions; under controlled laboratory settings compared to a natural environment. Both Gerstner and Cianfarani (1998) and Po et al. (2011) found higher mean chewing durations when chewing activity was monitored under a controlled laboratory setting rather than in a natural environment. These findings emphasized that performing a chewing task under a guarded surrounding such as being asked to focus and make a sensory judgement could potentially change human natural chewing behaviours. In the present study, ANOVA revealed that the mean values for the number of chewing cycles used were significantly higher when subjects were asked to perform the TDS task. Chewing frequency was the same throughout the task. Hence, the chewing rhythm was maintained, with an additional few cycles used. The number of cycles could be the more important mastication measure differentiating oral processing behaviours between the tasks. The finding is in agreement with Alexander (1998) where number of cycle iss a determinant factor before swallow. This finding suggested adaptation of the masticatory process to the mechanical properties in the mouth and more specifically the context (i.e. performing an unnatural task such as chewing and evaluating a sample using TDS).

Within each task, no distinctive chewing pattern was seen as from sample SP1 to SP5. It was expected that due to the higher solids content (i.e. sugar) potentially dissolved more rapidly and thus aiding particle size reduction, faster chewing frequency would be seen in the harder samples. However, present results are opposite of those commonly reported, where longer durations were needed to chew on hard products before forming a safe swallow-able bolus (Brown, Langley, Martin, & Macfie, 1994; Hiiemae & Palmer, 1999; Hough, Der Pilar Buera, Chirife, & Moro, 2001; Engelen et al., 2005; Foster et al., 2006). It must be taken into consideration that in these reported mastication studies, foods were crudely categorized into soft (cheese, salami) and hard (raw carrot, apple, biscuit) or food were altered to a range of hardness by additional food processing such as oven drying. The gels used by Peyron et al. (2002), Lassauzay et al. (2000), and Foster et al., (2006) were monoattributes. This may not be a representation of the influence of product properties (structures) on the chewing behaviours. These samples were also not mono-attribute so it is not possible to relate to any changes to hardness alone.

The present study focussed on the influence of structural properties of foods on chewing behaviours and model food systems were custom made to have increasing levels of food hardness. However, when a food sample is chewed, other attributes changed throughout a chewing sequence. Although no significant differences were found in chewing time and number of chewing cycles, the food properties had a significant influence on the chew frequency. Sample SP1 had a significant slower chewing frequency than samples SP3 and SP5. Both the samples were chewed with similar frequency. This may be due to their similar mechanical hardness although they were made using different sugar to fat ratios. The lack of dissolvability property (low sugar content) in sample SP1 may have created a drying sensation in the mouth and hence influenced the breakdown of sample (slow chew rate). It is deemed that the chewing rate was influenced by the state of the food (food structure) in the mouth, a result of differences in sample compositions. Peyron et al. (2002), Foster et al. (2006), and Cakir et al. (2011) also have shown that the structural properties of food played a key role in modifying oral processing behaviours and that mastication was highly responsive to changes food texture.

## 4.5 Conclusion

This finding confirmed the presence of two types of masticatory adaptation. The first is the adaptation of the masticatory process to the task performed. Adding a task to natural chewing (performing TDS task while chewing) does modify oral processing behaviours slightly (increase in time and number of cycles) although chew frequency remains constant. The second masticatory adaptation is to the altered textural properties caused by changing compositions and structure. Human mastication adapts to the changes in food structural properties by altering the chew frequency. These adaptations will be further explored in the later chapter by tracking the masticatory behaviour using instrumental methods.

# CHAPTER 5

# EVALUATION OF MODEL FOOD SYSTEM WITH SMALL STRUCTURAL DIFFERENCES USING TEMPORAL DOMINANCE OF SENSATIONS TECHNIQUE

## 5.1 Introduction

The Temporal Dominance of Sensation technique is used to assess changes in sensory perceptions of a food product throughout a chewing sequence, within a pre-defined period (Pineau et al., 2009) or after swallowing when after-taste perception is important (Labbe et al., 2009). However, many aspects of its methodologies are still yet to be understood, in particular its capability as a sensory method, its reliability to produce consistent sensory data, and its feasibility as a method to be used for relating sensory perception to chewing dynamics, which is the focus of this thesis. The technique needs to be explored for its ability to differentiate food with small structural differences and its suitability to be simultaneously analyzed with physiological data in future experiment.

A TDS evaluation usually starts when food is put into the mouth and stops when food is swallowed. Hence, a chewing sequence is defined from the time when the start and stop buttons are selected. However, the chewing period in a TDS curve is often standardized from the time when the first selection of a dominant attribute is made to the point of swallowing (Lenfant et al., 2009). The first part of this chapter explored the effect of using different time-axis standardization methods on TDS curves. In addition, the number of subjects required to produce reliable and representative TDS curves was also investigated. The second part investigated the capability of using the TDS technique for understanding the structural properties of a biscuit-based model system.

## 5.2 Materials and Methods

### 5.2.1 Model Food System

Three formulations of model food system were produced with the same manufacturing procedures as described in Section 3.1.2. The sugar to fat ratio increased from sample SP1 to samples SP3 and SP5. Hardness levels were determined by fracture test and values were reported as a critical stress intensity factor, $K_{IC}$, kPa $m^{1/2}$ (Table 5.1). Samples SP3 and SP5 had the same mechanical hardness despite differences in their sugar to fat ratios. Each biscuit was cut to 22 x 15 x 15 mm in size (approximately 3.8 g in weight), coded, and presented to subjects in triplicate (randomized blocks of three).

**Table 5.1: Results from single-edge notched bend (SENB) of each recipe (mean ± SD)**

| Samples | *Critical stress intensity factor ($K_{IC}$, kPa $m^{1/2}$) |
|---|---|
| SP1 | 18.8 ± 4.4 |
| SP3 | 69.9 ± 8.3 |
| SP5 | 68.0 ±14.6 |

*procedure for instrumental test is described in Section 3.1.3

### 5.2.2 Subjects

Twenty-four untrained subjects were recruited in this study (83% females, 17% males: mean age 29.7 ± 5.1 years) based on the criteria listed in Section 3.2.1.

### 5.2.3 Experimental Procedures

Subjects were introduced to the concept of temporality of sensations and were briefed on the attributes and definitions used in the study (Tables 3.2 in Section 3.2.2). All five attributes were presented simultaneously on a computer screen. Subjects were instructed to put the samples into their mouth and click on the *START* button to begin evaluation. During evaluation, subjects were asked to identify and select any of the five attributes that they perceived as dominant whilst chewing on the food. Subjects were also informed that they were allowed to choose the same attribute several times throughout an evaluation or conversely to never select an attribute as dominant. When final swallow occurred, subjects clicked on the *STOP* button.

### 5.2.4 Data Collection

The TDS data were collected using an in-house written program where the sequence of mouse clicks was recorded and the time stamped during evaluation (Labview 2009, National Instruments Corporation, Austin). Oral processing data (total chew duration and numbers of cycle taken to complete a chewing sequence) were observed and manually collected by the researcher.

### 5.2.5 Data Analysis

Temporal Dominance of Sensations Curves

For each sample evaluated and at each point in time, the dominance rates for each attribute were calculated. TDS curves were created for each attribute by plotting the calculated dominance rates against standardized mastication time. The x-axis of the TDS curves represented the mastication period from first bite or time when the first dominant attribute was selected (x=0) to final swallowing (x=100).

Prediction of Number of Subjects Required to Produce TDS Curves

The determination of the number of subjects required to produce reliable and representative TDS curves was performed using the sum of square calculation. The sum of square differences between the subsets of subjects and the average produced for all subjects (n=24) were calculated. Standard deviation was used as the criteria to determine the appropriate number of subjects to produce good TDS curves. A sum of square of less than 2 indicates good level of agreement among panellists.

## 5.3 Results

### 5.3.1 Variations in Mastication Durations

The average time before major swallowing occurred varied from 14.6 s to 48.2 s for sample SP1, 14.8 s to 44.1 s for sample SP3, and 14.1 s to 44.4 s for sample SP5 (Figure 5.1). It is well known that large variations among individuals exist for all parameters of mastication even when potential influencing variables such as age, gender, and dental status are controlled (Lassauzay et al., 2000). Different foods and replications could be another potential source of variations. Taking this into consideration, data from each subject are suggested to standardize according to individual mastication durations (Pineau et al., 2009).

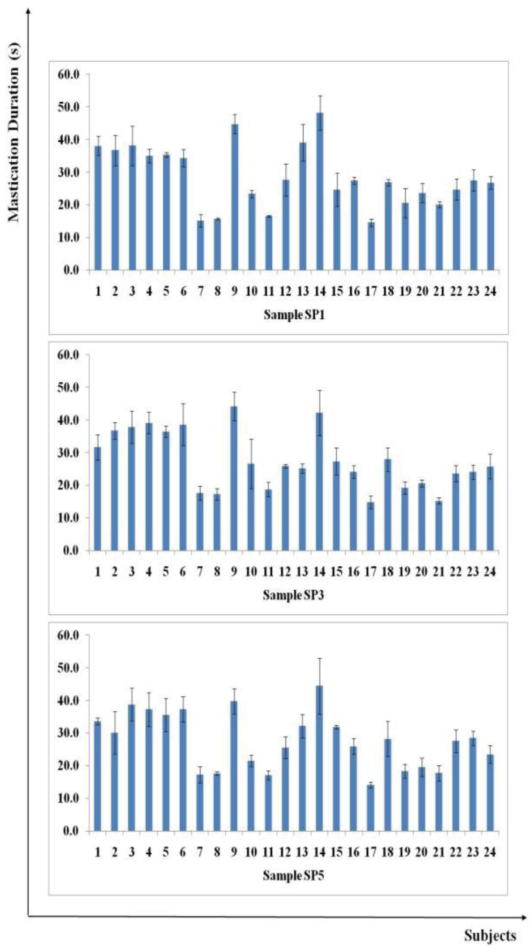



**Figure 5.1: Mastication durations for all subjects (n=24) chewing on samples SP1, SP3, and SP5 in triplicate**

### 5.3.2 Characterizing Model Food System of Different Structural Differences with Temporal Dominance of Sensations Technique

In general, sample SP1 was dominantly perceived as *crumbly*, *dry*, and *sticky*. A combination of all attributes was used to describe samples SP3 and SP5 throughout the mastication period, although sample SP5 was predominantly described as *hard* (Figures 5.2 A-C). Results showed that the variation in the initial hardness levels (structure) influenced the type of attribute perceived. A hard sample (SP5) was described as *hard* followed by *crunchy/crispy* whereas the least hard sample (SP1) was described successively as *crumbly* followed by *dry*. It is interesting to note that all samples were dominantly perceived as *sticky* towards the end of the chewing sequence (from 80% of the mastication stages).

The time an attribute was perceived as dominant also depends on the initial hardness levels. For samples SP1 and SP5, the dominance rate of at least one attribute became significant after food intake. Then, two to three attributes became dominant which differentiated the samples. A different phenomenon was observed for sample SP3. Although no attribute was perceived as dominant to a significant level, the sample was described using attribute *hard*, *crumbly*, and *crunchy/crispy* after food intake.

Standardizing the time-axis from either the first bite or first dominant attribute selection can potentially affect the sensory profiles, most apparent for sample SP3 (Figures 5.2 B and E). When the time-axis was standardized from first bite to swallowing, the attribute *hard* was not perceived at a significant level (significance level less than 0.28) in sample SP3. Conversely when the time-axis was standardized from first dominant attribute selection to swallowing (Figures 5.2 D), attribute *hard* appeared to be dominant for sample SP3. This is a direct consequence of this type of standardization method. In addition, this type of standardization method only represents the sensory perception that occurs from after the first dominant attribute was selected. The overall sensory perception is dynamic and occurs during all stages of the oral processing and hence the chewing sequence should be considered as a whole (from first bite to swallow). The over-lapping of attributes may also be caused by results collected from replicates of samples or different subject's interpretation of the attributes used.

The time an attribute was perceived as dominant depends on the standardization technique used. It can be either earlier or later during the mastication sequence. For example, attribute *crumbly* only became dominant at t=15 when the time-axis was standardized from first bite for sample SP1. However, the attribute dominated at the start of the time-axis (t=0) when time was standardized from first attribute selection. The time taken (at least 15% of the whole mastication period) to select *crumbly* from ingestion was not accounted for. This is in particular a disadvantage when the first bite is often of interest in many mastication studies. The first bite, which is part of the Stage 1 Processing in a mastication period, is often related to the preparatory stage (Foster et al., 2011). During this stage, sensory perception received may cover a wide range of textural features, which include attribute *hard* (Chen, 2009). In addition, first bite has been proven to be an exploratory procedure which is used for recognizing a specified attribute or to decide the subsequent chew strategy (Dan et al., 2007). It is clear that first bite is food-related which depends highly on the mechanical properties of the food and should be considered in studying human mastication.

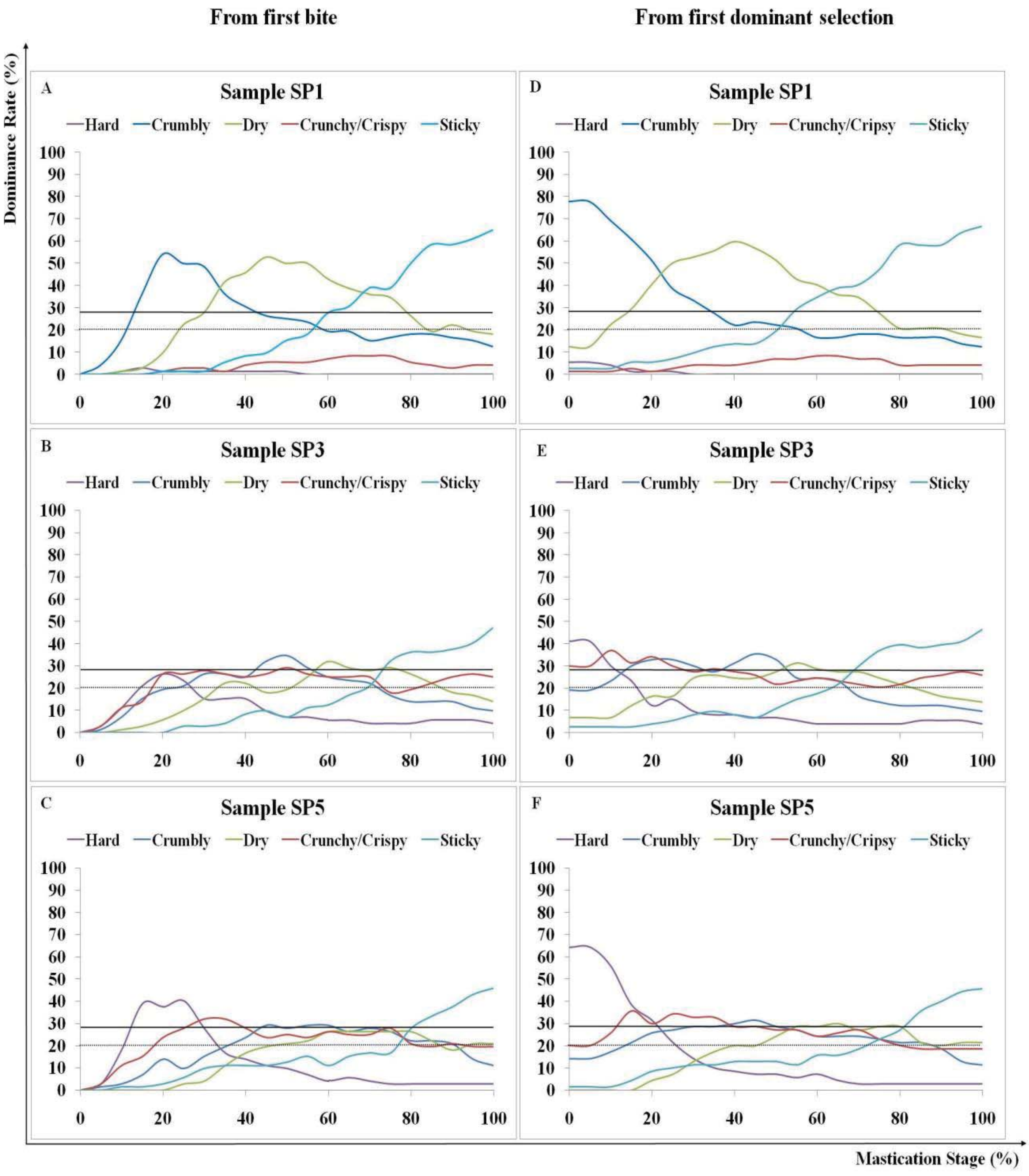



**Figure 5.2: TDS curves for samples SP1, SP3, and SP5 (dominance rate versus different method of standardizing time-axis for mastication period). Figures A-C defined time-axis from t=0 (first bite) to t=100 (swallow) and D-F defined time-axis from t=0 (first dominant attribute selection) to t=100 (swallow). Chance level is represented with dotted line and significance level is represented with solid line (n=24)**

Time to First Dominant Attribute Selection

The time when the first dominant attribute was selected is shown in Table 5.2. The time required to make the first selection for the least hard sample (SP1) was significant longer compared to the hardest samples (SP3 and SP5) ($X^2(2) = 9.194$, $p = 0.010$). These times corresponded to almost 19.8% of the total chewing time before first attribute selection for sample SP1, 16.3% and 16.2% for both samples SP3 and SP5, respectively ($X^2(2) = 7.528$, $p = 0.023$). The proportions of time corresponded to the first stage of a chewing sequence and therefore must be considered in order to capture a complete sample profile. The differences in the range of time used for the first dominant attribute selection between subjects are a key consideration not to standardized the TDS curves by the first dominant attribute selection. The range of time had a huge consequence on the method of standardization and further illustrates the importance of standardizing the time-axis from first bite.

It is worth noting that although the oral processing times were not different between samples, the time required to select the first dominant attribute significantly differed. This indicates that the different sample compositions did not influence the oral processing time but it affected the TDS performance. This is likely because the first dominant attribute was more obvious for the harder samples as compared to a softer and more crumbly sample.

**Table 5.2: Time to first dominant attribute selection for different sample compositions (mean ± SD and range of values) (n=24)**

| | SP1 | SP3 | SP5 |
|---|---|---|---|
| Time to first dominant attribute selection (s) | $5.5 \pm 3.9^a$ (1.0 – 22.1) | $4.3 \pm 3.5^b$ (1.0 – 23.1) | $4.0 \pm 2.6^b$ (1.3 – 16.7) |
| Average Oral Processing Time (s) | $28.5 \pm 9.6^a$ (13.1 – 54.3) | $27.5 \pm 9.1^a$ (12.6 – 48.6) | $27.6 \pm 9.0^a$ (13.6 – 54.3) |
| Proportion of time taken to make first dominant attribute selection (%) | $19.8 \pm 13.7^a$ (3.9 – 77.9) | $16.3 \pm 11.0^b$ (3.2 – 38.5) | $16.2 \pm 13.0^b$ (3.0 – 76.9) |

Different letters (a,b) indicate a significant difference between samples ($p < 0.05$)

### 5.3.3 Characterizing TDS Curves with Different Numbers of Subjects

Larger standard deviations from the sum-square differences were observed for small numbers of subjects (Figure 5.3). However, a long tail was observed with low variations (smaller standard deviations) from ten subjects onwards. Samples were tested in triplicate, hence a total of 30 observations were made. This means that it can be suggested than a minimum of ten subjects are required to reach a good level of agreement among panellists when samples are tested in triplicate.

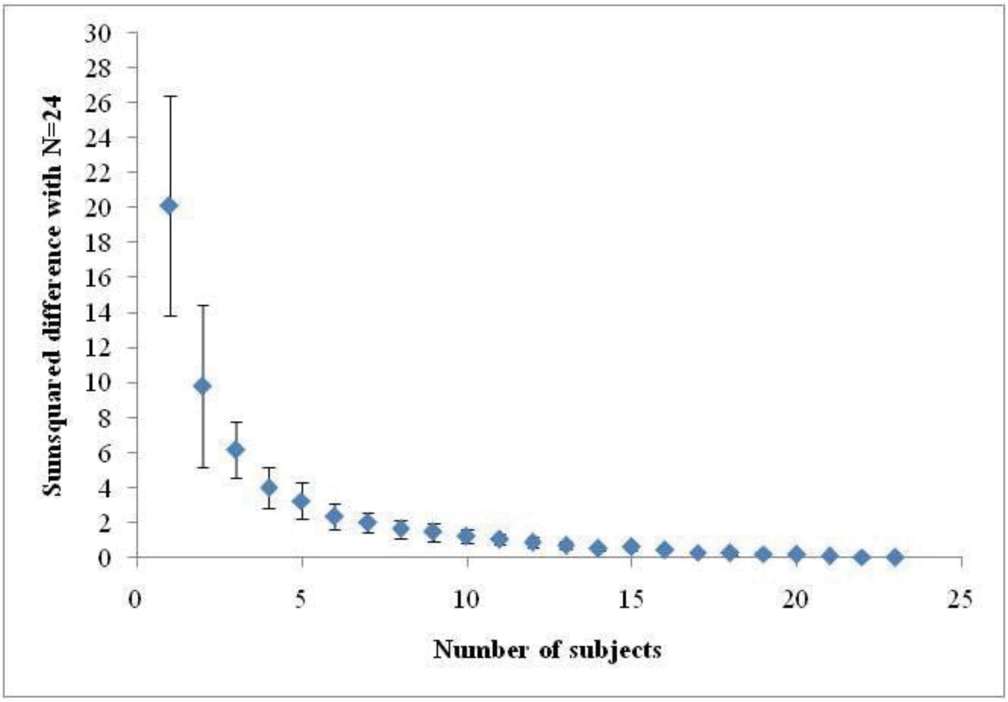



**Figure 5.3: Calculated sum of square from combinations of subjects for total number if subjects n ranging between 1 and 23 (mean ± SD)**

## 5.4 Discussion

Results have shown the effect of different time-axis standardization methods, in particular the time when an attribute was perceived as dominant. In general, all samples showed similar attribute transitions regardless of whether the time-axis was standardized from the first bite or the first dominant attribute selection. However, the key difference is that when time-axis was standardized from first selection to swallowing, higher dominance rates can be seen. This is a direct consequence of this type of standardization. Taking into consideration that a chewing sequence should be considered as a whole (from first bite to swallow), the characteristic of the overall sensory perception is dynamic, the range of times used to select the first dominant attribute are different between subjects, the oral processing times are different, and the number of replications used, the time-axis was standardized from first bite.

Most TDS studies standardized the time-axis when the first dominant attribute was selected (t=0) to swallowing (t=1) (Lenfant et al., 2009; Albert et al., 2012) and some standardized the time-axis based on evaluation time (Dinnella et al., 2012) although data acquisition started when food was taken into the mouth and ended when participants no longer perceived sensations. Clearly, the sensory perception from ingestion to first scoring was unaccounted for. Lenfant et al. (2009) attempted to describe the succession of the perceptual events that happen in the mouth by following the changes in texture attributes created from wheat flakes throughout chewing. Albert et al. (2012) assessed the sensory attribute evolution over the consumption of time of fish sticks. Standardizing time-axis from the first attribute selection seems sensible for liquid food but not solid foods. It is known that solid foods require more complex oral processing (van Vliet, 2002), and that longer time needs to be allocated for sample exploration in the mouth before a decision is made for the second and third bites (Heath, 2002). Because the time from first bite to first attribute selection can vary greatly per person, this means that the standardization can be different for different people.

When chewing and evaluating a sample, the early stage of a mastication sequence was mainly used to explore the sample characteristic. It was clearly shown that almost one fifth of a mastication sequence was used before an attribute was selected as dominant. Hence, it is important to take into consideration the time from first bite to first dominant attribute selection. Lenfant et al. (2009) have also indicated that an attribute became dominant just a few seconds after food intake. Present data showed a delayed in making the decision for dominant attribute selection. This may indicate that subject need to take a few chews to determine what was being perceived. The early stage (which includes the first bite) of a chewing process has been viewed as an exploratory procedure to determine how food behaves in the mouth and the mouth would respond to it (Bourne, 2004; Peyron et al., 2004b; Foster et al., 2006; Dan et al., 2007). The importance of considering the first bite as t=0 was further emphasized when the focus of two initial bites during the early stage of mastication has been of main interest in food texture researches. These bites were often used to correlate to data from instrumental methods such as TPA which supposedly mimic the first two chews of the human chewing (Bourne, 2002), but corresponds to only 2-10% of the total mastication time. But the present study showed that subjects are not making a decision until about 20% through a mastication sequence, which may be more than two bites into their chewing sequence. Hence, when more than the first bite is of interest, the data collected from TPA may not be practical for correlation. The validity of its information is of concern. It may also not be relevant given dominant sensations come after the first few bites.

In general, subjects appear to make their first attribute selection based on the hardness of samples. *Hard* is often defined as (1) the force required to rupture the food and (2) the force required to deform a food to a specific extent (Christensen, 1984). The former definition relates to first bite sensation and the latter relates to first bite and subsequent sensations. Although the definition of *hard* suggested that hardness can be perceived during the first bite and after the first bite, subjects sometimes did not select *hard* as the dominating attribute but *crunchy/crispy* or *crumbly* was used. These attributes appear to be closely related sensations (Brown et al., 1998b). It must be noted that in the present study, subjects were only given a definition for each attribute used before the TDS evaluation and this may have caused the use of these inter-related sensory sensations. Nevertheless, these attributes are related to force used to fracture a food, in particular fracturing of sample during first bite.

Sample SP1 had the lowest critical stress intensity factor which indicates the lowest measure of hardness. The sample was not perceived as *hard* but *crumbly* at the early stage of mastication. *Crumbly* is defined as a “mechanical textural attribute related to the force necessary to break a product into crumbs or pieces" (ISO 5492, 1992). Both samples SP3 and SP5 had different sugar to fat ratios but both shared similar levels of hardness, as shown by instrumental tests. However, these differing ratios were likely to lead to different breakdown pathways in the mouth (changes in structure). Sample SP3 was first perceived as both *hard* and *crunchy/crispy* whereas sample SP5 was firstly perceived as *hard.* Both attributes *hard* and *crispy/crunchy* were known to be highly correlated (Brown et al., 1998b) and hence the reason both the attributes were selected repeatedly. The critical stress intensity factor (Kc) has been found to be linearly related to sensory hardness and crunchiness perceptions for foods such as cucumber, carrot, celery, and apple (Vincent, Saunders, & Beyts, 2002; Harker, White, Gunson, Hallett, & de Silva, 2006).

After the initial chewing cycles, the crumbs or pieces produced may have prompted different sensations among subjects. If the sample reduced to many small particles thereby increasing the surface area, it may result in greater absorption of saliva and hence the sensation of *dry* being selected (sample SP1). If the sample reduced in size rapidly and had higher soluble solids (sugar), it may result in a sensation like *crumbly* being perceived (samples SP3 and SP5). The absence of moisture (saliva) may have caused the crumbs to adhere to the mouth or teeth, in which more time would be required to reposition and to remove sticky materials from the teeth. As a consequence, there is a tendency for *dryness* to dominate during mid-chewing.

In some cases, an equal level of dominance can be seen during mid-chewing. This was most obvious for samples SP3 and SP5 where some attributes were repetitively perceived as dominant during chewing. It should be noted that a mixture of sensations are continuously perceived throughout chewing and at some points in time, no attribute is more dominant than another. This may also be due to different interpretations of the texture terms amongst subjects indicating lack of understanding of the terms used or it may be a reflection of variations in chewing strategies and the state of food in the mouth or saliva production. The results clearly indicate that samples with different texture properties in the mouth can impact the sensory profiles and chewing strategies, and that hardness is not the only determining factor although it has been shown otherwise (Hiiemae & Palmer, 1999; Peyron et al., 2002; Ashida et al., 2007). In addition, samples of higher sugar ratios may have a glassy state which can be related to hardness or crunchiness of a product, whereas sample of higher fat ratio (low sugar level) have melting properties which may relates to the crumbly aspect of the sample. Towards the end of the chewing sequence, subjects tended to resort to only one attribute (*sticky*) as dominant until swallowing.

*Sticky* is defined as “the mechanical textural attribute relating to the force required to remove material that adheres to the teeth or to remove the teeth from the sample” (ISO 5492, 1992). Hence, it was expected that the selection of this attribute would be based on the need to remove the sample from the teeth or from anywhere in the mouth for swallowing. Solid food seems to be masticated until it reaches a defined texture before any major swallowing occurred (Peyron et al., 2011). Loret et al. (2011) have also observed similar features where all samples were described as *sticky* at the end of mastication for a series of cereal products with different textural properties. This led to the suggestion that the sensory property may be a signal for swallowing. The authors also found that although each subject had their own mastication strategy leading to a food bolus with different rheological properties and moisture contents, the particle size distribution in the bolus just before swallowing was comparable for all subjects (Hutchings et al., 2011; Peyron et al., 2011; Hutchings et al., 2012). This study further emphasized that the food bolus reaches a specific textural property in the mouth which may act as a trigger to swallow.

Hence, more experiments need to be conducted to study the effect of different textural properties in the mouth on sensory profiles and chewing strategies. The fact that subjects are different and vary among themselves, Stone and Sidel (2004) suggested that measurements should be repeated with enough subjects (often 20-50) to ignore all the variability. However, cost and time are of concern. Therefore, there was an attempt to demonstrate that TDS curves are reproducible with an appropriate number of subjects and replications. The number of subjects needed to compute a reliable and representative TDS curves was predicted to be of at least 10 subjects in triplicate (i.e. 30 observations). Some TDS studies have used a small number of panellists to as many as 43 panellists with one to four replications to describe foods of 5-10 dominant attributes. A panel of eight subjects was used in a TDS study conducted by Meillon et al. (2010) to evaluate five types of wine with different degrees of dealcoholization in triplicate (120 trials). Nine participants were used in a research by Albert et al. (2012) for evaluating the TDS profiles of six fish sticks. The evaluations were done in triplicate (162 trials). Le Reverend et al. (2008) used 12 subjects to perform evaluations on six hot beverages in duplication (144 trials) and Meillon et al. (2009) selected 16 consumers to perform the TDS task on red wines in three replications (384 trials). Meanwhile, Lenfant et al. (2009) performed 300 evaluations on six types of wheat flakes by recruiting 25 subjects and Labbe et al. (2009) conducted 387 TDS runs on nine gels with 43 panellists. These studies showed that TDS curves can be produced with a small numbers of subjects, provided with sufficient replications. A good level of agreement among panellists was seen from the calculated sum-square differences (based on the smaller standard deviation). This study demonstrates that a food sample with five attributes, to be evaluated in triplicate is reproducible using as few as 10 subjects. Thus provided with sufficient number of trials (number of subjects x number of samples x number of replications), a good and reliable TDS curve can be produced.

## 5.5 Conclusion

The study has demonstrated that the Temporal Dominance of Sensation (TDS) technique offers new opportunities to describe how the perceptual dynamics in the mouth change during oral processing as food structure changes. Although the texture of the samples was expected to be predominantly *hard* (mono-attribute), the term mono-attribute is not applicable as the food evolves during oral processing. The different structural properties produced in the mouth changes the sensory perception of samples, which potentially modifies the basic masticatory behaviour of the subjects. The study also indicates that hardness is not the only determinant of chewing strategy, but a range of different food structures are responsible and at different mastication points. This means that when a complete chewing sequence needs to be considered thoroughly (from first bite to swallow) and when the chewing variations between subjects are of key considerations, the time-axis for TDS curves are to be standardized by first bite. The technique has demonstrated its ability to differentiate food with small structural differences which may relate to changes in oral processing behaviours. In addition, the feasibility of using a small number of subjects to produce reliable and representative TDS curves has been shown. This is achievable provided sufficient replicates are done.

Although the capability of using the TDS technique for understanding how structural properties can be translated from sensory sensations perceived, the effect of subject training on the dynamic sensory technique needs to be explored. The feasibility of using subjects with no or minimal training could further emphasize the benefits of using the TDS technique in various food research areas, in particular food oral processing and sensory sciences.

# CHAPTER 6

# THE EFFECT OF SENSORY ATTRIBUTE TRAINING ON TEMPORAL DOMINANCE OF SENSATIONS SENSORY EVALUATION AND ORAL PROCESSING BEHAVIOURS

## 6.1 Introduction

The Temporal Dominance of Sensations (TDS) technique is a challenging procedure which requires subjects to focus on their sensory perceptions over a period of time. Instead of making a static or one-point judgement at a constant time like the conventional sensory methods, TDS is performed continuously throughout oral processing. During evaluation, subjects repeatedly select attributes that trigger the most attention from a list provided. Due to the real-time nature of the data collection, it is important for subjects to be familiar and understand the attributes used to avoid misinterpretation or misuse. TDS results from Chapter 5 (Figures 5.2 A-C) have shown the occurrence of equal levels of attribute dominance during mid-chewing, in particular for samples SP3 and SP5. This may be due to the lack of understanding with terms used in the study, indicating inadequate attribute training. Subjects may have made an incorrect selection or may have interpreted the terms differently from one another. It may also be that the attribute used appropriately represents their sensations and bolus properties, and that no attribute is more dominant than another.

The purpose of this chapter was to investigate whether attribute training has an effect on both the dynamic sensory judgments and oral processing behaviours. Attribute training was used as it has been shown to increase reliability and reproducibility (Ishihara et al., 2011), increased term agreement, provided familiarity to the technique, and reduced differences between panel members (King, Hall, & Cliff, 2001). However, training provided in the present research was minimized to ensure that subjects still behaved as close to a consumer panel as possible thus not negating one of the major benefits of the TDS technique. The term training in this study refers to introducing subjects to the sensory attributes and using commercial foods as references for these attributes.

## 6.2 Materials and Methods

### 6.2.1 Model Food System

A separate batch of biscuits with varying sugar to fat ratios ranging from 0.71 to 2.24 was prepared (Table 6.1). The ingredients used for the biscuits preparation are described in Section 3.1.2. The hardness levels of the biscuits were measured using the single-edge notched bend (SENB) test. Sample SP1 remained named as SP1. Samples SP3 and SP5 were renamed as SP7 and SP9 as their measure of hardness differed to that of SP3 and SP5 (in Section 5.2.1).

**Table 6.1: Results from single-edge notched bend (SENB) of each recipe (mean ± SD)**

| Samples | *Critical stress intensity factor ($K_{IC}$, kPa $m^{1/2}$) |
|---|---|
| SP1 | 26.71± 4.4 |
| SP7 | 46.24 ± 7.1 |
| SP9 | 56.22 ± 7.3 |

*procedure for instrumental test is described in Section 3.1.3

### 6.2.2 Subjects

Twenty subjects (80% females and 20% males), with mean age 27.9 ± 5.7 participated in this study. All subjects had healthy and complete dentition. Subjects did not have any major dental work such as braces, surgery, dental extraction or restoration carried out in the last six months prior to the experiment. None of the subjects reported any difficulty chewing any type of food. All subjects gave voluntary informed consent to take part in the study. Ten of the subjects were recruited from a previous TDS study and the other ten subjects were newly recruited with no experience in any sensory analysis study and were accepted as naïve (untrained) subjects.

### 6.2.3 Experimental Procedures

Experiments were conducted in two sessions; session 1 involved an introduction to sensory terms (referred to training), session 2 involved performing a sensory evaluation using the TDS technique. Sensory attributes used in this study were finalized by terms generated by trained panellists from Plant and Food Research, Palmerston North, New Zealand (in which none took part in the present experiment).

Session 1: Sensory Attribute Training

Subjects were asked to learn the given attributes used in this study by recognizing both the high and low levels of the relevant texture attributes using selected commercial biscuits (Table 6.2). For example, high level for attribute *hard* was described using ginger nut and super wine biscuit was used to describe a low level of hardness.

Commercial biscuits used to introduce attributes *hard*, *crumbly*, *crunchy/crispy*, and *dry* were selected based on results from a sensory profiling test from a separate group of trained panellists. Products used were also assessed for its suitability to relate to relevant texture attributes. Only foods used to describe the attribute sticky were based on the standard adhesiveness scales by Szczesniak, Brandt, and Friedman (1963) which recommend cream cheese and smooth peanut butter. During the introductory session, subjects were free to discuss or comment on the food used but all subjects agreed on the products used to associate with the terms introduced.

**Table 6.2: Food used for sensory attribute training**

| **Attribute(s)** | **Food Examples** | |
|---|---|---|
| | **Low level** | **High level** |
| *Hard* | Griffins Super Wine | Arnott's Gingernut |
| *Crumbly* | Griffins Super Wine | Arnott's Shortbread |
| *Dry* | Griffins Super Wine | Sanitarium Weet-Bix |
| *Crunchy/Crispy* | Griffins's Vanilla Wine | ABE's bagel crisps |
| *Sticky* | Kraft Philadelphia cream cheese | Sanitarium smooth peanut butter |

Session 1 was followed by a simple texture recognition test which used six other commercial biscuits (Table 6.3). The test required subjects to chew on the given food example, match it to its appropriate attribute. Subjects had to correctly describe the given food to its appropriate attributes, otherwise re-training was provided.

**Table 6.3: Commercial biscuits used with its related attributes**

| **Brand-Food** | ***Related Attributes** | | | | |
|---|---|---|---|---|---|
| | ***Hard*** | ***Crumbly*** | ***Dry*** | ***Crunchy/ Crispy*** | ***Sticky*** |
| Woolworths Select Pretzel Twist | √ | | | √ | |
| Unibic Sponge Finger | | √ | √ | | √ |
| Griffin's Ginger Nuts | √ | | | | √ |
| Pam's Butter Shortbread | | √ | | | √ |
| Unibic Almond Biscotti | √ | | | √ | √ |

* generated by trained sensory experts from the Plant and Food Research Institute, Palmerston North, New Zealand.
√ correspond to food's related attributes

Session 2: Sensory Evaluation with Temporal Dominance of Sensations (TDS)

Prior to Session 2, subjects were briefed on the concept of dominance and sequence before completing the sensory evaluation using the TDS technique. Subjects were asked to start the evaluation by clicking on the *START* button whilst food was put in the mouth. While chewing on the sample, subjects identified the dominant attribute that triggered the most attention and clicked on the buttons which corresponded to the associated attribute. Subjects were told they were free to select all attributes or to never select any during the evaluation. Subjects ended the evaluation by clicking on the *STOP* button (final swallow). Each subject evaluated samples SP1, SP7, and SP9 in triplicate (randomized blocks of three).

Feedback from Subjects via Email

Feedback from subjects was collated to get an insight as to how they made decisions on selecting the dominant attributes. This served as a confirmation that subjects understood and have used appropriate sensory attributes to describe the texture properties. An email was sent to every subject after a week from evaluation. Responses were voluntary. Subjects were provided with a copy of their TDS evaluation and the mean TDS curve. These feedbacks will be discussed in the following sections where appropriate.

### 6.2.4 Data Collection

TDS data were collected using an in-house written program where the sequence of mouse clicks was recorded and time stamped during evaluation (Labview 2009, National Instruments Corporation, Austin). During both sessions, the experimenter timed the total chewing duration and counted the number of cycles taken till swallowing. Chewing frequency was calculated from these data.

### 6.2.5 Data Analysis

Temporal Dominance of Sensations (TDS)

TDS curves were computed by considering each attribute individually for each point of time. The dominance rates from twenty subjects were calculated and were plotted against standardized mastication time. The analysis of TDS data has been comprehensively discussed in Section 3.4.1.

Oral Processing Behaviours

PASW Statistic (version 18, 2009) was used for statistical analysis. Normality tests were performed (Kolmogorov-Smirnov test). Oral processing parameters (chew duration, cycle number, and chew frequency) were analyzed using analysis of variance (ANOVA). General Linear Model procedure was used to study the effects of different factors on masticatory variables. When the F-test was significant, the Fisher’s least significant difference (LSD) was used to compare differences of means. When data was not normally distributed, a Friedman's Analysis was performed.

## 6.3 Results

The first part of this section presents data for all subjects (n=20) after sensory attribute training. The second part of this section presents the effect of sensory attribute training on TDS sensory evaluation and oral processing behaviours. For this purpose, results were compared to those obtained in Chapter 4 (oral processing behaviours) and Chapter 5 (TDS curves). During this comparison, data from the ten repeating subjects were extracted. Only data from sample SP1 was presented based on its textural similarity between experiments (Table 5.1, Section 5.2.1).

### 6.3.1 Evaluation of Samples with the TDS Technique

Temporal Dominance of Sensations (TDS)

Samples with variations in sugar to fat ratios showed distinctive attribute progressions (Figure 6.1). Sample SP1 was dominantly perceived as *crumbly*, followed by *dry,* and *sticky*. Sample SP7 was firstly perceived as *hard*, then *crumbly* and *dry* followed by *sticky* towards the end of the mastication stages. Sample SP9 was perceived as *hard* at the early chewing stages followed by *crunchy/crispy* and *sticky* towards the end of chewing stages.

In general, when the dominance rate of the first dominant attribute reduces, the dominance rate in another dominant attribute increases. Attribute *crumbly* in sample SP1 was apparent during early mastication but faded when the sample was chewed almost half way through mastication. The influence of attribute *hard* in both samples SP7 and SP9 was also reduced when chewed through the oral processing sequence. This was expected as a given attribute is dominant since it is selected until another attribute selection is done (Pineau et al. 2009). This is aligned with the instructions given to subjects for the TDS task.

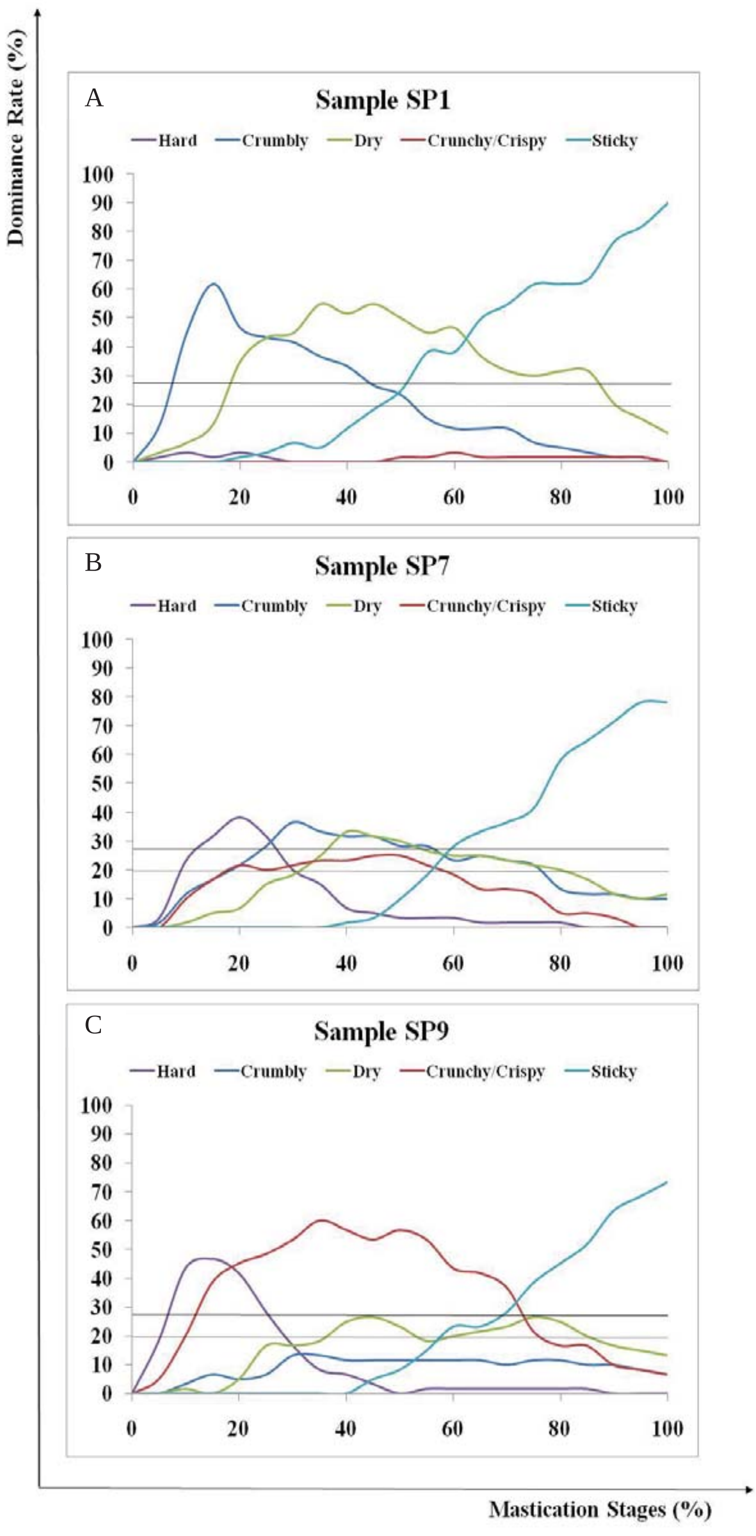



**Figure 6.1: TDS curves for sample SP1, SP7, and SP9 after sensory attribute training (n=20). Chance level is represented with dotted line and significance level is represented with solid line**

TDS results for all samples in present study were similar those discussed in Chapter 5 (Figure 5.2 A-C). Attributes used to describe the harder samples (SP7 and SP9) at the early stage of the chewing sequence were related to force used for chewing: *hard*. Hard also dominated for a medium-hard sample (SP7). Whilst this did not quite reach the significant level in the previous experiment, this dominance rate was well above chance level. The variation in sample material properties may have contributed to this observation. As expected, this stage was often targeted for hardness recognition (Dan et al., 2007). It is also during this stage that other attribute related to fracture properties may be selected: *crunchy/crispy*. Although literature have shown over-lapping of definitions in these terms (Brown et al., 1998b), subjects were able to differentiate the attributes. The definitions used in the present study focus on the amount of force required to compress a sample to describe *hard* and sound associated with breakdown by chewing (the noise) to describe *crunchy/crispy*. Some dialogue from subjects showed that the initial stage of mastication was mainly used for hardness recognition (force used) and that they were using the definitions provided as a cue to select the attributes:

*"Initial sensation was usually whether the biscuit was hard or not"*

*"When I first put the sample in my mouth and took first few chews, I was mainly assessing how hard/crunchy/crispy/crumbly it was with each bite"*

*"I first concentrated on crumbly, crispy, and hardness. I based crumbly on how the sample broke up in my mouth. I based crunchiness on the sound and hardness on how difficult it was to bite into the sample"*

*"For all biscuits, the first texture I sensed after putting it in my mouth was how hard the biscuit"*

*"I based hardness on how difficult it was to bite into the sample"*

*"I based crunchiness on the sound"*

*"Hard was the more prominent sensation"*

Further into a chewing sequence, sample SP7 was quickly dominated by attribute *crumbly* and *dry* during mid-chewing. Here, subjects may have used force to break the sample during the first few cycles but the ease of sample breaking down may have been more obvious. The easily shattered pieces around the mouth may have drawn out any saliva in the mouth, which may have prompted attribute *dry* being chosen as the dominating descriptor. Dryness may have been related to the need to produce saliva as opposed to how dry the sample was. Subjects relate attribute *crumbly* to how easy the sample breaks in the mouth and relate attribute *dry* to lack of saliva in the mouth (absence of water or hydration). Feedbacks collated from subjects have suggested they understood the terms thoroughly and have used the definitions provided to relate the attributes:

*"I based crumbly on how the sample broke up in my mouth"*

*"Crumbliness is sandy-like (small fragments)"*

*"I selected crumbly when I thought it was breaking down in a more powdery manner rather than shattering"*

*"The sample eventually became easier to break down to even smaller pieces…i.e. they just crumbled away"*

*"The sample was fairly crumbly and broke into smaller particles very quickly. I think perhaps the particles were much smaller at a given stage. As a result, the particles soaked up all my saliva more quickly so my mouth felt dry"*

*"After the biscuit had crumbled into lots of pieces, I could feel saliva being drawn from my mouth leading me to select dry"*

*"Dryness was based on how dry my mouth felt when eating the sample"*

*"Dry would have been selected if I felt that there was not enough saliva in my mouth"*

All samples were dominated by attribute *sticky* from approximately 50% of the masticatory sequence. This indicates that all samples undergo various structural changes in the mouth before reaching a definite state before swallowing, and that not a single attribute stays as dominant during the chewing sequence. The absence of water may have led to a high urge to produce more saliva to moisten the sample which increased bolus agglomeration and this may have increased the need to remove food materials from around the mouth before swallowing. Subjects would have used force to remove materials that adhered to the teeth or to remove materials from around the mouth, thus resulting *sticky* being chosen as dominant. This can be verified by the feedback collected via email interview:

*"Stickiness was pretty much how much it got stuck in my teeth"*

*"I just judged it whether the biscuits stuck on my teeth a lot or not"*

*"I found that eventually all the biscuits formed sticky 'goo' at the end before swallowing (sticky since I felt it stuck to my teeth). So hence, I ended all the chewing tests with sticky"*

*".....at the point that it felt like I wasn't trying to add lots of saliva and I was using my tongue to manipulate the food rather than my teeth, I selected sticky"*

It is also interesting to find that some subjects have indicated that stickiness was a cue to swallow:

*".....and then the chewed stuff became sticky before I finally swallowed it"*

*".....before finally becoming sticky"*

*".....followed by sticky quite close to the point of swallowing"*

*".....didn't swallow until I felt that the food was more of a paste (had sticky properties)"*

Time to First Dominant Attribute Selection

The times subjects took to select the first attribute were also examined (Table 6.4). Subjects were faster selecting the dominant attribute for the sample with the highest measure of hardness (SP9). One-way ANOVA showed that the time taken for the first attribute selection for sample SP9 was significantly different from the others ($X^2(2) = 16.63$, $p < 0.05$). In general, the range of times taken to make the first attribute selection varied between 1 s and 34.1 s, with smaller spread for the hardest sample.

The proportion of time used for first dominant attribute selection occurred at the early stage of oral processing (less than 20% of the whole duration on average). Depending on the samples, these times varied between 2.4% to 96.3% of the whole oral processing. In general, smaller range of time was observed for the hardest sample. It is interesting to note that some panellists used more than half of the chewing time (or greater) to select the first dominant attribute, most apparent for samples SP1 and SP7 (46.4% and 96.3%, respectively). The differences in the range of time are probably due to the different exposure time to the TDS technique. It must be noted that these results were obtained from combinations of subjects who had different levels of exposure to the TDS technique. Half of the subjects had more contact and familiarity in performing the TDS task from previous experiments, hence more experienced with the technique compared to those newly recruited. The following section further discussed these effects between subjects of different levels of exposure to the TDS technique.

**Table 6.4: Time to first dominant attribute selection for different sample compositions (mean ± SD and range of values) (n=20)**

| | SP1 | SP7 | SP9 |
|---|---|---|---|
| Time to first attribute selection | $3.3 \pm 2.6^{a}$ (1.0 – 12.8) | $3.8 \pm 4.8^{a}$ (1.0 – 34.1) | $2.1 \pm 0.9^{b}$ (0.8 – 4.8) |
| Proportion of time to first attribute selection (%) | 11.9 ± 9.7 (2.7 – 46.4) | 13.7 ± 13.4 (2.7 – 96.3) | 8.3 ± 4.4 (2.4 – 20.4) |
| Average oral processing time | 29.7 ± 7.5 (17.5 – 48.5) | 27.9 ± 7.5 (13.0 – 45.6) | 28.2 ± 8.1 (14.4 – 52) |

Different letters (a,b) indicate a significant difference between samples ($p < 0.05$)

Oral Processing Behaviours

The mean oral processing parameters after the sensory attribute training are presented in Table 6.5. When performing the TDS task, subjects used a longer time to chew on the least hard sample (SP1) compared to both harder samples (SP7 and SP9). A similar number of cycles were used for all samples. Although statistical analysis did not show significant differences for both these chewing measures ($p > 0.05$), calculated chewing frequencies showed that sample SP1 was chewed at a significantly slower pace than the hardest sample. These results indicated that only the chewing rates were affected by the sample sugar to fat ratios.

**Table 6.5: Effect of sensory attribute training on oral processing behaviours for samples of different sugar to fat ratios (mean ± SD) (n=20)**

| Samples | Chewing Duration (s) | Number of Cycles | Chewing Frequency ($s^{-1}$) |
|---|---|---|---|
| SP1 | 29.7±7.5[a] | 34.8±7.3[a] | 1.20±0.17[a] |
| SP7 | 27.9±7.5[a] | 34.3±8.1[a] | 1.26±0.20[ab] |
| SP9 | 28.2±8.1[a] | 36.1±8.9[a] | 1.31±0.20[b] |

Different letters (a,b) indicate a significant difference between samples ($p < 0.05$)

### 6.3.2 Comparing the Effect of Sensory Attribute Training for Sample SP1 (n=10)

Temporal Dominance of Sensations (TDS)

Figure 6.2 compares the TDS curves generated for sample SP1 before and after sensory attribute training for the ten repeating subjects. Similar trends were observed in both TDS curves, in which sample SP1 was firstly dominated by attribute *crumbly*, *dry*, and followed by attribute *sticky* towards the end of the masticatory sequence. The most notable differences are that the significant dominant attributes were perceived faster and with higher consensus after sensory attribute training (i.e. greater dominance rates).

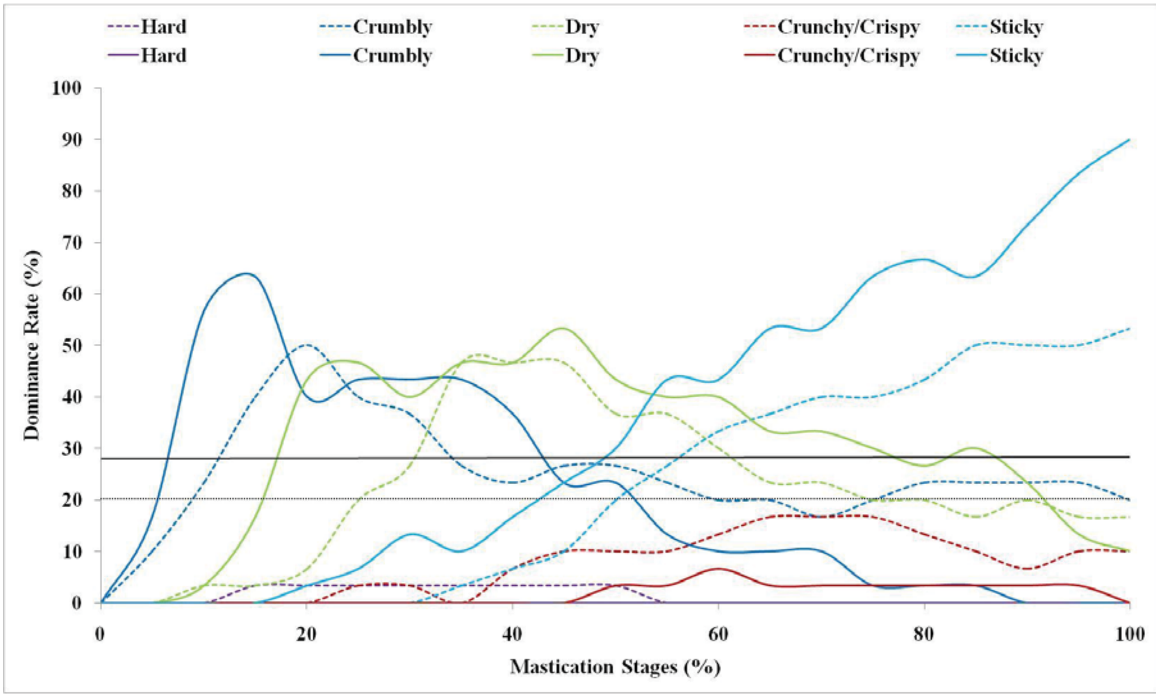



**Figure 6.2: TDS curves computed for sample SP1 for both TDS evaluation before training (in dotted coloured lines) and after training (in solid coloured lines) (n=10). Chance level is represented with dotted line and significant level is represented with solid line**

Oral Processing Behaviours

Figure 6.3 shows the mean oral processing data from the ten repeating subjects when sample SP1 was chewed with and without the TDS task. Subjects chewed for longer and with more chewing cycles when assessing samples using the TDS technique compare to no assessment being made. The range of chewing times used to chew on sample SP1 was between 13.8 to 59.8 s without any assessment made and 15.5 to 54.3 s with TDS task before training. Meanwhile, the range of number of cycles used was between 18 to 64 cycles without any assessment made and 23 to 66 cycles with TDS task before training. Chewing frequency showed no difference between the tasks. These results indicated that chewing strategy was affected by the task but not the sensory attribute training.

After sensory attribute training, the effect was only significant for the number of chewing cycles used. The number of cycles used to chew on sample SP1 reduced significantly after attribute training. Subjects may have struggled to perform the TDS task before attribute training hence the high number of chewing cycles used. These results indicated that subjects used different chewing strategy after sensory attribute training.

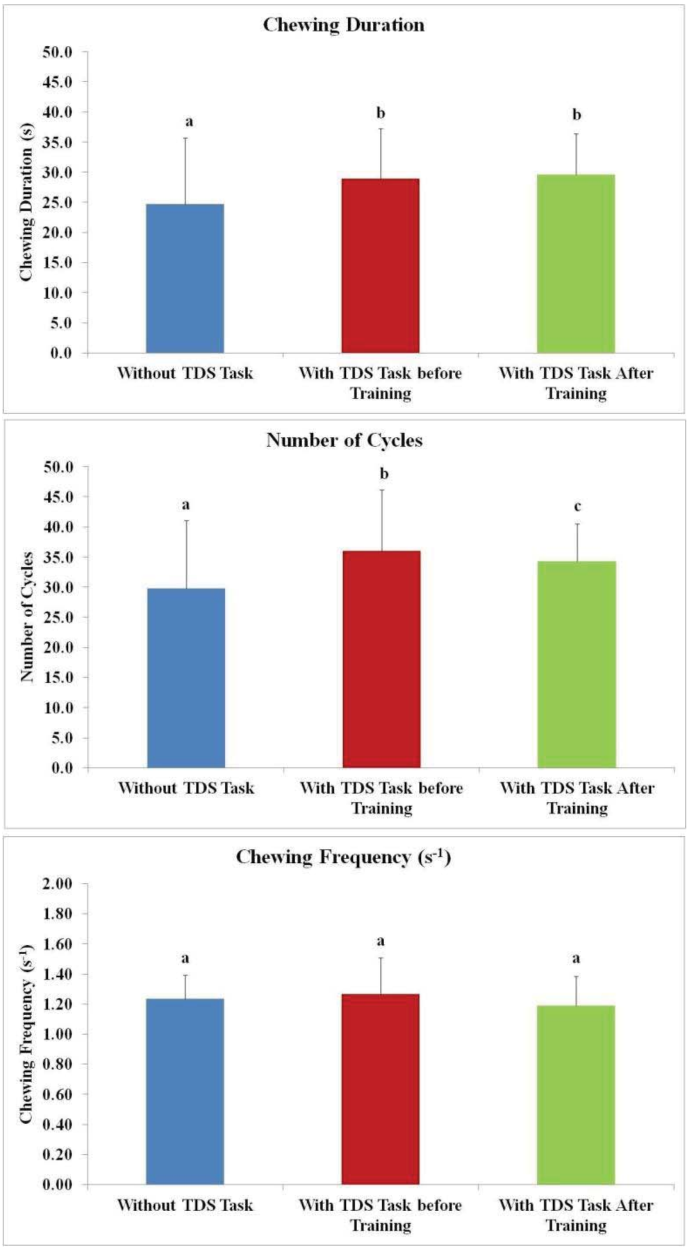



**Figure 6.3: Effect of sensory attribute training on oral processing behaviours for sample SP1 (n=10). Different letters (a,b,c) indicate a significant difference between tasks ($p < 0.05$)**

The effect of sensory attribute training was also significant on the time required to select the first dominant attribute (Table 6.6). Subjects were faster at making the first dominant attribute selection after training. Less variance was also seen in the standard deviation after training. The range of times taken to make the first attribute selection significant reduced from between 1.0 to 22.0 s before training to between 1.3 to 6.8 s after training. This further demonstrated that the training session helped subjects to recognize an attribute quicker.

The proportion of time used for first dominant attribute selection was expected to occur at an earlier stage after sensory attribute training. They were at least 10% earlier in making the first dominant attribute selection. The range of time to make the dominant attribute was reduced after training. This was expected as subjects were better able to recognize the texture attributes.

**Table 6.6: Time to first dominant attribute selection for sample SP1 (n=10)**

| | **Before Training** | **After Training** |
|---|---|---|
| Time to first dominant attribute selection | 5.8 ± 4.7[a]<br>(1.0 – 22.0) | 2.8 ± 1.5[b]<br>(1.3 - 6.8) |
| Proportion of time (%) | 20.9 ± 16.5<br>(3.9 – 77.9) | 10.6 ± 7.2<br>(3.2 – 32.8) |

Different letters (a,b) indicate a significant difference between tasks ($p < 0.05$)

## 6.4 Discussion

### 6.4.1 General Effect of Sensory Attribute Training on TDS Performance and Oral Processing Behaviours (n=20)

Results from Chapters 4 and 5 were included in this discussion. This was to assess the general effect of attribute training on changes in TDS performance and oral processing behaviours.

In general, all samples showed similar patterns of dominance before and after training but samples SP3/SP7 and samples SP5/SP9 (Figures 5.2 A-C and Figure 6.1 A-C) showed distinct differences in the TDS curves. The effect of food structure was clearly seen on the sensory sensations perceived. Subjects were able to differentiate the samples with small structural differences (Table 4.1 and Table 6.1). Three samples of different levels of hardness in the present study were distinctively described; two of the samples from previous study with similar measure of hardness were similarly described. Not only were they able to differentiate the samples, they were better in describing the samples, most apparent between samples SP5 and SP9. Sensory attributes used to describe these samples were more obvious after training, in particular during mid-chewing. Higher dominance rates were also observed for the attributes. In addition, they were also faster at recognizing the texture attributes after training. However, conclusion cannot be made as both these studies comprises of subjects with initial TDS experience and newly recruited subjects. Further discussions on these effects are in Section 6.4.2 with repeating subjects.

The present study demonstrated that chewing strategy changes with the changing properties in the mouth and that hardness is not the only property that is responsible. These samples of different compositions were chewed differently (Table 4.4 and Table 6.5). The only significant oral processing parameter was the chewing frequency, where slower chew rates were used for the least hard sample (SP1) as compared to the harder samples (SP3, SP5, SP7, and SP9). This was contrary to common conclusion found in literature. Peyron et al. (2002), Foster et al. (2006), and Woda et al. (2006a) reported constant chewing frequency with increasing of hardness of a food product but the present study found otherwise. This indicated that sample hardness was not the only factors determining the chewing strategy, but the sample compositions, which affected the breakdown of the products and bolus properties. There was a significant effect of sample composition on chewing frequency; sample with highest sugar to fat ratio was chewed faster than sample with the lowest ratio. The differences in sugar composition may have changed the structural properties in the mouth and thus the chewing strategy. High level of dissolving sugar composition in sample SP9 may have contributed to the property in the mouth (from addition of saliva). The property remained in the mouth for a long duration (almost the entire mid chewing process) till a sticky state was formed just before swallow.

### 6.4.2 Effect of Sensory Attribute Training on TDS Performance and Oral Processing Behaviours using Sample SP1 (n=10)

The effect of sensory attribute training on TDS performance and oral processing behaviours was better shown with ten repeating subjects. Subjects were faster at selecting a dominant attribute and the dominance rates for the dominant attributes were higher after training, most apparent for attributes *crumbly* and *sticky*. The effect can be emphasized by the reduction of times used in selecting the first dominant attribute. This indicates the great benefit of sensory attribute training for performing the TDS task and provided with sufficient attribute training, the use of untrained panellists to perform the TDS task show to be feasible. Previous TDS studies have used either trained sensory experts (Meillon et al., 2010; Albert et al., 2012; Bruzzone et al., 2013; Laguna et al., 2013; Paulsen et al., 2013) or untrained subjects (Lenfant et al., 2009; Pineau et al., 2009; Déléris et al., 2011b; Ng et al., 2012) to carry out the TDS task. These trained subjects have experience from other sensory work and may require less training but the untrained subjects were mostly non-expert that required long and intensive training. The training durations ranged from 4 to 12 different sessions with an average of an hour each. The extended period of training may not be practical, especially if required in a fast moving food company. In addition, the use of trained panels is not cost efficient and these panels can sometimes produce bias results due to their familiarity to a product (Cardello et al., 1982). Alternatively, consumer panels can be of benefit to perform the TDS task. Consumer panels (untrained subjects) are easily available and can be modified to certain levels of expertise and yet not negating the benefit of TDS evaluation. The present study showed that provided the sensory attributes used and their definitions are well understood, the use of consumer panels to perform the task has proven to be feasible.

Oral processing data from these ten repeating subjects showed that the chewing behaviours were affected when performing a TDS task and were slightly affected after attribute training. The number of cycles was the most influenced by the task and the sensory attribute training. There was an increased in cycle numbers when TDS task was performed before training and a decreased in the cycle numbers when TDS evaluation was performed after training. Although comparison could not be made between the three experimental samples, it is true that a specific chewing strategy may be used after numerous TDS evaluations. Subjects may have familiarized themselves with the samples and the TDS technique. When they are more familiar with the attributes and technique, chewing behaviours remained constant and subjects can better focus on performing the TDS task.

It is interesting to note that a TDS curve was achievable with low level of training provided. It is probably due to the uniqueness of the technique and the fact that the data collection is based on food evolution in the mouth. This demonstrated that the use of untrained subjects to perform the TDS evaluation can be practical and a good TDS performance can be obtained with minimal training. Training has shown to have low level of effect on chewing behaviours. Hence, it can be expected that the chewing behaviours will remained and subject's performance enhanced.

## 6.5 Conclusion

The biggest effect on the oral processing strategy was performing the TDS task and that sensory attribute training does not have any effect on oral processing behaviours, except for the number of cycles which reduces slightly. This study suggested that moderate training is important to enhance the TDS performance. This finding also confirmed that human mastication adapts to changes in food structural properties in the mouth and that these changes can be translated from the sensory sensations perceived. Although, in theory with an assumption that sensory attribute training did not much influence TDS performance and chewing behaviours, physiological measurement instruments like the electromagnetic articulograph (EMA) and electromyography (EMG) can be combined to provide an insight into the modulation of masticatory system that occurs in response to changing structural properties of foods. Despite the present findings, there is currently little data validating the TDS technique. TDS curves rely solely on the selection of an attribute as dominant or not. Although some TDS studies have also collected the intensity rating during the TDS evaluation, the scores are often not discussed. Therefore, the focus of the following chapter is to explore the benefits of collecting both the dominant attributes and intensity ratings during a TDS evaluation.

# CHAPTER 7

# DEVELOPMENT OF THE CONVENTIONAL TEMPORAL DOMINANCE OF SENSATIONS TECHNIQUE

## 7.1 Introduction

The focus of the TDS technique has always been monitoring the changing dominant attributes over the time of food consumption. Alternatively, the intensity of the attribute may be recorded at the same time (Meillon et al., 2009; Albert et al., 2012). However, the intensity scores are seldom included in literature (Pineau et al., 2009). This may due to the difficulty in interpreting the results.

The present study aimed to further explore the potential of collecting intensity information and to consider whether data analysis could be improved. The present study is one of the first in many TDS studies to perform TDS on paper. This method is similar to previously performed TDS evaluation, but now with additional intensity information collected at different points of mastication. For the sake of this thesis, the method is named Discrete Point TDS. In this method, subjects identify and indicate the perceived intensity of an attribute at four different stages of mastication using a paper questionnaire; first compression, initial chewing, throughout chewing, and at swallow point.

## 7.2 Materials and Methods

### 7.2.1 Model Food System

Samples SP1, SP7, and SP9 of increasing hardness levels were used (differing in sugar to fat ratios). The samples were the same to those used in Chapter 6 and have similar manufacturing process (as described in Section 3.1.2). Results of hardness properties are as presented in Table 6.1 (Section 6.2.1).

### 7.2.2 Subjects

Twenty subjects (80% females and 20% males) from previous experiment (as described in Section 6.2.2) participated in this study.

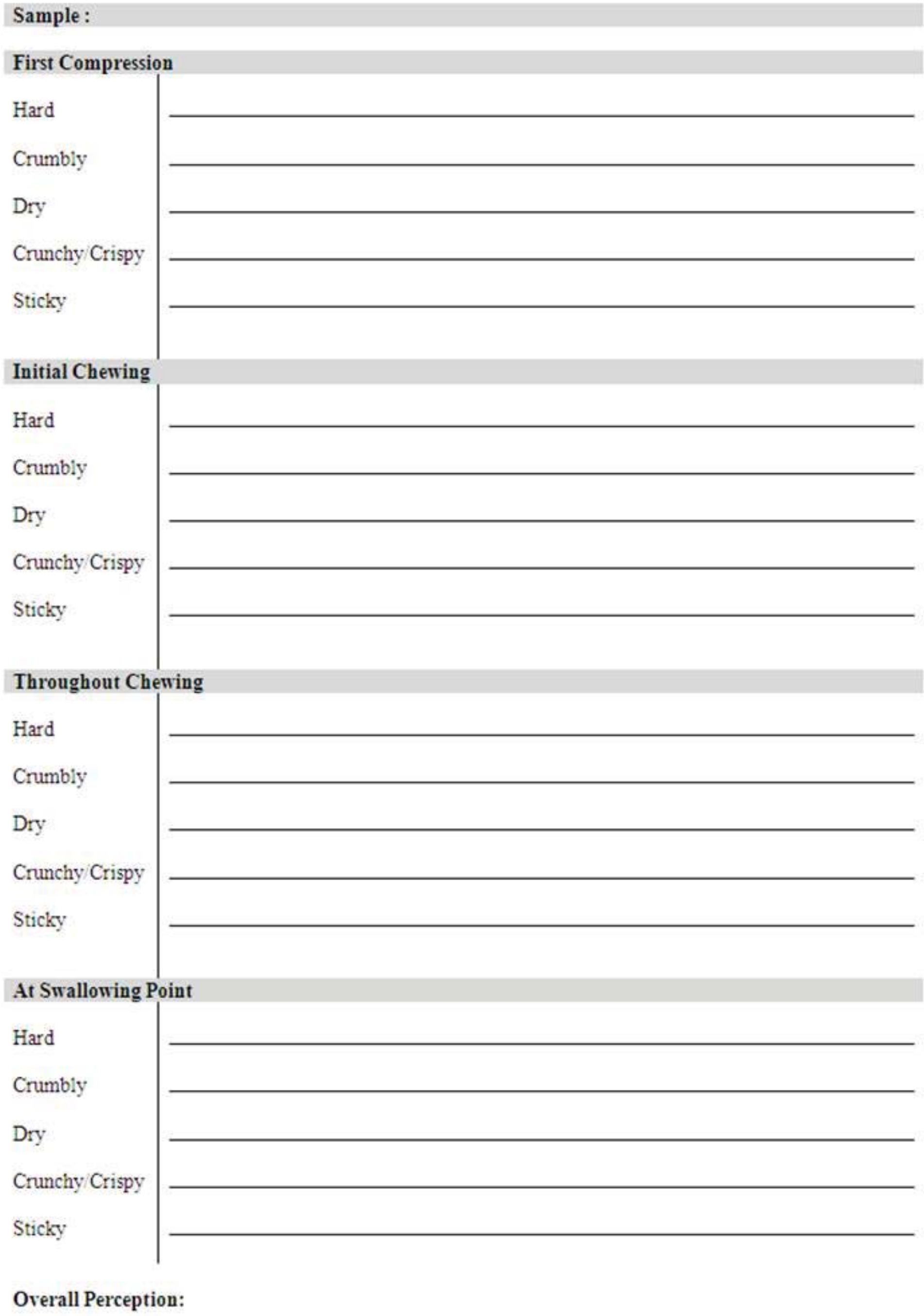
### 7.2.3 Experimental Procedures

Discrete Point TDS

Subjects were asked to rate the five attributes given (on the paper provided) for each sample at four specific times of their mastication period (in triplicates). The four main mastication times involved were first compression (first bite of sample between molar teeth), initial chewing (at approximately 2 to 3 cycles of mastication), throughout chewing (in the middle of mastication which was self assessed), and at swallow point (at swallowing point).

### 7.2.4 Data Collection

The intensity of each selected attribute was rated on a paper questionnaire with a 15cm unstructured line scale. All scales were presented on the same page (Figure 7.1). Subjects placed a mark on the scale to indicate their perceived intensity for each attribute. Subjects were told that the line was scaled from least to most for each attribute. During evaluation, definitions of attributes and chewing stages were given to subjects and they were permitted to evaluate the samples for as long as it takes. Subjects also have an option to provide information on the overall perception perceived.



**Sample :**

**First Compression**

Hard
Crumbly
Dry
Crunchy/Crispy
Sticky

**Initial Chewing**

Hard
Crumbly
Dry
Crunchy/Crispy
Sticky

**Throughout Chewing**

Hard
Crumbly
Dry
Crunchy/Crispy
Sticky

**At Swallowing Point**

Hard
Crumbly
Dry
Crunchy/Crispy
Sticky

**Overall Perception:**

**Figure 7.1: Page layout of the Discrete Point TDS evaluation**

### 7.2.5 Data Analysis

Discrete Point TDS

Two types of data were collected from this current method; the total number of selections and the intensity scores. These results were discussed separately. The total number of selections for each attribute was used to calculate the dominance rate using the same formula as described in Section 3.4.1. Using the same concept, these rates were obtained by dividing the number of selections of an attribute (in triplicates) by the number of subjects and the number of replications. Results were plotted against four mastication points of time (x-axis) with dominance rate as the y-axis. These points of time correspond to the first compression (assuming that in 10% of their mastication time, the subjects have already make first compression and made first dominant attribute selection), initial chewing (assuming the time used to make 2 to 3 cycles), throughout chewing (from 30% to 90% of the mastication period), and at swallow point (90 - 100% of the mastication stages). These TDS plots were also added with significance and chance lines to facilitate reading of the chart. As described in previous chapters, an attribute is considered to be dominant if the calculated dominance rate was higher than the significance level (in this study = 0.28) (Lenfant et al., 2009).

Intensity scores

Perceived intensities for each attribute were converted into a 10-point scale from the 15 cm lines. Zero was anchored at the left end of scale and was given to the attributes not selected (absent). Ten points was anchored at the right end of scale and correspond to the attributes that were perceived at intense level. Attributes that have scores less than 2 were considered as being perceived only at weak level and may not be analyzed. Analysis of variance (ANOVA) with samples, subjects, and stages as factors was performed for each attribute and at each mastication stage to determine whether the samples could be discriminated by the subjects. The Fisher's least significant difference (LSD) was computed to determine if differences between selected pairs of samples were significant. The confidence level was set to 95% for both ANOVA and LSD tests. In a separate analysis, sensory intensity scores were average across subjects and replicates and data were subjected to principle component analysis (PCA).

## 7.3 Results

### 7.3.1 Discrete Point TDS Curves

The TDS data collected using the paper format was plotted at four points on the time-axis as shown in Figure 7.2B. At first compression, sample SP1 was dominantly perceived as being *crumbly* and *hard*. Then, the sample was perceived as *crumbly* and *dry* during initial chewing before being perceived as *crumbly*, *dry,* and *sticky* throughout chewing. The sample was then dominantly perceived as *sticky* at swallowing point. Sample SP7 was firstly perceived as *hard* and *crumbly* at first compression. During initial chewing, the sample was perceived as *hard*, *crumbly*, *dry,* and *crunchy/crispy*. Throughout chewing, attribute *sticky* took over attribute *hard* as the dominant attribute with other attributes remained perceived as dominant. *Sticky* remained dominant until the swallowing point. Sample SP9 was mainly perceived as *hard* and *crunchy/crispy* at first compression, *hard*, *crunchy/crispy*, and *crumbly* at initial chewing, *crunchy/crispy*, *crumbly,* and *sticky* throughout chewing and *sticky* at the swallow point.

When samples were firstly compressed between the molars, attribute *hard* was considered the dominant attribute in all samples at the first compression. The sugar to fat ratios corresponded to the dominance rates; the higher the sugar composition (Table 6.1), the higher the dominance rate. It is interesting to note that sample SP1 was perceived as being *hard* at a significant degree (> 28%) using Discrete Point TDS, it was less apparent using conventional TDS. The attribute may have been overshadowed by a more dominating attribute (*crumbly*) during the TDS evaluation using the electronic method. During conventional TDS evaluation, subjects were only asked to focus on the dominant attribute during chewing whereas Discrete Point TDS requires subjects to focus on all attributes listed at each stage of the mastication (it is up to the subjects to determine which attribute was the most dominant if multiple dominant attributes were perceived). Depending on the sample hardness, *crumbly* (in samples SP1 and SP7) and *crunchy/crispy* (in sample SP9) were also perceived as dominant.

During initial chewing, *crumbly* remained as the dominant attribute in samples SP1 and SP7. Meanwhile, c*runchy/crispy* remained as dominant for sample SP9. At this stage, other attributes were also perceived at a significant level (*dry* for SP1; *dry* and *crunchy/crispy* for SP7; *crumbly* for SP9). Subjects were likely two to three cycles into their mastication period in which they may still be using their molars for chewing. This showed that the attributes which were related to fracture properties were still perceived. Food was likely broken down rapidly into smaller particles which scattered around the mouth increasing the surface area which prompted saliva production. The process may have prompted *dry* being perceived. However, these dominating attributes decreased throughout chewing for all samples when attribute *sticky* emerged as the most perceived attribute. At swallowing point, *sticky* appeared to be the only dominant sensation for all samples while other attributes became less apparent (did not reach a significant level).

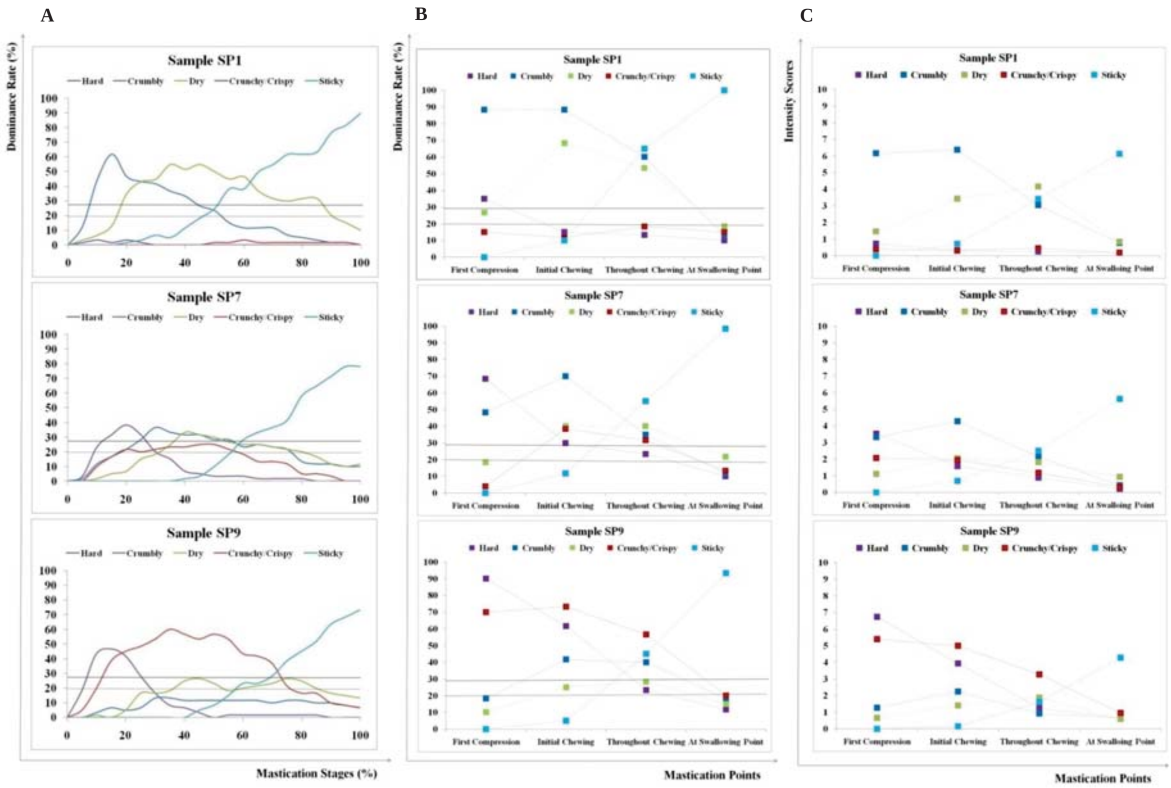



**Figure 7.2: (A) TDS curves against mastication period (%) as described in Section 6.3.1; (B) Discrete Point TDS curves, and (C) mean intensity scores at four main mastication points for samples SP1, SP7, and SP9**

### 7.3.2 Intensity Score

Plots of Intensity Scores

Mean intensity scores of each attribute were also plotted at the four main points of mastication for all samples (Figure 7.2C). At first compression, *crumbly* was rated with the highest intensity in sample SP1, *hard* and *crumbly* in sample SP7, *hard* and *crunchy/crispy* in sample SP9. Other attributes were only scored at a weak level (score less than 2) or at threshold (score of less than 1). No scoring was made for *sticky* at this stage. During subsequent stage (initial chewing), the scores for attributes either increased (*crumbly* and *dry* for samples SP1; *crumbly* for samples SP7 and SP9) or decreased (*hard* for sample SP7; *hard* and *crunchy/crispy* for sample SP9). All other attributes remained scored at weak level or threshold. Throughout chewing, scores for all dominating attributes continued to reduced or remained scored the same. It is interesting to note that at this stage, *sticky* was scored. At swallowing point, all attributes were perceived at weak or threshold level, except *sticky* which was scored at highest intensity.

Intensity Scores for Samples with Different Sugar to Fat Ratios at Four Main Mastication Stages

Overall, the different stages of mastication played a role in the sensory texture perception of the three samples of different compositions. Figure 7.3 illustrates the splitting of samples SP1 and SP9, with SP7 in the between. Sample SP1 seems to be *crumbly* and *dry*; Sample SP9 is *hard* and *crunchy/crispy*. The plots showed that there was a relation between the different stages of mastication and the sensory intensity scores. During the early stages of mastication (first compression and initial chewing), attributes scored the highest for sample SP9 were related to fracture properties. Attributes mainly rated for the sample were *hard* and *crunchy/crispy* from first compression through chewing. Meanwhile, intensity scored for attributes used to describe sample SP7 were in between samples SP1 and SP9. The least hard sample (SP1) was mainly described as *crumbly* and *dry* during the early stages of mastication. Intensity scores for attribute *sticky* were the highest at the swallow point for all samples. This stage separated from other mastication stages on the axis, which accounted for

60.7% of the variations. These results showed that the Discrete Point TDS was able to attain similar results from previous experiments using the conventional TDS technique, hence validating the approach of solely focusing on the selection of an attribute as dominant or not.

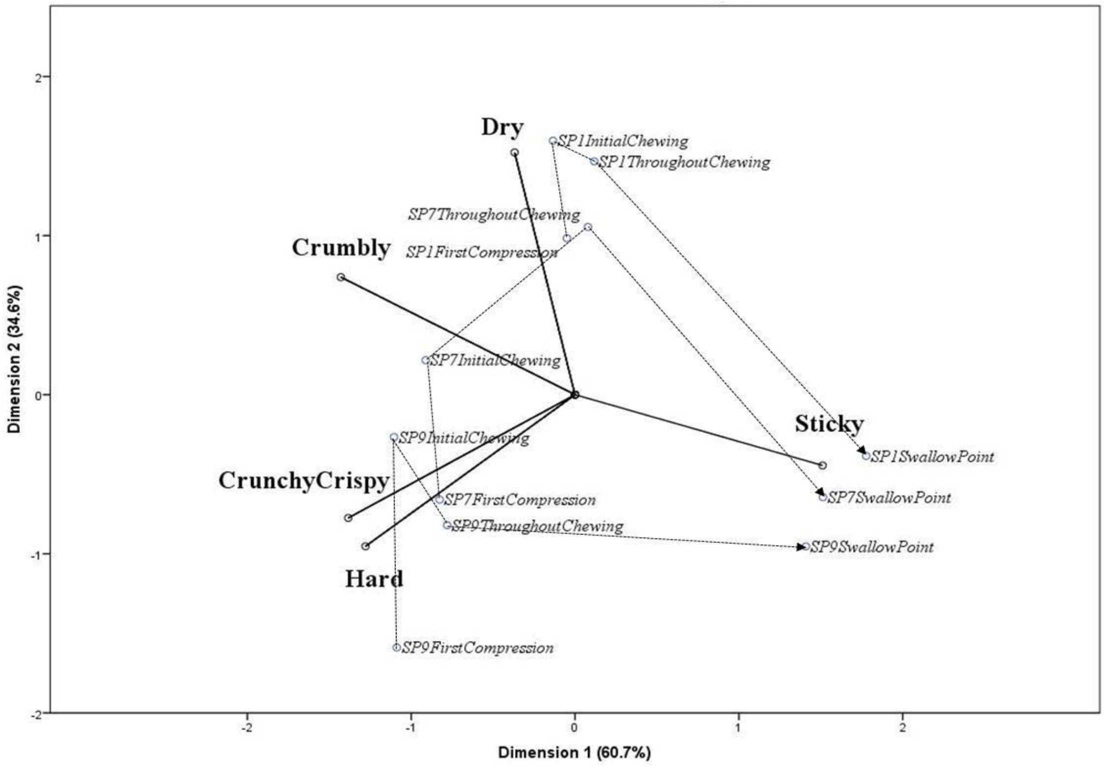



**Figure7.3: Biplot representing the intensity scores of three samples of different sugar to fat ratios over four main mastication stages**

## 7.4 Discussion

In general, both methods used to collect TDS data resulted in closely matched attribute patterns. Both methods were capable of differentiating samples with different compositions. TDS curves via conventional measurement are useful in providing information on the most dominating attributes throughout a chewing sequence but not at each specific stage during oral processing. Some TDS results discussed the sensory results based on the durations of time an attribute was dominant (Meillon et al., 2009; Pineau et al., 2009; Saint-Eve et al., 2011) or vaguely describe the overall differentiating attributes between samples (Ng et al., 2012). Although there was attempt to discuss the TDS results by dividing the mastication sequence into few stages; the first half of the mastication process, from 50% of the mastication onwards, at final stages of mastication (Albert et al., 2012), the division of these stages were unclear. It is important to collect information through each important stage of a chewing sequence (first compression, initial chewing, throughout chewing, at swallow point) to obtain a sensory profile at different point in time. Brown, Foegeding, Daubert, Drake and Gumpertz (2003) have also mentioned that sensory perception at these points of times was equally important.

Data collection using the paper form does provide new information that the typical TDS curves could not. The main difference is that all attributes were focused at each evaluation point. Assuming that the main mastication points in TDS plots correspond to 10%, 30%, 60%, and 90% of a mastication period in the TDS curves, results collected from the two methods can be compared. It must be noted that these points are estimation and may not be true. Results from this chapter discovered that an attribute that was selected a greater number of times may not mean that it will be perceived at a higher intensity. Using *hard* as an example in sample SP1, this attribute was not apparent in the conventional TDS curves but was selected at a significant degree in the Discrete Point TDS curves. *Hard* may have been an important attribute that had been neglected during former evaluation. This may be true when *hard* was an attribute of interest in samples of varying hardness levels. However, the intensity score for the attribute clearly showed that hard was not as important (scored below threshold). Present method has shown that the Discrete Point TDS can be an alternative cheaper and faster method in the field of sensory science.

## 7.5 Conclusion

These results obtained from the TDS paper format and TDS software techniques were very similar. The Discrete Point TDS method is an alternative to other expensive conventional sensory techniques. By adding in intensity scoring, more information can be collected and it complements the standard TDS data which focuses on only the selection of dominant attributes. The present study also highlighted that a more frequently selected attribute which affects the dominance rate may not mean that the attribute was perceived at a higher intensity. This indicated that attributes that catch the most attention may not be the most intense. Hence, measuring the dominant attributes which are the key characteristics of a product are more beneficial to the food industry. Analysis has also shown that different mastication stages may be responsible for the different sensory sensations. By using the same concept, the method can be used to further explore the selection of dominant attributes at different stages like first compression, initial chews, throughout chewing, and at the swallow point.

# CHAPTER 8

## SIMULTANEOUS RECORDINGS OF SENSORY PERCEPTION AND ORAL PROCESSING BEHAVIOURS FROM FIRST BITE TO SWALLOWING

### 8.1 Introduction

Food texture research can only be better understood from combining the monitoring of human mastication in response to food structure (Chen, 2009). The changes in food structure during chewing have been studied using sensory evaluation and physiology measurements. However, these techniques have always been studied in isolation. Hence, there is a need to investigate how these techniques can be combined simultaneously to advance the understanding of how oral processing is altered in response to changes in texture.

In previous chapters, the use of an additional tool (a sensory software) to measure the perceived sensory responses had shown no obstruction to natural chewing functions. In this chapter, the effect and suitability of combining the masticatory recording devices whilst performing the TDS task during human mastication was performed, in particular the effect of attaching sensors and electrodes on subjects. Electromyographic activity (EMG) and mandibular movements using an articulograph system (Carsten AG500) were the techniques chosen to be used in this study. EMG measurements are performed by temporarily attaching electrodes to the jaw closing muscles and mandibular measurements are performed by tracking the mandibular movements by attaching sensors to the incisors.

Ioannides et al. (2009) and Gonzalez et al. (2004) agreed that EMG measurements were effective and stable to be used in relating muscle activities with food properties. Agrawal et al. (1998) combined the EMG measurements from the left and right temporalis muscles and found that the food properties had a significant relationship with neuromuscular activity during human mastication. Proschel and Hofmann (1988) traced the incisor movements during chewing of food and showed that the varieties of jaw movements are dependent on the resistance of food. Peyron et al. (1996), Lassauzay et al. (2000), Peyron et al. (2002), Peyron et al. (2004a), and Foster et al. (2006) recorded EMG muscle activities and traced the movement of incisor during chewing of various foods and found both the EMG and jaw movement parameters were clearly affected by the food texture.

Investigators have emphasized that the tracking equipments were non-invasive and cause very little interferences to normal chewing behaviours (Peyron et al., 1996; Gonzalez et al., 2001b). Takada et al. (1996) combined the traces from incisor movements and measurements of muscle activities to study the relationship between jaw and lip muscles activities and human jaw movement. The authors confirmed that there were no noticeable differences in jaw movement trajectories when observations were performed with and without electrodes assembled in the mouth. However, Po et al. (2011) claimed that by altering subjects' conditions (i.e. attachment of surface EMG electrodes on skin during chewing) may affect habitual chewing activities. The positioning of sensors and electrodes may possibly interfere with chewing action causing discomfort to subjects hence impeding natural chewing function. Hence, more research is needed to investigate the effect of these tracking equipments on human natural masticatory function and how they can be used to understand the link between sensory perceptions and chewing dynamics.

## 8.2 Materials and Methods

### 8.2.1 Model Food System

A new batch of biscuit model samples of increasing sugar to fat ratios was manufactured for this work. The samples were manufactured using a similar process as described in Section 3.1.2. The measures of hardness are expressed in critical stress intensity factor as shown in Table 8.1.

**Table 8.1: Results from single-edge notched bend (SENB) of each recipe (mean ± SD)**

| Samples | *Critical stress intensity factor ($K_{IC}$, kPa $m^{1/2}$) |
|---|---|
| SP1 | 25.09 ± 3.33 |
| SP7 | 44.61 ± 2.83 |
| SP9 | 65.90 ± 8.17 |

*procedure for instrumental test is described in Section 3.1.3

### 8.2.2 Subjects

Ten female subjects (with mean age 29.5 ± 3.4) participated in this work. Seven of the subjects were recruited from the previous TDS study and the other four subjects were newly recruited with no experience in any sensory analysis and were accepted as naive (untrained) subjects. These untrained subjects were recruited based on the criteria listed in Section 3.2.1. Only female subjects were recruited in the study, hence gender-related differences in masticatory movement can be ignored (Nagasawa, Yanbin, Tsuga, & Abe, 1997).

### 8.2.3 Experimental Procedures

Preliminary Trial

A preliminary study (with two female subjects) was conducted to determine the practicability and the best procedures of simultaneous operation for the Temporal Dominance of Sensations (TDS) technique, electromyography (EMG), and articulograph (EMA). Preliminary trials were performed to ensure the experimenter was proficient in operating the instruments and data analysis. Steps to assess and minimise noise, to minimise movement of the reference sensors (critical for normalising jaw movement) and to minimise interference were conducted.

From preliminary results, it appeared that the masseter showed larger and clearer onset and offset during jaw closing. There was virtually no EMG activity with less clear bursts of activity for the temporalis during chewing. Therefore, only the masseter muscles were selected for further exploration. Preliminary trials also showed that it was feasible to add the measurement of movements related to jaw closing (the vertical, lateral, and anterior-posterior displacements).

Day 1: Familiarization Session

The definition of texture, dominance, and sequence were explained to the subjects. The definitions of attributes used (*hard*, *crumbly*, *dry*, *crunchy/crispy*, and *sticky*) were introduced using commercial food products as outlined in Table 6.2 (Section 6.2.3). The session continued with familiarization of the systems used. This session was conducted to ensure subjects were comfortable with the environment, the experiment protocols and the software. Experiments were done in a quiet and private room; this was to reduce any potential embarrassment for the subject and to avoid disturbances whilst performing the assessment.

Then, subjects were seated comfortably in the centre of the EMA cube. They were asked to refrain from moving their head during recordings. During an evaluation, a subject was instructed to put food into the mouth and click on the *START* button when signalled by the researcher (this is to simultaneously start the other two instruments by the researcher). To create a reference position for analysis, subjects were asked to hold onto the samples on the centre of the tongue (teeth fitted together) for 3 seconds before taking their first bite to start chewing and evaluation. During chewing, subjects performed the TDS task (selecting the attribute that triggers the most attention) and clicked on the *Swallow* button if intermediary swallows occurred. When the major swallow occurred, subjects were instructed to click on the *STOP* button and were instructed to hold for 5 seconds (occluding their teeth). This was used as the baseline for data analysis (to normalise jaw movements). They were also warned that they were to signal if they sensed any discomfort such as the likelihood to cough, so the recording could be aborted. Each subject evaluated samples SP1, SP7, and SP9 once.

Day 2: Formal Assessment

Formal assessments were conducted between 2-4 days after the familiarization session. Similar procedures during familiarization session were performed. Data were collected by simultaneously operating the three instruments, sensory software (TDS), the electromyograph (EMG), and the electromagnetic articulograph (EMA). Each recording involved the subject chewing on one sample (from any of the three samples with different levels of hardness). Subjects chewed and evaluated eleven model food samples (two warm up samples and nine research samples) whilst chewing behaviours were monitored. The research samples were presented in randomised blocks of three. Data for the first two samples were discarded. Breaks were imposed between recordings to overcome fatigue. Assessment resumed once subjects had enough rest and were confident to continue. During a break, subjects were free to move, speak, and drink water. Each session took about an hour.

### 8.2.4 Data Collection

Temporal Dominance of Sensations (TDS)

TDS data were collected using an in-house written program where the sequence of mouse clicks was recorded and time stamped during evaluation (Labview 2009, National Instruments Corporation, Austin). The collection of TDS data has been comprehensively discussed in Section 3.3.1.

Recording of Mandibular Movements

Jaw movements were recorded using a three-dimensional magnetic sensing system called an articulograph (Carstens AG500, Carstens Medizinelectronik, Lenglern, Germany) which measures the movement of sensors fixed to the participant's teeth within a magnetic field.

Prior to the recording, sensors were coated in Plasty-late one night earlier. The EMA machine was warmed up for at least two hours ahead of the experiment. Prior to experiment, three sensors were temporarily glued to subject's teeth (lower right and left canine, and one on lower middle incisor) and two references sensors were fixed behind the ears. Kitchen paper was used to dry the teeth before an appropriate amount of glue (Cyano Veneer adhesive glue, Hager & Werken GmbH & Co. KG) attached to the sensor and it was secured on the marked location by tweezers. The ground wire was then clamped to one wrist of subject. The articulograph's sensors were placed slightly apart and were glued closer to the gum to prevent subjects chewing on the sensor's wire. An adequate amount of wire was allowed between the teeth and mouth to facilitate chewing (excessive wires coming out from the mouth were securely attached to the chin with adhesive tape).

During the experiment, jaw movements data were collected with mc5recorder from the AG500 program package (http://articulograph.com/). When the experiment finished, the sensors were carefully removed and disinfected by submerging in 95% ethanol for 30 minutes. Collected data were analysed with the AG 500 program package running under windows (CalcPos, HelpCalcpos, NormPos, Bin2ASII) (http://articulograph.com/).

Recording of Muscle Activities

Muscle activities were measured by surface electromyography (EMG) (Powerlab/4SP (ML750), ADInstruments Pty Ltd, Australia). The data acquisition system recorded the electric activities and data appeared as a series of bursts which correspond to the muscle activities. EMG signals were recorded from both the left and right masseters.

Prior to the recording, skin around the left and right masseters were cleaned with alcohol swabs (Alcohol Swabs: MLA1094, ADInstruments) and electrodes (Disposable EMG electrodes: MLA1010B, ADInstruments) were attached with an abrasive gel (Abrasive Gel: MLA1093B, ADInstruments). Positive and negative electrodes were placed on the muscle, and a ground electrode was placed on a bony piece of the body (the collarbones) for reference. The site of electrode placement was established by palpation during jaw clenching. To maintain good contact, adhesive tape was added for support.

During the experiment, muscle activities are collected using computer software (Chart v5.5 for Window, ADinstrument).

### 8.2.5 Data Analysis

Temporal Dominance of Sensations (TDS)

TDS curves were computed by considering each attribute individually for each point of time. The dominance rates from twenty subjects were calculated and were plotted against standardized mastication time (procedures have been comprehensively described in Section 3.4.1.

Oral Processing Behaviours

Combined EMA and EMG recordings were exported to Matlab (the MathWorks, Inc., US) for further data analysis. The chewing durations were the time from the beginning of the first mouth opening (first cycle) to the end of the last closing of the mouth, including swallowing (determined with data from EMA and EMG). The numbers of chewing cycles were counted and were defined as total number of cycles used to masticate a sample during the sequence. A complete cycle was defined from a complete jaw opening and jaw closing. A cycle was considered as irregular if it did not reach the *resting* position (very close to baseline). Chewing frequency was calculated from these data (number of chewing cycles/chewing duration).

EMA Signals

Raw data were processed (removal of head movements by means of reference sensors) to correspond with the three coordinate planes of motion; x (anterior-posterior), y (superior-inferior), and z (lateral-medial) and two angles; φ (tilt) and υ (yaw) (which were not used). The corrected data was then processed following instructions provided by the manufacturer (Carstern, 2012). The x-coordinate corresponds to the front and back movement of the jaw; y-coordinate corresponds to the left and right movement of the jaw and z-coordinate corresponds to the up and down movement of the jaw. These coordinates were plotted against mastication time. The y-axis shows the displacement of the mandible in millimetres (mm) and the x-axis shows time in seconds. The start of first cycle was manually marked from the beginning of the z-coordinate (down movement of the jaw). Each sequence was analyzed separately for each subject. The jaw displacements were measured with reference to a baseline created from after the *STOP* button was selected; when there was no more food left in the mouth and hence smoother reference line. The line created after the *START* button was not used for this purpose. The line may have cause confusion in data analysis because there was occurrence of uneven jaw displacement when the teeth were held together. This was probably caused by improper occlusal (holding teeth together) from the holding of samples in the mouth at the start of an evaluation.

EMG Signals

The raw EMG signals were high-pass filtered with 15Hs cut-off frequency and rectified using the root mean square. The start and the end of each EMG burst throughout a masticatory sequence were identified (manually by researcher).

## 8.3 Results

### 8.3.1 Temporal Dominance of Sensations (TDS)

Figure 8.1 shows that from the first bite, the least hard sample (SP1) exhibited distinctive textural perceptions compared to the two harder samples. Attributes *crumbly* was mainly selected for samples SP1 and SP7 and *hard* was used to describe sample SP9 during the early stage of a mastication sequence. During mid-chewing, both sample SP1 and SP7 were clearly described as *dry* and sample SP9 was described as *crunchy/crispy*. At the clearance stage, the attribute *sticky* was the main descriptor. These results were similar to TDS profiles collected in previous experiments, simply indicating that subjects were consistent and able to distinguish between the samples of different compositions.

### 8.3.2 Oral Processing Behaviours

Tables 8.2 shows masticatory parameters extracted from the simultaneous EMG and EMA recordings for subjects chewing on samples SP1, SP7, and SP9. Statistical analysis indicated that there was no effect of sample's hardness on the mastication parameters, indicating subjects used similar chewing strategies for all samples.

**Table 8.2: Mastication parameters extracted from the simultaneous recording of samples SP1, SP7, and SP9 (mean ± SD) (n=10)**

| Samples | Chewing duration(s) | Number of cycles | Chewing frequency (1/s) |
|---|---|---|---|
| SP1 | $32.2 \pm 11.5^a$ | $33.0 \pm 9.2^a$ | $1.06 \pm 0.21^a$ |
| SP7 | $32.0 \pm 11.0^a$ | $34.6 \pm 11.4^a$ | $1.10 \pm 0.23^a$ |
| SP9 | $32.3 \pm 11.0^a$ | $36.0 \pm 11.8^a$ | $1.14 \pm 0.30^a$ |

Same letter (a) indicates no significant difference between samples ($p > 0.05$)

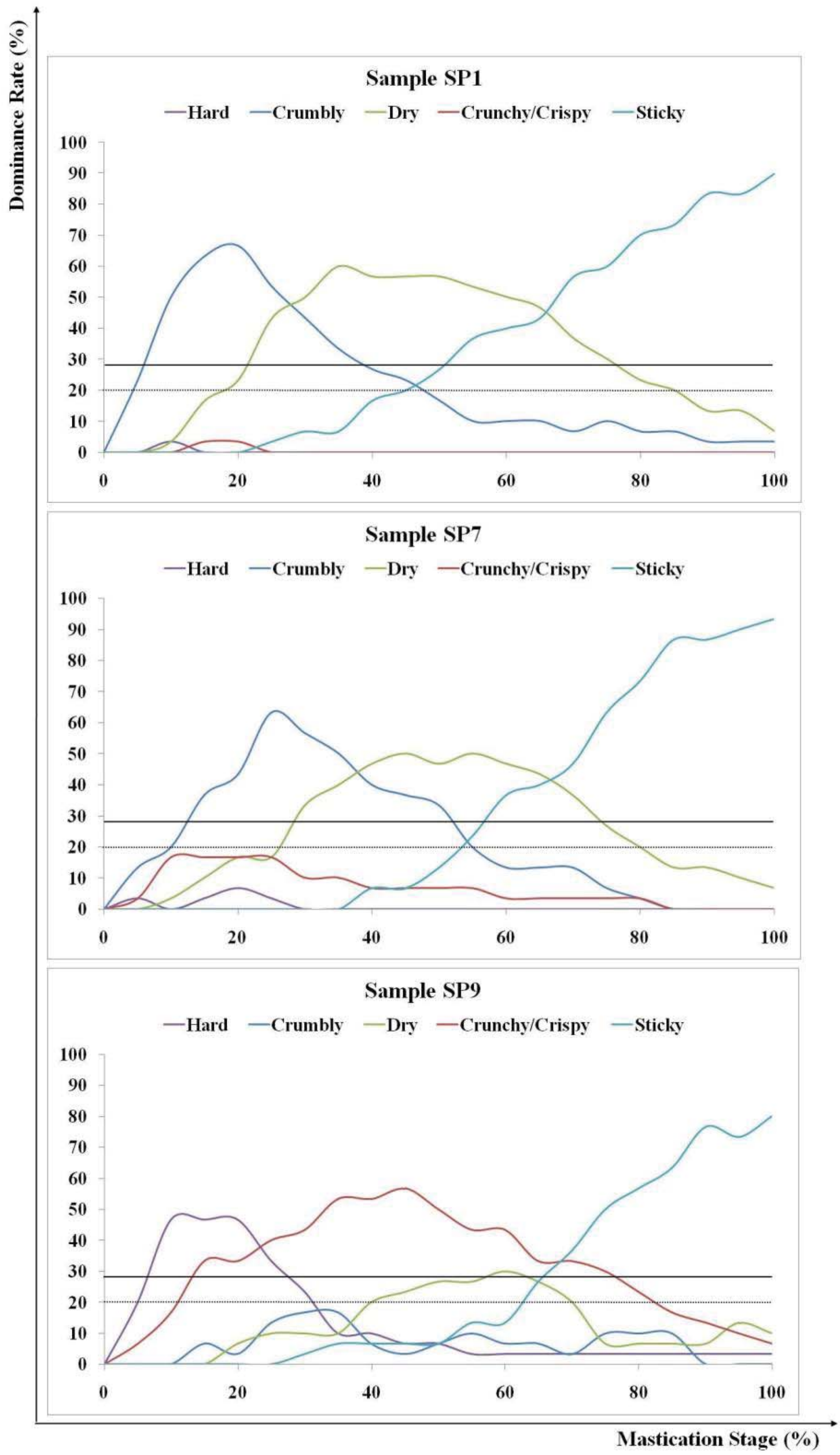



**Figure 8.1: TDS curves computed for samples SP1, SP7, and SP9 during simultaneous recording of TDS, EMG and EMA. Chance level is represented with dotted line and significant level is represented with solid line (n=10)**

### 8.3.3 Effect of Simultaneous Recordings on Sensory Perception and Oral Processing Behaviours (n=7)

Data from simultaneous recordings (combinations of TDS, EMG, and EMA) were extracted to investigate the effect of attaching electrodes and sensors to subjects on the oral processing behaviours and TDS performance. From the ten subjects who participated in this experiment, only data from seven repeating subjects were extracted to study this effect. These seven subjects participated in all experiments outlined in Chapter 4, 5, 6, and 7. Hence, there was oral processing data available when not performing any task, when performing the sensory evaluation after minimal and moderate training and also when muscle activity and jaw movement information were collected. With regards to the sensory evaluations, there was data after minimal and moderate training of attributes. Only data from sample SP1 were compared due to its textural similarity for all previous experiments. It should also be noted that results analysed using data from the seven repeating subjects may be different if more subjects were involved.

Temporal Dominance of Sensations (TDS)

TDS curves produced after minimal and moderate training showed that *crumbly* was significantly dominate (significance level > 0.28) at 10% of the mastication stage, followed by *dry* at 30% and *sticky* at 55% of the mastication stage (Figure 8.2). However, the appearance of these was earlier (*crumbly*, *dry*, and *sticky* at 5%, 25%, and 50% of the mastication stages, respectively) during simultaneous recordings. In addition, higher dominance rates were also observed. This indicated that subjects were able to draw the same conclusion although TDS was performed under different circumstances. It also suggests that the subjects became more confident in their selections over time as they made earlier dominant attribute selections.

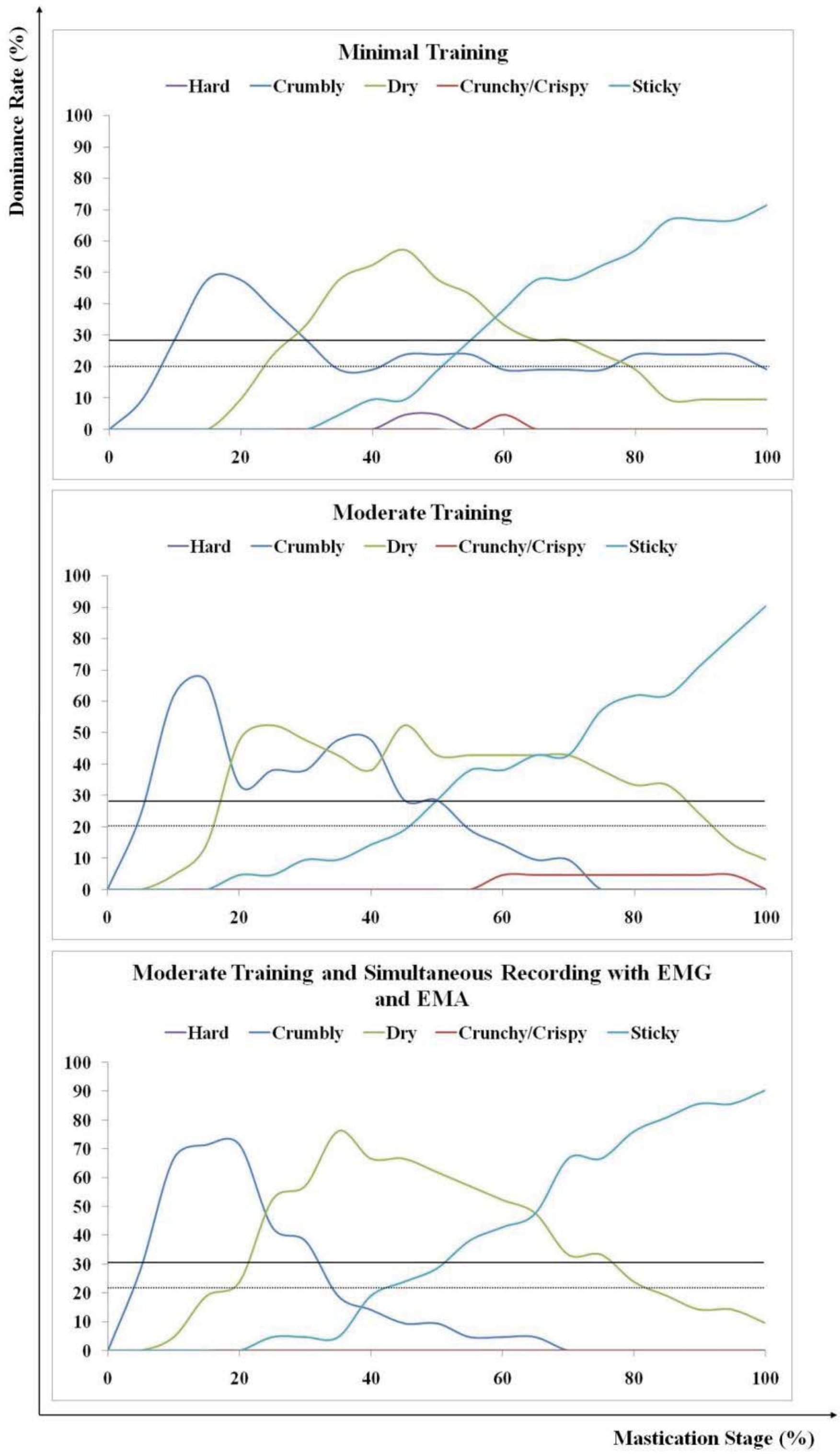



**Figure 8.2: TDS curves computed when subjects chewed on sample SP1 while performing TDS task (minimal training, moderate training, and during simultaneous recording) (n=7)**

Oral Processing Behaviours

No effect of simultaneous recordings of TDS, EMG, and EMA was observed (Table 8.3). This indicates no effect of attaching the electrodes and sensors to subjects during oral processing. Although no significant effect was indicated by statistical analysis, it is worth noting that longer chewing durations were used during simultaneous recordings. The mean masticatory parameters form the seven repeating subjects when they chewed on sample SP1 were similar across previous experiments. This indicates that similar chewing strategies were used although subjects were placed in a different environment.

**Table 8.3: Effect of simultaneous recording on oral processing behaviours on sample SP1 (mean ± SD and range of values) (n=7)**

| Experiments | Chewing duration(s) | Number of cycles | Chewing frequency (1/s) |
|---|---|---|---|
| Minimal training | 30.6 ± 9.3[a] (20.3 – 54.3) | 37.6 ± 11.8[a] (23 - 66) | 1.23 ± 0.17[a] (0.85 – 1.58) |
| Moderate training | 31.5 ± 7.4[a] (17.5 – 45.7) | 36.1 ±6.4[a] (25 - 45) | 1.18 ± 0.22[a] (0.85 – 1.60) |
| Moderate training and simultaneous recordings | 34.9 ± 12.3[a] (16.8 – 67.2) | 36.7 ± 8.1[a] (20 - 53) | 1.10 ± 0.22[a] (0.74 – 1.61) |

Same letter (a) indicates no significant difference between experiments ($p > 0.05$)

An effect of moderate training with simultaneous recordings on the time to select the first dominant attribute was observed (Table 8.4). One-way ANOVA analysis showed that the time to select the first dominant attribute when minimal training was given was significantly longer than the times required after moderate training and during simultaneous recordings. Subjects were faster at deciding the first dominant attribute after moderate training (2.8 s) and during simultaneous recordings (3.3 s). The range of times used was reduced significantly after the training session. This is probably due to subject being more familiar with the techniques, attributes and definitions. This indicates the importance of familiarization sessions to the experiment environment.

**Table 8.4: Effect of simultaneous recording on TDS performance on sample SP1 (mean ± SD and range of values) (n=7)**

| Experiments | Time to first attribute selection(s) | Proportions of time to first attribute selection (%) |
|---|---|---|
| Minimal training | $6.3 \pm 5.2^{a}$<br>(1.8 – 22.1) | $22.1 \pm 18.0^{a}$<br>(4.0 – 77.9) |
| Moderate training | $2.8 \pm 1.5^{b}$<br>(1.3 – 6.8) | $10.2 \pm 8.1^{b}$<br>(3.2 – 32.8) |
| Moderate training and simultaneous recordings | $3.3 \pm 1.5^{b}$<br>(0.8 – 6.5) | $10.2 \pm 5.2^{b}$<br>(3.7 – 22.2) |

Different letters (a,b) indicate a significant difference between experiments ($p < 0.05$)

### 8.3.4 The Mastication Sequence

Figures 8.3 to 8.5 show different chewing strategies were used for samples of differing levels of hardness throughout a mastication sequence. Different chewing strategies were used in chewing samples with different levels of hardness. This observation was expected as chewing behaviours have shown to be influenced by food properties (Peyron et al., 1997; Kohyama et al., 2004; Foster et al., 2006).

In general, the first chew was commonly represented with two incomplete cycles. This was probably due to the exploratory phase at this stage. Subjects may have explored the sample with first bite before deciding on the strategy for the next chew. Wider jaw movements were also observed during the early stages of mastication, which may have induced slower chewing rates. Opening and closing pathways tended to be widely separated when chewing on hard samples than the least hard sample. During mid-chewing, the cycles were quite rhythmical (consistent vertical displacements) with closer and sharper jaw cycle. Towards the end of mastication, odd jaw movements were observed, in particular when swallowing occurred.

Most chewing work was done on food during the late closing of the jaw. When the jaw closes, the masseter muscles were activated during the mechanical reduction of food particle size by generating masticatory forces between the teeth. Larger vertical amplitudes had larger masseter muscle activities, and vice versa. It is interesting to note that at some points there were no EMG activities recorded but vertical movements can be seen. At these points, other jaw movements such as anterior-posterior and lateral movements became dominant. These movements activated other adjacent muscles responsible for the movements that may not have been recorded. This explained why there were no EMG activities recorded at some points of time during a mastication sequence.

Higher EMG signals responded to harder samples and the sensory terms used mainly associated to fracture mechanism. The first bite is mainly used to fracture a sample and hence a higher shift in the lateral movements can be seen in some subjects during this stage. Prior to the first bite, subject repositioned the sample to the molars to begin chewing. It was at the first cycle that higher side to side movements were noticeable as an effect from repositioning food. In addition, some subjects portrayed larger lateral movements than others to some extent. This may be due to the food size some subjects may find it too big to be consumed. Häggman-Henrikson and Eriksson (2004) suggested that a small shift can be seen before the start of the first chewing cycle (known as the preparatory head extension) when chewing on larger food size. It was also observed that subjects used at least two cycles to fracture a sample before selecting the first dominant attribute. These initial cycles can sometimes be divided into two lower peaks, in which the second peaks were often shorter or equal to the first peak. Hence, these initial cycles can be concluded as the exploratory cycles targeted for sensory judgement (Dan et al., 2007). Hence the repositioning of food and exploratory phase of a sample may have induced wider jaw movements.

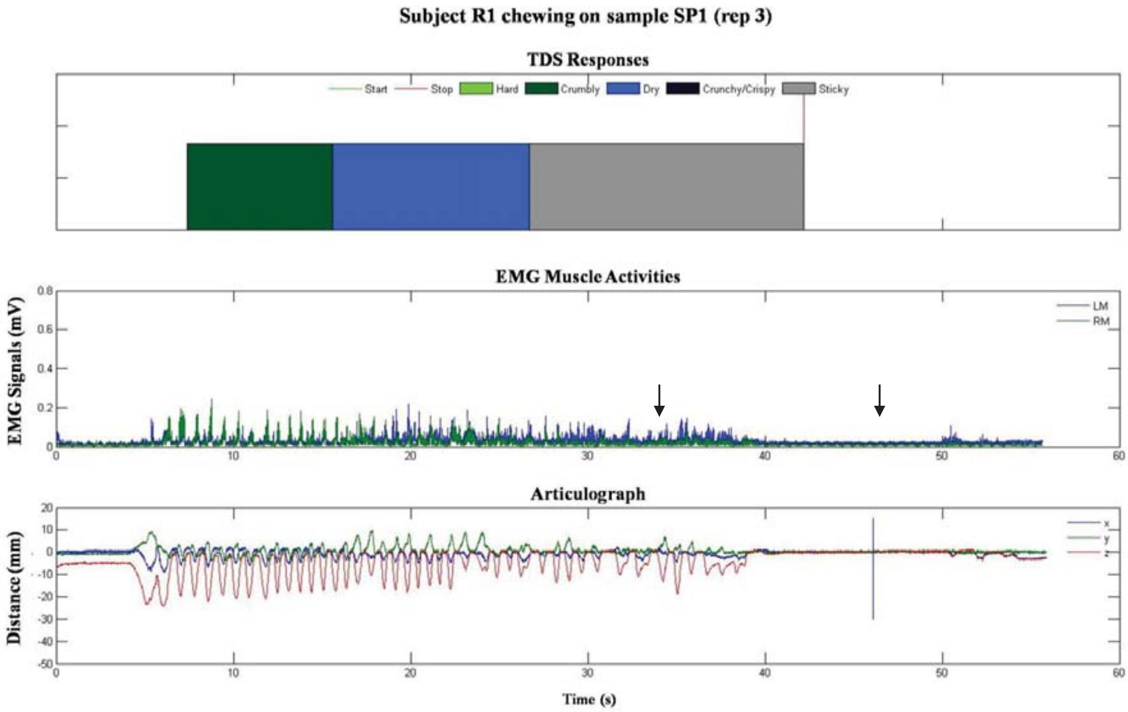



**Figure 8.3: Example chosen to show results from simultaneous recording of TDS, EMG (LM: Left Masseter; RM: Right Masseter) and EMA (x: anterior-posterior; y: horizontal; z: vertical) for sample SP1. Arrows indicating swallows**

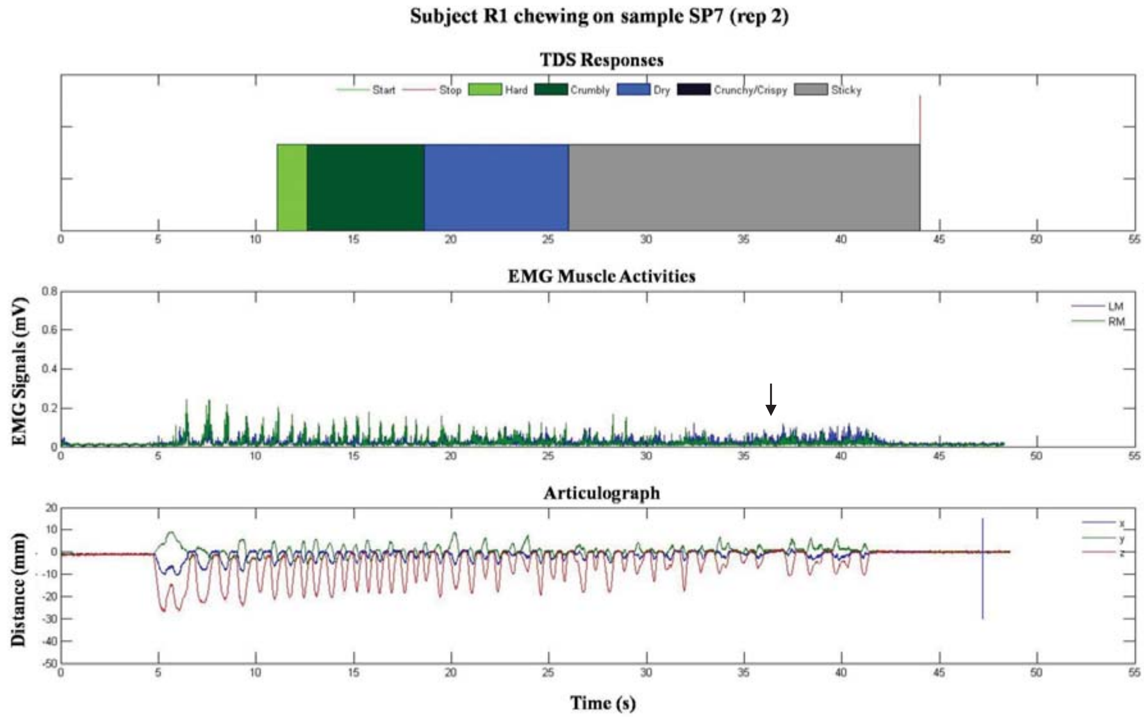



**Figure 8.4: Example chosen to show results from simultaneous recording of TDS, EMG (LM: Left Masseter; RM: Right Masseter) and EMA (x: anterior-posterior; y: horizontal; z: vertical) for sample SP7. Arrow indicating swallows**

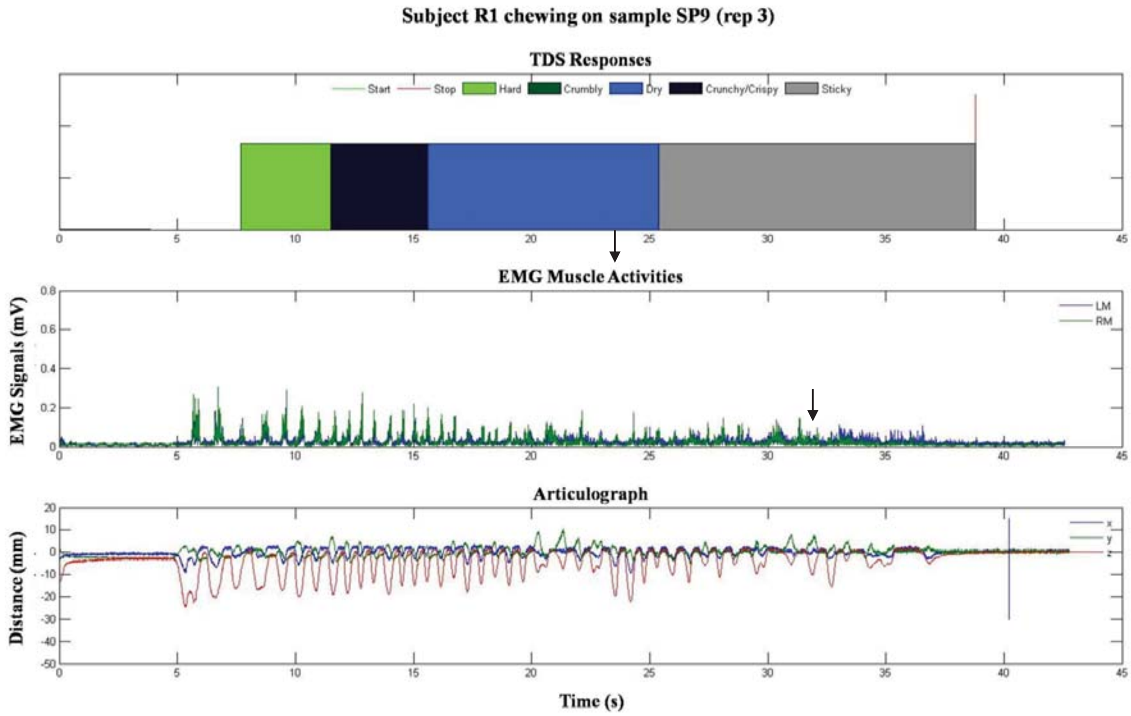



**Figure 8.5: Example chosen to show results from simultaneous recording of TDS, EMG (LM: Left Masseter; RM: Right Masseter) and EMA (x: anterior-posterior; y: horizontal; z: vertical) for sample SP9. Arrow indicating swallows**

## 8.4 Discussion

### 8.4.1 The Combined Recording of TDS, EMG, and EMA: Interaction between Changes in Structural Aspects of Food with Sensory Perception and Oral Processing Strategies

No change in subject's oral processing behaviours were observed after minimal or moderate training and chewing with simultaneous recording of jaw movements and muscle activities. No effect was indicated by statistical analysis. This also indicates that there is no time limit for the simultaneous recordings.

The figures indicated variations of chewing strategies used in masticating samples of different compositions. During first bite, the jaw was first opened for first compression of the sample, followed by closing movement of the jaw where the masseter muscles were activated. During this stage, attributes related to fracture properties like *hard* and *crunchy/crispy* were selected for samples SP7 and SP9, and *crumbly* was often selected for sample SP1.

It is also interesting to note that shorter time was taken to make their first attribute selection during the simultaneous recordings. Previous results have also indicated that subjects were faster at selecting their first dominant attribute after moderate training compared to minimal training. In addition, better subject's performance was also obtained after moderate training. Hence, subjects were expected to be better and quicker in terms of first attribute selection during simultaneous recording of TDS, EMG, and EMA. Therefore this is not likely to be an effect of the attachments of EMG and EMA sensors but rather due to increased familiarity with the technique and foods.

The most obvious variations can be observed by comparing consecutive cycles. Despite a standard complete jaw opening-closing, high variability can be seen by comparing consecutive chews. The anterior-posterior (x), lateral (y), and vertical (z) movements differed from cycle to cycle. There could be two reasons for these variations of jaw movement, 1) there is random variation in natural jaw movement (Gerstner & Cianfarani, 1998) and 2) there is an ongoing modulation by sensory feedback (Agrawal et al., 1997; Brown & Braxton, 2000; Foster et al., 2006). Human has no standard chewing cycle and there are never two chewing cycles that are exactly the same. This could be the reason why jaw movements vary from chew to chew. In fact, Kobayashi, Shiga, Arakawa, Yokoyama and Nakajima (2009) managed to classify human mandibular movement into 3-8 patterns, Proschel and Hofmann (1988) proposed eight patterns of classifications and Kobayashi et al. (2009) identified seven different types of chewing cycle in a group of 100 healthy subjects chewing on softened chewing gum.

Further into a chewing sequence, the cycles became more regular and rhythmical compared to judgemental biting (Dan et al., 2007). The number of cycles used in this stage largely depends on the food state, volume and individual chewing pattern, which varies between foods and the time that a sample is progressively processed in the mouth (Heath, 2002). At this stage, combinations of several attributes such as *crunchy/crispy*, *crumbly,* and *dry* were used to describe all the samples. Sensory terms evaluated after several cycles tend to be less predictable based on the physical properties of food (Brown et al., 2003; de Wijk, Engelen, Prinz, & Weenen, 2003). Samples SP7 and SP9 tended to produce larger lateral deviations than the less hard sample (SP1). The harder samples were also chewed more rhythmically and consistently, probably because of the crushing of food sample for particle size reduction. In each cycle, the sample was compressed towards the cheek, palate and tongue, and coated with saliva and then reformed into a new bolus between the teeth when the jaw opened. It is during this stage where sensations were perceived from the bolus surface that contributed to the textural perception perceived (Peyron, et al.2002). It is also during this closing phase where larger lateral movements can be observed. Rilo, Fernandez, Da Silva, Martinez and Santana (2001) have shown positive correlations between the closing phase of a cycle to the lateral path of the jaw. Food that required more chewing effort and greater pathway excursion often prompted *hard* and *crunchy/crispy* being selected. Food that required less chewing effort and was relatively soft and easy to be broken down was often described as *crumbly*. This increased the surface properties and therefore fast absorption of saliva often prompted the sensation of *dry*. When more saliva was likely needed to moisten a food (an absence of water), *dry* was often the dominant attribute selected.

As the chewing progressed, the continuous reformed bolus was being picked up around the tongue and the palate to form cohesive aggregates. The act of picking up clusters and pasty bolus particles from around the mouth can be observed from the emerging of irregular cycles and odd jaw movements (cycles that do not go back to resting position) towards the end of a mastication stage. This explained the need for slower and wider cycles to bind those particles together with fluid (mostly saliva) into a cohesive aggregate so that the food bolus can be swallowed (Prinz & Lucas, 1997). At this stage, the force required to remove the adhering material may have driven the selection of *sticky*. Reduction in vertical movements (approximately 20mm) and muscle activities (approximately 0.2 mV) with increased of odd cycle were observed at this last stage of mastication. Kohyama and Mioche (2004) observed similar gradual decrease of muscle activities and vertical movements at the last stage of the mastication process when subjects were asked to chew foods with different textures.

Overall, larger EMA amplitudes and EMG bursts corresponded to a fracture mechanism in which attributes such as *hard*, *crunchy/crispy*, and *crumbly* were perceived. The emergence of irregular movements corresponded to the preparation of the bolus for safe swallowing, in which attributes such as *dry* and *sticky* were mainly perceived. It can also be concluded that the start of irregular movements may be the cue for selecting attribute *sticky*. When irregular movements occurred, there was almost no EMG activity and minimum vertical movement but more lateral and anterior-posterior movements which means the food materials were being manipulated in the mouth to prepare for swallowing. Foster et al. (2006) demonstrated that sticky plastic caramel products required greater lateral movements than less sticky elastic gel based products.

## 8.5 Conclusion

Combinations of results from simultaneous recording of TDS, EMG, and EMA have shown to be of benefits for studying human mastication in food texture research. This confirmed that to better understand human mastication and food textural perception, a combination of techniques is required to capture the dynamic nature of food oral processing. Conclusions from the experiment were that 1) the analysis was possible because of the integrated system of TDS, EMG, and EMA, 2) the technique was reliable and practical and can be used in studying how changes of structural aspects of food interact with oral processing strategies and sensory perceptions from first bite to swallowing, 3) there was no time limit in monitoring the whole mastication sequence using the simultaneous recordings, 4) almost every type of solid food can be tested, extending the range of products in food texture research.

This study also confirmed that oral processing behaviour is altered in response to the changes of texture in the mouth. An increase of hardness in samples required increase of muscle activity and jaw movements during the early stage of a chewing sequence. Chewing patterns were more rhythmical and varied depending on the state of food in the mouth throughout oral processing. During chewing of sticky samples towards the end of a chewing sequence, jaw movements were irregular; wider vertical movements were required to remove materials from the oral surfaces.

# CHAPTER 9

## GENERAL CONCLUSIONS AND RECOMMENDATIONS

The simultaneous recordings of sensory perception and oral processing behaviours are relatively simple and reliable for understanding the relationship between eating physiology, food structure, and sensory responses.

Temporal Dominance of Sensations (TDS) technique has shown its capability to describe the perception dynamics in the mouth is determined by the product structure and physical breakdown in the mouth. It is noted that a sample of different compositions (complexity in terms of ingredient) starts off with only one attribute and will end with only one attribute.

Although TDS is suitable to be used in dynamic sensory evaluation of solid food system, the complexity of a product can be clearly seen during mid-chewing (from TDS curves). It was thought that this may be due to lack of attribute understanding. Sensory attribute training has proven to enhance TDS performance and have no effect on the oral processing behaviours (except for the number of cycles). Subjects were better in recognizing the texture and has proven that the attributes were used appropriately representing their sensations and bolus properties. Since subjects have interpreted the attributes correctly, this can only mean that no attribute is more dominant than other attributes during mid-chewing.

This study indicates that the early chewing stage is dedicated to the fracture mechanism (the mechanical properties), the mid chewing is dedicated to effort used to masticate a food into a safe swallow bolus which includes effort to reduce food particle sizes and effort to remove food materials from around the mouth whereas the end of a chewing sequence is often dedicated to the effort to remove food materials from around the mouth for swallow. These results suggested that instead of chewing a food until it is broken up thoroughly, perhaps the food should be chewed until it sticks together before swallow.

With information of how the sensory task may affect oral processing behaviours and the benefit of providing sensory attribute training, this study confirmed the multi parameters nature of texture and that a complete understanding of texture can only be obtained through collaboration among different disciplines; the simultaneous measurements of jaw-muscle electromyography, and jaw movements during mastication. Two main attributes, structural and physical properties at different stages of a mastication sequence had key impact on the breakdown properties.

The present study has collected a considerable amount of data on model food system and suggested that food hardness is not the only property responsible for chewing strategy, but a range of different food structures at different mastication points are responsible. The variation of chewing series which involves cycles of repetitive jaw movements (vertical, horizontal, and lateral directions) and masticatory muscular activities (masseter) constantly change throughout the chewing sequence in response to the changing physical and chemical properties of foods in the mouth.

However, the information on the effect of food structure on oral processing behaviours and sensory sensations is only limited to one bite size of sample. Therefore, future research is crucial to determine the effect of not only the next bite of food, but multiple bite sizes. Then, experiments could extend to how the mastication parameters are affected during consumption of a full meal. The "real" everyday food with complexity may present a much bigger problem. Food is taken into the mouth in various quantities and sometimes, the second bite is taken even before the first bite of food is fully masticated or swallowed.

Next, it may be worthwhile investigating the potential of the technique in product development and optimization. One of the main interests during this process is to detect and understand the differences between products and how these relate to consumer liking or acceptability. The TDS technique has the potential of not only detecting the differentiating (dominant) attributes, but an effective method to determine point at which the changes develop. This serves as a basic for making claims about a product performance for a food company.

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# APPENDIX A

## Screening Questionnaire

***Relating sensory perception to chewing dynamics and the production of a bolus***

## SCREENING QUESTIONNAIRE

Please note that all information provided will be treated as confidential.
Please not that information in this screening questionnaire may be forwarded to other researcher within this research team.

**Participant's Information**

| | |
|---|---|
| Code No | |
| Age | |
| Gender | |
| Height (m) | |
| Weight (Kg) | |

**Health and Safety Question:**

| | | |
|---|---|---|
| Are you in general good health? | Yes | No |
| Are you pregnant? | Yes | No |
| Do you smoke? | Yes | No |
| Are you taking any medication which affects muscle function or saliva flow? | Yes | No |
| *Do you suffer from any blood borne or infectious disease?* | *Yes* | *No* |
| Are you allergic to any of the ingredients on the list below?<br><br>1. Flour<br>2. Sucrose<br>3. Oil/Margarine<br>4. Salt<br>5. Baking Powder<br>6. Emulsifier [sodium stearoyl lactylate (SSL) or ester of monoglycerides (Datem)]<br>7. Water<br>8. Vanilla essence<br>9. Spices (mixed spices, cinnamon)<br><br>* Please check carefully the ingredients listed above in case you have allergies to or are intolerant to any of the ingredients. | Yes | No |
| Are there any foods you have difficulty chewing?<br>If yes, what is it? _________ | Yes | No |

**Dental Health Question:**

| | | |
|---|---|---|
| Do you wear dentures or have any prosthetic teeth? | Yes | No |
| Do you have any teeth missing or loose?<br>If yes, How many? ________<br>Tooth Type? __________ | Yes | No |
| Have you had any major dental work carried out in the last six months? | Yes | No |
| Do you generally chew on one side of the mouth?<br>If yes, which side? _______ | Yes | No |
| Are you right or left-handed? | Right | Left |
| Do you grind your teeth? | Yes | No |
| Do you feel any tooth pain when eating foods? | Yes | No |

## APPENDIX B

**Massey University Human Ethics Committee: Southern A (Application 10/13)**

MASSEY UNIVERSITY

19 April 2010

Jean Ne Cheong
IFNHH
**ALBANY**

Dear Jean Ne

**Re: HEC: Southern A Application – 10/13**
**Relating sensory perception to chewing dynamics and the production of a bolus**

Thank you for your letter dated 19 April 2010.

On behalf of the Massey University Human Ethics Committee: Southern A, I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

A/Prof Hugh Morton, Acting Chair
**Massey University Human Ethics Committee: Southern A**

cc Dr Kylie Foster, Dr John Grigor
IFNHH
ALBANY

Mr Marco Morgenstern
IFNHH
ALBANY

A/Prof John Bronlund
SEAT
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